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Meeting Micronutrient Requirements for Health and Development

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Preface

In the past two decades, micronutrients have been recognized as having a more prominent position in the global effort to control and overcome hunger and malnutrition. The three micronutrients whose deficiencies have the greatest prevalence and have attracted the greatest attention from international agencies and as well as institutions and not-for-profit agencies are vitamin A, iodine and iron. Recently, zinc has received much attention for its role in the treatment of diarrhea and its preventive potential for childhood diarrhea and pneumonia. Even with this increased attention and enhanced research, a number of important questions remain. What is the importance of these micronutrients and others, including folate and vitamin B₁₂, as limiting nutrients in growth and development and their potential for preventing stunting and global malnutrition? What are the forms and doses of these micronutrients that may be required for the prevention of stunting and malnutrition or the treatment of moderate and severe malnutrition? What are the effective interventions including individual or multiple micronutrient interventions, and what are the effective methods of addition of these micronutrients to the food supply and in the form of fortification? In which form are these micronutrients most effective and most appropriately or most effectively absorbed and bioavailable? Are there any potential safety concerns? Do interactions restrict potential coadministration? And perhaps most importantly, what are the appropriate target populations for interventions in treatment or prevention?

To address the current knowledge in these and other related questions, Nestlé Nutrition Institute invited three nutrition scientists to organize a symposium with other leading scientists knowledgeable and active in the field of micronutrient nutrition. These scientists were invited to give their presentations at the meeting in Cebu, Philippines, in April 2011, along with an invited group of knowledgeable leaders in the fields of pediatric nutrition and micronutrient nutrition from all parts of the world. The papers that were presented at this meeting and a summary of the discussions form the substance of this publication and report. We hope that this publication is another milestone in the path
towards a better understanding of the importance of micronutrients in nutrition and development, and that it will contribute to the growing urgency for the control of childhood malnutrition.

Zulfiqar A. Bhutta
Richard F. Hurrell
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Foreword

Meeting macro- and micronutrient requirements during pregnancy and early childhood (‘the first 1,000 days’) is crucial for short- and long-term health and cognitive function. Recent surveys on the timing of growth retardation and stunting indicate that the onset is maternal undernutrition before and during pregnancy. Inappropriate weaning food and frequent infections hamper growth and development during the ‘critical window’ of the first 24 months after birth. Stunting (178 million children worldwide) and severe acute malnutrition (wasting –19 million) are associated with micronutrient deficiencies. Epidemiology indicates that the most common micronutrient deficiencies – vitamin A, iron, zinc, and iodine – could be related to an estimated 1 million child deaths per year and 9% of global childhood DALYs [1]. Meta-analyses confirm that supplementation or fortification of food with the ‘big four’ is efficacious to reduce risk of infectious disease, improves growth and cognitive outcome. More recently, folate and vitamin B₁₂ deficiencies during pregnancy have been shown to be associated with poor neurodevelopmental outcome and childhood obesity. Because of the high prevalence of micronutrient deficiencies in populations from developing countries, the challenge is to develop the right intervention strategies. Governmental agencies and international non-governmental organizations need strong support from scientific institutions, and industry should cooperate with all relevant stakeholders.

The 70th Nestlé Nutrition Institute Workshop, which took place in March 2011 in Cebu, Philippines, was the third to address micronutrient needs. Two previous NNI workshops addressed the needs during the first months of life (NNIW 52) and the weaning period (NNIW 54). It became clear during the last years and was addressed at this workshop that maternal and fetal deficiencies can induce inadequate metabolic programming in the offspring with increased risk for non-communicable diseases later in life. In order to answer questions and lead scientific discussions, we asked world-renowned experts in the area of health science and nutrition to clarify the pathogenesis of micronutrient deficiencies in pregnancy and childhood, preventive methods and strategies, and opportunities for treatment.
To discuss the most recent findings and outcome strategies, leading experts in the fields of epidemiology and nutritional intervention met with those in genetics, epigenetics, and metabolic outcome. We would like to warmly acknowledge the excellent program conceived by the chairpersons – Prof. Zulfiqar A. Bhutta, Pakistan, Prof. Richard F. Hurrell, Switzerland, and Prof. Irwin H. Rosenberg, USA. We are also indebted to all the renowned speakers giving the presentations, and discussants leading the debates of this important topic. We thank all experts who came from across the globe to review and discuss the importance of the micronutrients in child life and the opportunity to meet them.

Finally, we wish to thank Dr. Marco Turini and Dr. Grace L. Uy with their teams from the Nestlé Nutrition Institutes in South East Asia – Philippines for excellent logistic support and hospitality which allowed the participants to enjoy both the scientific program and the wonderful cultural spirit of Cebu.

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Global Micronutrient Deficiencies in Childhood and Impact on Growth and Survival: Challenges and Opportunities

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Abstract
Despite numerous advances and improvements in child health, malnutrition still remains as one of the main public health challenges of the 21st century, particularly in developing countries. It undermines the survival, growth and development of children, and is associated with almost 35% of all deaths in children under the age of 5 years worldwide. An estimated 178 million children are stunted globally, and an additional 19 million children have severe acute malnutrition (wasting). These conditions are very often associated with concomitant micronutrient deficiencies, and among these, vitamin A, iron, zinc and iodine deficiencies are the most prevalent in childhood. Vitamin A and zinc deficiency is associated with an estimated 1 million child deaths and 9% of global childhood disability-adjusted life years. Recent data on the timing of growth retardation and stunting in infants suggest that the onset is commensurate with inappropriate complementary feeding and potentially compounded by maternal undernutrition and intrauterine growth retardation, and that the first 24 months represent a critical window of opportunity for intervention. Given the wide prevalence of multiple micronutrient deficiencies in malnourished children in developing countries, the challenge is to implement intervention strategies that combine appropriate infant and young child feeding with micronutrient interventions at scale. Emerging data from community intervention trials now provide evidence that this is both tangible and can lead to alleviation of childhood undernutrition. Some of these recent findings will be discussed.
and development of children, and is associated with almost 35% of all deaths in children under the age of 5 years worldwide [3]. An estimated 178 million children are stunted globally and an additional 19 million children have severe acute malnutrition (wasting) [3]. Many of these conditions are associated with concomitant micronutrient deficiencies, and among these, vitamin A, iron, zinc and iodine deficiencies are the most prevalent in childhood. Vitamin A and zinc deficiency is associated with an estimated 1 million child deaths and 9% of global childhood disability-adjusted life years [3]. Recent data on the timing of growth retardation and stunting in infants suggest that the onset is commensurate with inappropriate complementary feeding and potentially compounded by maternal undernutrition and intrauterine growth retardation, and that the first 24 months represent a critical window of opportunity for intervention [4].

The relationship between micronutrient deficiencies and increased risk of infections and mortality is well established [5–7]. Several micronutrients play an important role in the immune system, and are critical in determining the outcome of host microbe interactions [8, 9]. Infections on the other hand are a risk factor for malnutrition as during an episode of infection, there is a general decrease in nutrient intake, increase in losses (e.g. GI losses, fluid losses, etc.) and altered metabolic pathways [9]. This condition is more prevalent among poor children whose micronutrient status is already marginal, and they also have a high frequency of infectious disease. This leads to a vicious cycle where malnutrition is a health outcome as well as a risk factor for disease and exacerbation of malnutrition [10]. The resulting complex and mutually adverse interactions with infections constitute the major determinants of childhood morbidity and mortality among the underprivileged preschool children in several developing countries [11].

Knowledge of prevalence of micronutrient deficiencies and their role in childhood morbidity and mortality is of great importance in planning comprehensive strategies to promote child health and survival in developing countries [5]. The purpose of this chapter was to summarize the current knowledge on micronutrient deficiencies and their role in reducing morbidity and mortality during childhood. A literature search was conducted on PubMed, Cochrane library and WHO and UNICEF databases. We discuss the epidemiology, effective intervention and challenges and opportunities for vitamin A, zinc, iodine, iron and other micronutrients.

**Vitamin A**

Vitamin A has been termed an anti-infectious agent [12, 13], and it plays an important role in the visual system [14]. Vitamin A deficiency (VAD) impairs numerous body functions, and can lead to many adverse health consequences including xerophthalmia (dry eyes), infectious morbidity, mortality, suboptimal
Micronutrient Deficiencies in Childhood and Impact on Growth and Survival

physical growth and anemia [12, 15]. VAD is a major nutritional public health problem in the developing world. According to the latest report of the WHO, globally about 190 million preschool-aged children and 19.1 million pregnant women are vitamin A deficient (i.e. serum retinol <0.70 μmol/l) [16]. This corresponds to 33.3% of preschool-aged children and 15.3% of pregnant women in populations at risk of VAD. According to current estimates, 122 countries are classified as having a moderate to severe public health problem based on biochemical VAD in preschool-aged children, and 88 countries based on biochemical VAD in pregnant women [16]. Xerophthalmia, resulting from VAD, is the primary preventable cause of blindness, and of the world’s children with xerophthalmia, nearly half reside in South or South-East Asia, of whom more than 85% live in India [17]. About 5.2 million preschool-aged children and 9.8 million pregnant women suffer from night blindness, which represents 0.9 and 7.8% of the population at risk of VAD, respectively. The estimates show that Africa and South-East Asia contain the highest proportions of preschool-aged children and pregnant females with biochemical VAD and night blindness [16]. Evidence from community trials has shown that vitamin A supplementation (VAS) reduces all-cause mortality and diarrhea- and measles-specific mortality [18–21]. It also reduces incidence of measles and diarrhea infection [21]. VAS has also been shown to reduce prevalence of xerophthalmia (RR 0.31, 95% CI 0.22–0.45) and night blindness (RR 0.32, 95% CI 0.21–0.50) [21]. VAS as an adjunct in the treatment of measles has been shown to reduce mortality (RR 0.18; 95% CI 0.03–0.61), pneumonia-specific mortality (RR 0.33; 95% CI 0.08–0.92) and incidence of croup (RR 0.53; 95% CI 0.29–0.89) [22].

World Health Organization recommends two annual high-dose supplements of vitamin A for every child at risk of VAD [23]. Administering vitamin A is a simple act that can be performed by a trained health worker, community worker or volunteer. It is one of the most cost-effective large-scale child survival interventions [24]. VAS is inexpensive on a per-child basis, with the cost of single capsule approximately USD 0.02 [25]. The total cost of two annual doses varies according to the region, and it has been estimated that supplementing each child with two doses per year, it costs USD 1.20 per child per year for South Asia and sub-Saharan Africa, with relatively higher costs for Central Asia and Latin America [26].

Since 1998, large VAS programs are in place in about 193 UNICEF-targeted countries to deliver the required dose of vitamin A [27]. Figure 1 shows the progress of increase in VAS from 1999–2007. It can be noted that in 1999, just 16% of children in these countries received full VAS, while in 2005 this number increased to 72% [28]. In 2007, the rate of coverage dropped to 62% partly because India increased its target group from 3 to 5 years of age [28]. An important point to note is that least developed countries showed the most impressive progress in achieving two-dose coverage [29] (fig. 2). While these figures are encouraging, significant gaps in VAS coverage remain and continue to
undermine children’s health [30] (fig. 3). An effort should be made to improve the current strategies of vitamin A delivery to achieve at least 80% coverage on persistent basis. Special efforts should be made to cover the hard-to-reach children through complementary strategies, such as special outreach programs, to reach the final 20% who have not been reached through regular programs. To maintain the sustainability, resources should be mobilized in national budgets, and VAS programs should be made part of integrated delivery strategies, with continuous monitoring and tracking of progress.

**Zinc**

Zinc is the second most abundant transition metal in humans after iron. It is an essential part of about 100 specific enzymes and also serves as structural ions in transcription factors [31]. The association of zinc deficiency in children with
growth retardation and hypogonadism was first described in 1963 in a study from Iran [32], and it is now well established based on animal and human studies demonstrating that zinc plays a critical role in cellular growth, cellular differentiation and metabolism [33], and in turn promotes immunity, resistance to infection, and the growth and development of the nervous system [31]. Although zinc deficiency is increasingly being recognized as a widespread problem, there is very limited nationally representative data on the magnitude and severity of this deficiency [34]. Some of this is due in part to the lack of reliable biomarkers of zinc status. Recently, Wuehler et al. [35], using data from national food balance sheets compiled by the Food and Agricultural Organization, estimated that 20% of the world’s population is at risk of low zinc intakes. The global prevalence of low intakes by region indicates that 26% of population in South Asia and 28% in Sub-Saharan Africa are at risk of deficiency [35].

Evidence from community trials has shown that both therapeutic and preventive zinc supplementations are effective in reducing morbidity and mortality in children <5 years of age [36–40]. Therapeutic zinc supplementation as an adjunct in the treatment of diarrhea has been shown to reduce the duration of acute diarrhea by 0.5 days and that of persistent diarrhea by 0.68 days [37]. Preventive zinc supplementation reduces incidence of diarrhea by 20% (RR = 0.80; 95% CI, 0.71–0.90) and that of pneumonia by 15% (RR = 0.85; 95% CI, 0.75–0.97) [36]. Preventive zinc supplementation has been shown to reduce the rate of stunting as well [36, 41, 42].
In 2004, the WHO and UNICEF formulated a new recommendation to administer zinc for 10–14 days as an adjunct treatment for diarrhea, along with low-osmolarity oral rehydration solutions and continuation of feeding [43]. Since then, the WHO and UNICEF in collaboration with USAID and Johns Hopkins University has worked to ensure the availability of zinc products [44], and about 91 million tablets had been provided in the year 2008 [45]. Despite all these efforts, zinc supplementation is not part of national programs around the globe. Figure 4 shows that only 46 countries have adopted zinc policy as part of their national child health policy [45]. It is therefore required to scale up the zinc supplementation, and it should be incorporated into national diarrhea management policy. Adequate funds with possible public and private partnerships should be sorted out, and general awareness about the effectiveness of zinc supplementations should be raised through media campaigns.

**Iodine**

Iodine is the key element required for thyroid hormone synthesis, and is also important for brain development during fetal and early years of life [46]. Iodine deficiency is the primary cause of preventable mental retardation and brain damage and also increases the chance of infant mortality, miscarriage and stillbirth.
Children born to iodine-deficient mothers may appear normal at birth, but might have suffered brain damage and loss in IQ points, affecting their ability to develop to their full potential. These seemingly normal children will later have difficulty learning in school and staying in school. It has been shown that in communities where iodine intake is sufficient, average IQ is on average 13 points higher than in iodine-deficient communities [46]. According to an estimate, about 2 billion people have insufficient iodine intake around the globe and about 31.5% of school-age children (266 million) have insufficient iodine intake (fig. 5) [47].

Salt iodization is one of the exemplary success stories of food fortification offering great benefits for the intellectual health of nations that have embraced it [48]. The number of countries where iodine deficiency disorders were a public health concern has been reduced by more than half from 1993 to 2007 [47]. Thirty-four developing countries have achieved the universal salt iodization goal, and an additional 38 countries are considered ‘on track’ for elimination of iodine deficiency disorders [48]. These are countries that have either shown increases in coverage of at least 20% over the previous decade or that have reached between 80 and 89% coverage with no indication of possible decline [48]. Despite this progress, many countries are lagging far behind. Twenty-four countries have experienced no growth in coverage rates or have even experienced a decline since the mid-1990s [48]. In 12 countries, less than 20% of the population are consuming adequately iodized salt [48].

Fig. 5. Prevalence of iodine deficiency in school-aged children [47].
In order to achieve universal salt iodization, country level legislation should be done, and adequate funds should be ensured to enforce it. At the same time, media campaigns should be launched for general awareness of the importance of salt iodization. Population monitoring systems should be strengthened so that program adjustments can be made as habits and diets change over time.

Other Micronutrients

Iron is an essential mineral for human development and function. It is required for hemoglobin and is critical for motor and cognitive development in childhood, and for physical activity in all humans [49]. Iron is also critical to the health of a pregnant mother and her unborn child. A woman needs more iron during pregnancy because the fetus and placenta both need additional iron. Iron supplementation during pregnancy lowers the risk of maternal mortality due to hemorrhage, the cause of more than 130,000 maternal deaths each year [27]. Supplementation also helps to lower the risks of premature birth and low birthweight. Studies have shown that infants with anemia caused by iron deficiency have lower mental scores and lower motor scores than infants without anemia [50]. Ensuring sufficient iron levels in the first months and years of life is, therefore, critical.

Conclusions

Micronutrients like vitamin A, zinc, iodine and iron are important for growth and survival of children. Given the wide prevalence of multiple micronutrient deficiencies in malnourished children in developing countries, the challenge is to implement intervention strategies that combine appropriate infant and young child feeding with micronutrient interventions at scale. Emerging data from community intervention trials now provide evidence that this is both tangible and can lead to alleviation of childhood undernutrition.

References


Micronutrients in the Treatment of Stunting and Moderate Malnutrition

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Abstract
Linear growth retardation or stunting may occur with or without low weight-for-age, but in both cases stunted or moderately malnourished children are deficient in micronutrients. Pregnancy and the first 2 years are critical periods. Dietary deficiency of zinc, iron, calcium, and vitamin A are especially common and often occur together. Zinc is essential for adequate growth, and supplements have been shown to increase intrauterine femur length and to prevent stunting. However, in general, supplements which provide a mixture of micronutrients have been more successful in preventing stunting and are simpler to take and distribute. Multiple micronutrients together with energy and macronutrients are also needed for the management of moderate malnutrition. Multiple micronutrients may be delivered as medicinal-like supplements, but may also be combined with food, for instance in milk drinks, in fortified dried cereal mixes used to supplement complementary foods or in lipid nutrition supplements. The latter also provide essential fats necessary for growth. Micronutrient powders for home fortification are effective in preventing anemia, but present combinations do not prevent stunting. Improving the diets of infant and young children is also possible, and increased intake of animal source foods can improve growth.

Introduction
Infant and child nutritional status is expressed as a weight-for-age, height-for-age, and weight-for-height z scores in relation to the median (50th centile) of a reference population. Linear growth retardation leads to stunting, which is defined as length or height-for-age z score less than –2. Stunting is a chronic process that represents a personal history of deprivation and has lifetime consequences [1]. Low weight-for-age can be a consequence of acute malnutrition associated with loss of fat and lean body mass resulting in thinness (low weight-
for-height) or result from chronic low growth rate when it is often associated with stunting and intrauterine growth retardation. Moderate malnutrition (MM) is defined variously as weight-for-age z scores between –2 and –3 SD [2] or weight-for-length-/height-for-age (wasting) between –2 and –3 SD [2]. Children with MM are at risk of long-term adverse consequences [3].

The new WHO growth reference standards have provided a benchmark against which population and individual child growth and nutritional status can be compared [4], and the strikingly similar growth pattern of children from different ethnic and geographical conditions adds to existing evidence that in the first 5 years it is possible to use a single reference to compare growth across the world.

While normal growth seems similar across populations, the pattern of growth of children in different low resource situations is not. Figure 1 is an example of the considerable difference in early growth between babies born in South Asia compared with babies in South and Central America. In the latter area, the most common growth deficit is stunting in the absence of low weight, whereas in South Asia low birthweight is common, and more than 40% of young children are underweight. Treatment and prevention guidelines need to take this into consideration, and I will consider the role of individual micronutrients in the prevention and treatment of stunting and MM separately.

**Stunting**

Linear growth retardation often begins in utero, but is most marked during the vulnerable period of complementary feeding, the transition from a diet of breast milk to the family food [5]. While dietary intakes of breast milk plus
complementary foods usually meet protein requirements, energy intakes may be low, and diets are almost always low in critical micronutrients, especially calcium, iron and zinc, both in absolute terms and in terms of nutrient density. Vitamin A is also deficient in some areas, and dietary deficiency of niacin, riboflavin, thiamine, B₆, B₁₂, vitamin C, vitamin D, magnesium, phosphorus and potassium have been reported [6–9]. These micronutrients, except vitamin C, are typically associated with animal source foods (ASFs). ASFs are especially good sources of minerals such as iron, zinc and calcium because of relatively high concentrations and bioavailability. Inhibitors, such as phytates and fiber, present in cereals and legumes, reduce the dietary utility of minerals from plant sources. Diets that are high in cereal content and low in ASFs have been associated with stunting [10, 11].

**Zinc**

Zinc is essential for many physiological processes, and severe zinc deficiency leads to dwarfism [12]. Zinc deficiency is common in many populations [13]. Two out of three recent meta-analyses [14–16] of clinical trials of zinc supplementation in children report a large and significant effect of daily oral zinc supplements on linear growth especially in stunted children and in developing countries. Imdad and Bhutta [16] reported the greatest impact when zinc was given alone, and estimated that in children under 5 years there was a net gain of 0.37 ± 0.25 cm for a dose of 10 mg zinc daily for 24 weeks.

Antenatal zinc supplementation has been shown to increase fetal long bone growth [17], of particular relevance because nutritional stunting is due to short femur length [18]. A study in Nepal showed a small but significantly greater height in school children whose mothers received antenatal zinc supplements [19], but a large study of antenatal zinc supplements reported no effect on postnatal growth, although the offspring of supplemented mothers had reduced prevalence of diarrhea and other infections [20]. Zinc supplements have been repeatedly shown to prevent respiratory complications and diarrhea in infancy, themselves associated with stunting. The benefit of zinc may be limited by the continuing infection or by the presence of other nutrient deficiencies. A study combining zinc and antiparasite treatment reported a positive effect of zinc on linear growth that was reduced by the presence of *Giardia lamblia* and *Ascaris lumbricoides* [21].

**Other Single Micronutrients**

No other micronutrient has been so well studied in relation to linear growth as zinc, but large scale trials assessing the importance of other single micronutrients such as vitamin A and iron have often measured growth.
Clinical vitamin A deficiency is associated with poor growth, but although a few studies report increased height gain when children with severe deficiency of vitamin A were given supplements [22], most studies have not reported any significant effect on linear growth or weight gain [15].

Iron deficiency is common, and the hematological and cognitive effects are sufficient reason for supplementation. The impact of iron supplementation on linear growth has been inconsistent. A systematic review by Ramakrishnan et al. [23] reported a nonsignificant tendency towards a positive effect in children who were stunted at baseline, and a review by Sachdev et al. [24] reported significant effects only in some subpopulations: children older than 5 years, in malaria hyperendemic areas and those who received the supplement for longer than 6 months. An update of recent evidence [25] concluded that growth was neither positively nor negatively affected by iron supplementation.

Calcium is essential for bone formation and often deficient in diets low in ASFs, especially when dairy products are absent. Trials of calcium supplementation as a single nutrient have tended to focus on school-aged children rather than infancy when linear growth faltering is maximal. A review of randomized trials concluded that calcium supplements had no impact on growth [26].

Many other micronutrients have a role in normal growth and potentially may contribute to stunting. Severe iodine deficiency for instance causes cretinism, a form of dwarfism, but since important deficits in cognitive development occur even in moderate deficiency, this has been the primary outcome of intervention trials, and linear growth has not been measured.

Other vitamins commonly deficient in cereal-based diets include vitamin B₁₂, folic acid, thiamine, niacin and vitamin D, and because of probable dietary deficiencies these are often included in multi-micronutrient supplements, but their role in preventing stunting has not been established.

**Multiple Micronutrients**

Given that micronutrient deficiencies rarely occur in isolation, attention has turned to multimicronutrient supplements as an alternative approach. This brings challenges because of interactions, product stability and acceptability, but this approach is very attractive to programs since the logistics and cost of attempting multiple separate daily supplements is prohibitive.

Multiple micronutrients (MMNs) have been assessed in clinical trials in different parts of the world. A review in 2003 [27] of community-based micronutrient supplementation trials concluded that supplements that contained zinc, vitamin A and iron could have positive effects in deficient populations. A meta-analysis in 2004 [23] also reported overall positive effects of MMN supplements on growth in contrast to single nutrient supplements. A recent review by Allen et al. [28] was able to include a further 13 studies. The analysis included studies
that had compared MMNs against single nutrients, mainly iron. This analysis concluded that overall length and height were improved with an effect size of about 0.13 (95% CI: 0.055–0.21). Some studies reported positive effects only in subgroup analyses; for instance, in Mexico a multinutrient beverage was only effective in children under 12 months [29], and in Vietnam [30] the subgroup of children who were stunted at the start of the study showed the largest effect size. MMNs were delivered in a variety of ways, but the analysis was unable to detect whether any particular delivery mode was better than another.

In conclusion, MMN supplements given to infants and young children seem to have a fairly small but positive effect on length or height, and two recent analyses suggest that this is greater than the effect of single micronutrients.

Given that birthweight is associated with postnatal infant growth, micronutrients given antenatally would be expected to reduce infant malnutrition at least during the first few months. A Cochrane review [31] reported that prenatal MMNs were no more effective than iron and folic acid alone in reducing low birthweight and small for gestational age. However, a recent cluster-randomized trial of the INIMMAP MMN prenatal supplement in Niger significantly reduced low birthweight and increased average birthweight but only by 67 g [32]. An effectiveness trial in Vietnam [33] showed an impact on birthweight and also on the height of 2-year-old children including 10% less stunting in the communities where the prenatal supplements had been used. A more recent combined analysis of original data from 12 randomized trials concluded that compared with iron-folic acid, MMNs resulted in a small increase in birthweight and a reduction of low birthweight by about 10% [34]. Information about the infant’s postnatal growth was not included.

Thus, it seems that MMNs are an effective intervention to prevent or treat stunting in infants and young children, but more needs to be studied about means of delivery and the potential impact of prenatal supplementation, particularly in preventing the growth retardation in the first 6 months that has been revealed by the WHO growth reference [35].

**Moderate Malnutrition**

Moderate malnutrition may arise either because a previously ‘normal’ child has lost weight or failed to gain weight and crosses ‘growth centiles’ or because a low birthweight baby continues to track along a low ‘centile’ corresponding to less than 2 SD below the norm. These two scenarios are very different in clinical terms, but cannot be distinguished by a single measurement, and for this reason in evaluating individuals longitudinal growth data whenever available should always be examined. Thus, moderate malnutrition, in contrast to stunting does not necessarily represent a chronic dietary inadequacy; however, recovery from moderate malnutrition will always require an increase in body mass implying
increased energy and protein needs. On the other hand, the treatment of stunting with additional calories, especially in the context of Latin America where stunting is often associated with above average weight-for-height, may potentially lead to increased overweight or obesity, and should be carefully controlled.

A recent World Health organization call for action and consultation on the management of moderate malnutrition in children under 5 years of age has addressed the issues related to managing moderate malnutrition and provides an excellent review of all the issues involved in rising to the challenge of treating these children [2]. The nutrient needs of children have been considered and a number of nutrients including protein, potassium, phosphorus as well as energy, essential fatty acid and MMNs are considered important. In terms of micronutrients, there is emphasis on the need to ensure sufficient intake of all type 2 micronutrients, those associated with growth. Although single micronutrients such as iron [24, 25], vitamin A [22] and zinc have been associated with weight gain, since children with moderate malnutrition are especially likely to have multiple nutrient deficiencies, the role of single micronutrients may be less significant, and MMNs have usually been considered; in fact, the expert consultation went as far to say that ‘approaches putting emphasis on single nutrients are misguided and should be abandoned’ [2].

There is considerable debate over the amounts of micronutrients that should be included in multi-micronutrient mixes whether in food or as supplements, and the expert consultation suggested that the requirements be set between the daily recommended intakes for eutrophic children and the contents of the F100 diet designed for management of severe malnutrition with the latter providing a safe upper limit. The same consultation provided detailed background and thoughtful recommendations not only on the content of foods but also relevant foods and ingredients including anti-nutrients, and the reader is referred to this report for further information [2]. In addition, attention is given to the important complementary activity of dietary counseling [2].

**Delivery of Multiple Micronutrients**

The evidence so far suggests that both for the treatment and prevention of stunting and for moderate malnutrition MMNs are indicated. So what options are available for delivery of these micronutrients, and what are the issues involved?

Many different formulations have been tried in clinical trials of micronutrients, but there are some emerging leaders in the field. For more than 30 years, food assistance programs have distributed food aid for the prevention and treatment of malnutrition and for emergencies as fortified food blends [2]. These are intended to replace or add to local traditional complementary foods. These mixtures usually contain blends of cereals such as corn, soy beans, sugar and oil and are distributed by international agencies. In many countries there are
also local products such as Incaparina of Guatemala, and Mi Papilla in Ecuador. These products can be successful in reducing anemia, but have had less impact on stunting or moderate malnutrition. Ecuador children attending participating health facilities who were offered counseling and ‘mi papilla’ had highly significant reductions in anemia compared with those attending control facilities who only received counseling, but differences in height were not significant when confounding variables were controlled [36]. Studies in India, Mexico and Perú have shown less encouraging results with no effect on linear growth faltering, and a review of the PL480 title II foods program included in the moderate malnutrition consultancy [2] illustrated some of the problems of low acceptance and intrafamily sharing of the product that reduced the nutritional benefit to the target child.

Two other categories of fortified foods: complementary food supplements and micronutrient powders are extensively reviewed by the Ten Year Strategy to Reduce Vitamin and Mineral Deficiencies, Maternal, Infant and Young Child Nutrition Working Group: formulation subgroup of the moderate malnutrition consultancy [2]. Complementary food supplements are food based and contain macronutrients and micronutrients designed to complement, rather than replace, other foods prepared in the family. Micronutrient powders are mixtures of vitamins and minerals that are added to traditional foods. They do not include macronutrients in significant dietary amounts.

**Complementary Food Supplements**

These supplements include the highly nutrient-dense spreads described by Briend [37] also known as ready to use therapeutic food, and now being successfully used for the treatment of severe and moderate malnutrition [2]. These lipid nutrient supplements (LNS) are based on high-fat products such as peanut butter or soy beans with added micronutrients with or without milk. They have the benefit of being highly palatable with good acceptability. They provide an excellent medium for micronutrients, and essential fatty acids can be added, and because of the low water content, bacterial growth is inhibited. A study in Malawi confirmed better weight gain in moderately malnourished children compared with a cereal product [38], but there was no difference in linear growth. These products are now being developed for use as a food complement in smaller doses and with micronutrients adjusted for treatment of stunting and moderate malnutrition. In addition, a trial of multi-micronutrients delivered as micronutrient powders, LNS and crushable tablets in Ghana showed that while all three supplements reduced anemia and resulted in improved motor function, only the LNS improved linear growth [39]. Cost is a concern with LNS, but it is hoped that local production will address this problem.
Micronutrient Powders

Micronutrient powders presented in small single-use sachets to add to a serving of complementary food have proved successful in preventing and treating anemia [40, 41]. The potential for adding other micronutrients such as zinc has been explored, but as yet no formula has been shown to prevent stunting or promote linear growth.

Improving the Infant and Young Child’s Diet

Another alternative to improve stunting and moderate malnutrition is to improve the child’s normal diet by increasing the micronutrient content and availability. Two approaches have been tried: reducing inhibitors to absorption to enhance bioavailability and increasing consumption of ASFs.

Complementary foods can be processed, for instance by fermentation to reduce phytate content. In Tanzania, processed complementary food reduced phytate but did not improve growth compared with usual household preparations [42]. Another attempt to use maize that had been selected for low phytate content to improve growth was not successful in infants in rural Guatemala [43]. The reduction of inhibitors to mineral absorption may be seen as a complementary strategy to improve complementary foods, but has not yet been shown to enhance growth.

Enhanced nutrition education through the health services in a population where low-cost ASFs are available, resulted in higher intakes of ASFs associated with prevention of stunting [44]. Intake of ASFs was associated with better height gain in Kenyan school children, and weight gain and increased lean body mass was associated with increased meat intake, but only milk intake resulted in height gain [45].

Milk deserves special mention because of its potential role as a vehicle for fortification. Fortified milk given to Indian children resulted in increased linear growth [46] compared with unfortified milk. Milk is of particular interest because it stimulates IGF-I secretion which stimulates growth. Further research is needed to explore milk, fortified or not in programmatic conditions.

Discussion and Future Directions

The evidence suggests that MMNs can reverse to some extent the impact of linear growth retardation in infants and young children whose dietary intake is insufficient. MMN combinations may be more effective than single nutrients and have logistic advantages. New strategies are encouraging, and many programs and countries are now considering how best to implement MMN
supplementation at scale. The combination of micronutrients makes it possible to tailor interventions to the particular needs of different populations addressing the multiple effects of inadequate dietary intakes. We need to take a holistic approach that also includes the health hazards of both deficiency and excess, especially where increasing rates of childhood obesity coexist with stunting and anemia.

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Discussion on Micronutrient Requirements

Setting the Terms of Discussion for the Workshop
The 70th Workshop of the Nestlé Nutrition Institute was entitled 'Micronutrient Requirements for Health and Development'. Two distinguished scientists with experience in developing countries with important micronutrient deficiencies: Dr. Zulfiqar Bhutta and Dr. Mary Penny took part in this workshop. The interaction of nutrition, growth and child survival are prominent public health issues in both the South Asian subcontinent and the Andean region, providing ample opportunity for the two speakers in the inaugural session to gain experience and synthesize their reflections.

One of the important terms of reference in the Workshop title is that of 'micronutrient requirements'. For the introductory talks, and throughout the Workshop, however, randomized trials with single or multiple micronutrients formed the evidence for a discussion of a requirements theme. Most often, levels of nutrients well above the 'requirement' (daily recommended intake) amounts are delivered in such experimental settings. The variance in health and developmental outcomes that can derive from improving the micronutrient offering from dietary interventions with high-quality foods and beverages was only rarely alluded to. Such a perspective would have been more consonant with the explicit terms of reference in the title of the Workshop. To her credit, Dr. Penny was one of the speakers who used the prism of dietary variation, although the title and message of her presentation dealt with treatment – of stunting and of severe malnutrition.

Another term of reference is health. Impaired health is associated with altered nutrient requirements, usually a selective increase in the needs for macro- and micronutrients. Dr. Penny's discussion of prescription of nutrients to assist what is called in the title as 'moderate malnutrition' (underweight), derives from her years of experience in defining the safe and effective rescue, recovery and rehabilitation of children with clinical syndromes of undernutrition. An opportunity was missed in this session, and the subsequent program, to examine the term 'health' in terms of nutrient requirements. What are established as reference intakes by bodies such as the UN Systems Food and Agricultural Organization and World Health
Organization or by the Food and Nutrition Board of the Institute of Medicine in the USA are explicitly applicable to ‘healthy populations’. Children in low-income settings are more often than not infested by protozoan and helminthic parasites and beset by recurrent communicable diseases affecting the respiratory and gastrointestinal tracts. These alter intermediary metabolism and can interfere with nutrient intake and provoke excessive nutrient wastage. Are the environmental and epidemiological correlates of living in disadvantaged circumstance a motive for adjusting the recommendations to compensate for the ambient stress?

The nutrients most associated with growth and survival, as mentioned in this session, were vitamin A, iron, zinc and iodine. Severe deficiency of all four nutrients is, indeed, associated with decreased survival, and even marginal vitamin A deficiency increases mortality from common childhood infections. Where both Dr. Bhutta and Dr. Penny produced a potential disconnect across the title topic was in the arena of what degree of recovery of linear growth we should expect from nutrient exposures across the full panorama of intervention possibilities. Almost any nutrient at severe enough restriction will cause livestock, poultry or laboratory rodents to falter in their growth; this is the basis for the classical demonstration of specific food factors as essential nutrients. Profound effects on linear growth in free-living populations can only be related to zinc and iodine, with vitamin A and iron producing limited growth faltering. In places like Afghanistan, Angola, and the Horn of Africa, and in my region of Guatemala, the accumulated deficit in height at 5 years of age reaches 10 cm or more. However, when it comes to preservation in linear growth, effect sizes of only a few cm are the maximal outcomes even in the most successful single and multiple micronutrient interventions on record. It may not be the dosage or combination of micronutrients that limit this effect, but rather the intrinsic biological nature of short stature. Evidence can be mobilized from livestock management and experimental science that inflammation and the stress of living in an unsanitary environment are important determinants of growth faltering, and will not be overcome by any amount of additional nutrient exposures.

Salient Points of the Session’s Presentations
The titular theme of Dr. Bhutta’s presentation involved ‘global micronutrient deficiencies’; using maps, he illustrated the estimated prevalence of vitamin A, zinc and iodine deficiency. Not surprisingly, the concentration of micronutrient deficiency problems aligned with the poorest nations of Latin America, Africa and Asia. He accompanied this geography lesson with a narrative on the impacts on health and development of the deficiency of these micronutrients in early life, pointing out that vitamin A and zinc deficiency is associated with an estimated 1 million child deaths and 9% of global childhood disability-adjusted life years. In this respect, he harkened to the 50-year-old paradigm of the interaction of nutrition and infection introduced by Nevin Scrimshaw, noting that child health is an outcome as well as a risk factor.
He reinforced the relevance of the period from conception to 24 months of life, the so-called 1,000 days’ window of opportunity, involving maternal health in pregnancy and the adequacy of complementary feeding. He also mentioned that other nutrients beyond the primary three, namely iron, was also associated with limitations of human development, especially with an onset in infancy. The opportunity to mention vitamin D deficiency as an impending public health problem was missed both in this session, and throughout the Workshop.

On repeated opportunities in the presentation, the pediatrician from Pakistan cited the challenge as being ‘to implement intervention strategies that combine appropriate infant and young child feeding with micronutrient interventions at scale’. There is a sense of detachment or dissociation of the appropriate feeding and the interventions. One wonders if the concentration on what are appropriate estimates for required intakes of nutrients from young-child diets in low-income societies would not obviate the need for large-scale interventions with micronutrient supplements.

The treatment of moderate undernutrition involves correction of inappropriate weight deficit. Although energy and protein are the primary nutritional considerations when repleting body tissue mass, the British pediatrician in Peru, Dr. Penny, pointed out how micronutrients could not be ignored in this therapy. The broad consideration of micronutrients, beyond those of primary public health interest, is exemplified in the comprehensive therapeutic approach to moderate malnutrition. In fact, this led to a discussion of multiple micronutrients administered in combination. Surprisingly, again in light of the title of the Workshop, if we take the whole program and its 14 invited presentations into perspective, only Dr. Penny’s piece in the Introduction, the two presentations in the pregnancy session (Dr. Darnton-Hill; Dr. Bhutta), and the fortified food topic of Dr. Allen put the emphasis on multiple micronutrients in combination. The other 11 are monotonic for single nutrients.

The treatment of stunting or short stature is also addressed by Dr. Penny, and she invokes both single-nutrient and multi-nutrient formats. To her credit, she provides a balanced review of the literature, and shows the mixed – and not overwhelming – evidence for efficacy of reversing linear growth faltering with single or combined nutrients in stunted populations. The most interesting insight into the biology of stunting is that the length of the head, neck and torso of stunted children is equivalent to that of a normally grown peer. The growth faltering is largely the result from the failure of elongation of the long bones of the legs. This could point to some disruption of the hormonal cascade that supports bone growth at the epiphyses.

Summary of the Question and Answer Participation
In the question and answer discussion period, Dr. Bhutta was challenged on several points. Since both issues of bodyweight (underweight) and diverse micronutrient deficiencies seem to predict and predispose to under-five mortality,
how does one parse and separate out the specific impact of the different deficiency conditions, and how does one translate that into effective programmatic actions. The discussion moved to the inconsistencies related to the impact of zinc interventions in achieving improvements in survival, disease resistance and other functional benefits. One factor is the (as yet imperfect) approaches to assessing the existence of zinc deficiency due to lack of a specific biomarker of deficiency. Regarding the query of why vitamin D deficiency has yet to be incorporated into the global micronutrient deficiency agenda, it was admitted that the so-called ‘sunshine vitamin’ can be deficient in populations of the tropics. Insofar as this realization is of recent origins, we are only at the incipient level of inquiry into vitamin D deficiency, beyond overt rickets, in the global statistics of mortality, morbidity and intrauterine growth failure.

Dr. Penny extended her discussion of animal protein food in meeting requirements from dietary sources in response to questions as to whether milk was also in this category. Two characteristics of milk that could produce positive trophic effects in children were identified. The first is that bovine milk has been found to contain insulin-like growth factor-I, which may be absorbed intact and function as a hormone in the milk consumer. Another thought was that of surreptitious and serendipitous correction of an unrecognized iodine deficit by milk. Due to the iodine-containing antiseptic solutions used to disinfect the cows’ udders prior to industrial milking, residual iodine remains in the dairy items derived. By repleting iodine status, milk consumption might positively impact those short children with prior iodine deficiency. Rounding out the discussion on nutrients from actual foods was a focus on the limitations to obtaining adequate micronutrient nutrition during the critical 6–12 months of life covering transition from exclusive breastfeeding to mixed feeding. In this period, the critical quest is getting complementary foods with a high enough nutrient density to provide the residual needs of vitamins and minerals with a low allotment for additional calories. This is where animal protein foods are indispensable because of their generally more nutrient dense composition.

The remainder of the discussion centered on deriving useful insight on the Workshop topic from published trials. The question of how the various study designs included together in a review of intervention studies are casting light on micronutrients and growth was raised. Obvious issues such as the nutrient dosages, the mixtures of nutrients, the duration of the trial, the age and status of the children, and the degree of preexisting stunting in the trial populations are all features that could influence the findings of benefits. The issues of nutrient–nutrient interactions in multiple nutrient interventions were raised within this context. That interactions might be responsible for the variability of results from trial to trial was raised. Most of the interest centered on the competitive interaction of iron and zinc. The possibility of certain interactions having the effect of accentuating the actions of the nutrients was also raised as a theoretical possibility when multinutrient combinations are at play.
The status of parasitosis with protozoan and helminthic pathogens in the various intervention trials was advanced as another issue of potential relevance to the interpretation of the findings. Parasite loads vary from setting to setting depending upon residual infections in the population and ecological factors supporting parasite transmission and propagation. One commentator opinion was that multicellular pathogens are much more common as agents of severe gastroenteritis episodes and malabsorption than has been previously recognized. Finally, an interesting suggestion on transgenerational effects, i.e. that attention to micronutrients in the mother will have beneficial effects for her daughters and her granddaughters, was raised in the discussion.

Conclusion of the Discussant
The issue of micronutrient requirements from the connotation of diet has become divorced from the thrust of the field research on nutrient supplementation conducted over past quarter of a century. These years have allowed for the proliferation and collation of well-conducted and statistically powerful field trials, now coalesced into systematic reviews and meta-analyses. By their very design and nature, they allow for pharmacological actions of high-dose nutrients to be misinterpreted as nutritional functions of the nutrients and produce imbalances that can distort the normal metabolism of nutrients taken in from the diet itself. For all of their citation by the lead-off speakers and by the majority of presentations to follow, their capacity to inform us regarding ‘micronutrient requirements’ should be viewed with healthy skepticism. Aside from the question of the long-term sustainability of intervention programs as public policy, their limitations in eradicating the survival, growth, health and developmental issues may derive from the simple reality that other, nutrient-independent environmental assaults are influencing or even determining the outcomes. An alternative view might be to let the best quality diet do what diet can do, and address the collateral causes of poor health and development with a comprehensive array of complementary actions directed at the residual ecological origins.

Noel W. Solomons
Zinc Requirements: Assessment and Population Needs

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Abstract
Reliable estimates of zinc requirements have assumed greater priority as the global public health importance of preventing zinc deficiency has gained increasing recognition. On a global public health basis, our first most evident goal is reliable estimates of average population requirements. Despite expectations of rapid advances towards simpler and more sophisticated strategies, estimations of zinc requirements continue to depend on a factorial approach. Since the Dietary Reference Intakes (DRIs) were published, there have been important advances in techniques for the factorial approach but also confusion resulting from the subsequent publication of conflicting ‘international’ estimates. The reasons for these differences have now been fully elucidated, removing an obstruction to continuing progress and refinements of our knowledge base. A key advance has been the development and validation of a model that can be simply applied to determine the inhibitory effects of phytate on zinc absorption. Better understanding of maternal and young child zinc requirements continues to present a challenge of special importance.

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Introduction
Zinc deficiency now has an established place among the small group of micronutrient deficiencies having acknowledged public health importance of global dimensions. This recognition has underscored the need for reliable, broadly accepted estimates of dietary needs for all population groups consuming a broad variety of diets. Though there have been expectations for some years of new successful approaches for the assessment of zinc requirements, perhaps especially, zinc status-related changes in one or more of the zinc absorption transporter proteins or corresponding mRNA, these have yet to be validated. A factorial approach, therefore, remains not only the strategy of choice but the
only available method. Fortunately, though data remain limited, new concepts and effective modeling over the past decade have resulted in quite fundamental advances in the effectiveness of the factorial approach. The specifics and implications of these advances as well as the resolution of conflicting estimates are the focus of this review. Though a principal focus of this workshop is on development, this paper will be directed to adult requirements. Reliable estimates for all women of child-bearing age are important for the in utero development of their offspring. Estimates of zinc requirements for young children depend primarily on extrapolation from adult estimates. Therefore, reliable estimates of adult zinc requirements provide an essential cornerstone for understanding requirements during all stages of development. These are also an essential starting point for our understanding of the effects of inflammation and chronic/recurrent disease on zinc requirements.

The factorial estimation of average zinc requirements of populations depends on reliable data that can best be considered under three major headings. These are: (1) physiological requirements (PR) defined as the quantity of zinc absorbed daily (TAZ) that is just adequate to match inevitable excretion of endogenous zinc by all routes together with any zinc retention required for new tissue (fig. 1); (2) the estimated average daily zinc requirement (EAR) of bioavailable zinc necessary to achieve absorption of the PR, and (3) the bioavailability of the ingested zinc which is determined at least primarily by the quantity of dietary phytate ingested. This paper will review each of these aspects including the resolution of recent disparities in estimates of zinc requirements. The accurate determination of zinc absorption requires measurement over a period

Fig. 1. Schematic of total body zinc homeostasis.
of a minimum of one day rather than single meal studies, and all data covered in this review are expressed in terms of mg Zn/day.

**Physiological Requirements**

Typically, except during early lactation, the intestine is the major route of excretion. The quantity of zinc excreted via this route is regulated in response to current zinc absorption and to an undefined but probably limited extent by ‘zinc status’. Other routes of excretion of endogenous zinc are via the kidneys (also regulated but with relatively minor implications for requirements), reproductive system, integument, and, during lactation, the mammary gland. The demands of new tissue add to the PR during childhood and pregnancy, though these may be offset during pregnancy, as may the extra excretion of zinc via the mammary gland, by up-regulation of absorption [1, 2] and, possibly, by down-regulation of endogenous zinc excreted via the intestine [1].

A limitation in concept of human zinc homeostasis in one case and errors in determining the relationship between endogenous fecal zinc (EFZ) and TAZ in another resulted in mistaken estimates of PR in two of three relatively recent publications considered in this review, each of which has had and continues to have an impact globally. In the first of these, in 1996 [3], it was concluded, during this initial (and notable) endeavor to provide a strong evidence base for zinc requirements, that EFZ and, to a small extent, urine varied with adaptation to recent intake or/and ‘status’. This led to the concept of ‘basal’ (fully adapted) and ‘normative’ (non-adapted) PR. However, in both cases these levels were based on low zinc intakes and were regarded as ‘static’ numbers which did not increase with increasing absorption of zinc. This resulted in extremely low and erroneous estimates of PR [4] in what was otherwise an outstanding contribution to the evolution of emphasis on an evidence base for assessing zinc requirements.

A key advance was contributed by the Institute of Medicine (IOM) in 2001 [5] with recognition of the occurrence and significance of a strong positive correlation for EFZ versus the quantity of TAZ which, importantly, was apparent at levels of TAZ well below the PR [6]. The implication is that regulation of EFZ is imperfect, as zinc excretion increases with increasing TAZ when the latter is still below that needed to meet physiologic requirements. This concept and supporting data resulted in estimates of PR for adult males [5] that were more than threefold those estimated by the WHO [3]. The validity of this approach has not been questioned, though relatively minor numerical adjustments are likely as the current limited data base is enlarged, especially with studies of zinc homeostasis while ingesting habitual diets.

Publication of the IOM estimates [5] was followed 3 years later by ‘international’ estimates [7]. It should be noted that these ‘international’ estimates
were based on studies of human zinc homeostasis in the USA or UK with one study of ours in China [8] being the only exception. Two errors in these estimates, both related to the determination of the relationship between EFZ and TAZ, were primarily responsible for major underestimates of PR [9]. Because these estimates have been widely disseminated and utilized globally, they have been the cause of much confusion and have inhibited progress in our quantitative understanding of zinc homeostasis and requirements for several years. Correction of these errors together with using the same reference data for adult size and correction of an IOM overestimate of menstrual zinc losses results in close reconciliation [9]. The very small residual differences (fig. 2–4) are attributable to the use of different datasets, notably with IOM utilizing male data only to define the relationship between EFZ and TAZ [5] and International Zinc Nutrition Consultative Group (IZiNCG) [7] using data from both genders. The closeness of the reconciliation is remarkable in view of the limited database, the interpretation of which remains complex for females [10] and the range of laboratory techniques and strategies that have been used.

Fig. 2. Discrepancies between adult PR for zinc estimated by the IOM in 2001 [5] and IZiNCG in 2004 [7].
Estimated Average Zinc Requirements for Populations

The EAR is determined from the intercept between the PR and the model describing the relationship between TAZ and ingestion of bioavailable zinc. The conceptual advance that has occurred subsequent to the publication of the DRIs by the IOM is that this relationship is best fit by a saturation response model [4]. This is not surprising as the absorption of zinc by the enterocyte has long been known to be a saturable process in mammals [11]. Recognition of the appropriateness of this model has not only provided the best fit for data, but has proved a major visual advance in our understanding of human zinc homeostasis including advancing our understanding of the relationship between the quantity of bioavailable zinc ingested and the efficiency of zinc absorption. As with the regulation of EFZ, it is apparent that the regulation of zinc absorption is not a completely efficient phenomenon [4] (fig. 5). If it were, the model would coincide with the line of equality between dietary zinc and TAZ until the PR is met and then remain horizontal. The gap between the line of equality and the saturation response model is consistent with the abundant evidence that human

Fig. 3. Residual discrepancies in estimates of PR for zinc after correction of identified errors in estimates.
zinc deficiency, from the very mild to severe, does indeed occur and is no surprise. As up- and down-regulation of proteins involved with zinc absorption via the enterocyte occurs within a short time span, it is concluded that the less than perfect efficiency of absorption at levels of ingestion of bioavailable zinc less than EAR occurs despite maximal up-regulation of transporters and other proteins involved with zinc absorption. Despite this limitation on adaptation, fractional absorption is quite favorable at levels of ingestion less than EAR, averaging approximately 40%. It is noted that the estimated average requirement approximates half maximal absorption of zinc, below which absorption is relatively favorable. Also of note is the low and increasingly poor fractional absorption of zinc when ingestion of bioavailable zinc exceeds the estimated average requirement.

While search continues for the ‘holy grail’ of a sensitive, reliable biomarker of zinc status, we should not underrate, especially at the population level, how much we can learn about status from reliable determinations of dietary zinc intakes and, from there, the percentage of the population with zinc intakes

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**Fig. 4.** Effect of selection of different adult weight reference standards in estimating excretion of endogenous zinc via the integument. Minor residual differences between estimates attributable to use of different datasets.
below the EAR [12]. The only substantial additional information required is the bioavailability of the ingested zinc, a topic to be addressed in the next section. For the individual, biomarkers are certainly needed as is adequate information to provide a reliable measure of the variance around the EAR.

**Bioavailability**

Phytate is the one dietary factor recognized to have a major effect on the absorption of ingested zinc apart from the quantity of ingested zinc itself. The inhibitory effect of phytate on zinc bioavailability is notable and of most profound importance in populations largely dependent on plant products, especially unrefined cereal grains and beans. In developing the DRIs for zinc, the IOM concluded that, at that time, there were insufficient data to reliably estimate the quantitative effect of phytate on daily absorption of zinc. Subsequent trivariate modeling of TAZ as a function of both daily zinc and daily phytate intake appears to have quite successfully addressed the estimation of the quantitative effect of different quantities of dietary phytate on zinc absorption [13] over the spectrum of intake of dietary zinc. Though minor modifications of this model remain in progress as the effects of other dietary components are examined, the quality of both the parameter estimates and fit of the model to the data are reassuring. Moreover, it has been validated independently [14]. Based on this

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**Fig. 5.** Estimates of daily requirements for ingested zinc for adult males. A: line of equality between ingested zinc and absorbed zinc; B: saturation response curve fitted to adult male data utilized by IOM [5]; C: predicted effect of 1 mmol of daily dietary phytate. Vertical lines from intercept of estimated PR and saturation response curves indicate estimated average requirements for ingested zinc. Adapted from Hambidge et al. [17].
model, the effect of realistic quantities of dietary phytate on estimated requirements of ingested zinc necessary to meet physiological zinc requirements in adults is illustrated in figure 5. This model has been used, for example, to predict the level of enhanced zinc absorption achieved by zinc biofortification of wheat [15]. It is apparent that existing and future strategies to reduce the phytate content of meals prior to consumption, without equivalent reductions in zinc, can have a profound effect on zinc requirements and the prevalence of zinc deficiency in populations dependent on high-phytate diets.

**Infants and Children**

Data on zinc homeostasis in infants and young children, the most vulnerable to the effects of zinc deficiency, are limited. It is known that the same saturation response modeling as for adults serves to fit existing experimental data well [16]. In line with lower physiological zinc requirements, the models have lower levels of absorbed zinc and lower estimates of maximal zinc absorption [16, 17]. It has been noted that if these models, even for premature infants, are adjusted for differences in length of small intestine, they fit closely with the adult model [16]. However, direct measurements of the inhibitory effect of phytate in young children remain pending at this time, and measurements of endogenous zinc losses are limited.

**Conclusions**

At least 15 years ago, there was a perception that stable isotopic investigations were outdated, almost before their application had begun and long before we knew how to use them optimally in estimating zinc requirements. There was a palpable feeling that molecular techniques, in particular, would sweep all before them. In fact, we need these ‘out-of-date’ techniques more than ever and depend on them to estimate zinc requirements with reasonable precision. However, the necessary data are still remarkably limited, and the variance remains uncertain. Moreover, existing data continue to be evaluated, and it is apparent that additional modifications of current understanding of human zinc homeostasis and requirements will follow [Miller et al., unpubl.]. This underlines the importance of building onto an existing base that is as reliable as current data permit.

The greatest challenge is presented by those for whom we need it most. At this time, we still depend to a very large extent on extrapolation from adults to estimate zinc requirements in young children. Maternal estimates are complicated by changes in zinc homeostasis that include regulation of absorption and probably of intestinal excretion of endogenous zinc and, during the entire course of pregnancy, by the interrelationships between the maternal, placental and fetal regulation of zinc homeostasis.
References

Role of Zinc in Child Health and Survival

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Abstract

Zinc deficiency has been estimated to result in more than 450,000 child deaths annually by increasing the risk of diarrhea and pneumonia mortality. Trials of daily supplemental zinc have shown preventive benefits in childhood diarrhea with a 20% reduction in incidence. Use of zinc in treatment of diarrhea has also been successful in shortening the duration of the episode by 10% and reducing the number of prolonged episodes. The World Health Organization recommends that zinc supplements be used for 10–14 days for every episode of childhood diarrhea along with oral hydration and feeding. Large-scale effectiveness trials of these recommendations in Bangladesh and India have found a reduction in hospitalizations due to diarrhea and pneumonia and in child mortality. Trials have also demonstrated a reduction in the incidence childhood pneumonia with zinc supplements and some, but not all, studies have found a therapeutic benefit of zinc as adjunctive treatment along with antibiotics as well. Preventive zinc also improves the growth of children in developing countries and reduces total deaths in 1- to 4-year-old children by 18%. Zinc supplementation is an intervention with proven effectiveness and broad application to address pneumonia and diarrhea, the two most important childhood infectious diseases globally.

A large proportion of the world’s population is at risk of zinc deficiency which has serious consequences for health and survival, especially in children under 5 years of age [1]. The initial descriptions of severe human zinc deficiency in adolescent boys included stunting of growth, immune compromise, high rates of infectious diseases and substantially reduced survival [2]. More recently, it has been demonstrated, largely through controlled trials of zinc supplementation, that even less severe zinc deficiency in preschool children is associated
with growth faltering, increased risk for serious infectious diseases and excess mortality.

**Childhood Growth and Development**

Stunting of linear growth in early childhood, a good overall measure of child health, occurs in a third of children worldwide [1]. Zinc deficiency is one contributor to stunting. Most meta-analyses of trials demonstrate that zinc supplementation enhances growth [3], and subgroup analyses suggest that the effect is greatest in growth-retarded children in less developed countries [4]. A number of trials of zinc supplementation also find benefits on child activity and developmental measures, although the evidence is more limited and less clear than for the effects on growth [4].

**Childhood Diarrhea**

Diarrheal diseases remain one of the top two causes of child death globally in spite of reductions over several decades associated with improved nutritional status and increased implementation of preventive and therapeutic interventions [5]. Children with zinc deficiency are more susceptible to infections causing diarrhea, and the loss of zinc during diarrhea exacerbates dietary inadequacy of zinc, contributing to a vicious cycle of infection and nutritional deficiency.

Numerous trials comparing oral zinc supplements, usually given daily, with placebo have been done and summarized in meta-analyses. The most recently published of these included 33 comparisons and more than 16,000 child participants [3]. This analysis found an overall effect of zinc supplementation of a 20% lower incidence of diarrhea. One of the initial trials of zinc supplementation reported 27% lower rate of diarrhea in children 12 months of age or more but no effect in infants [6]. The meta-analysis likewise found a 27% (95% confidence interval, CI: 13–39%) lower diarrheal incidence in children with an initial age greater than 12 months and no effect in infants [3]. The effect of zinc was also found to be significantly greater in children who were stunted at enrollment.

Diarrhea treatment with oral rehydration solution has become a mainstay for controlling diarrhea mortality, but this has no effect on diarrheal duration. In contrast, zinc supplementation has been found to reduce the duration and severity of the episode. Since the first definitive trial of zinc for treatment of diarrhea [7], there have been numerous trials in low- and middle-income countries. There have been several meta-analyses of these trials, demonstrating benefits with regard to episode duration, proportion of episodes persisting beyond 7 days and stool frequency. The most recently published systematic review found that zinc in treatment of diarrhea reduced the mean duration by 19.7%
[8]. There is heterogeneity in the trial results, and the benefits have been found in some but not all studies in infants 1–5 months of age.

Several early trials of treatment of diarrhea provided zinc for 2 weeks during and following the episode. These trials were summarized in a pooled analysis to show that there was a 34% (95% CI: 17–48%) reduction in the prevalence of diarrhea and a 26% non-statistically significant reduction in the incidence of pneumonia in the 2–3 months following the diarrheal episode [9].

In addition, there have been two large trials in which health service areas were randomly assigned to provide for children with diarrhea ORS or ORS plus daily zinc supplements for 14 days during and after diarrhea. In Bangladesh, children in the zinc intervention service clusters had a 24% (95% CI: 10–35) shorter duration and 15% (95% CI: 4–24) lower incidence of diarrhea than children in the comparison group [10]. Admission to hospital for diarrhea was 24% (95% CI: 2–41%) lower in the intervention group and admission for pneumonia was 19% lower, but this was not statistically significant. The rate of non-injury child deaths in intervention clusters was 51% (95% CI: 6–75%) lower. In India, children in the zinc intervention clusters had lower 24-hour prevalences of diarrhea and acute respiratory infections than children in the comparison group [11]. All-cause, diarrhea and pneumonia hospitalizations were reduced in the intervention areas by 59, 31 and 71%, respectively (all statistically significantly different from comparison areas).

**Childhood Pneumonia**

Pneumonia is the leading cause of child mortality, and zinc deficiency is a risk factor contributing to the incidence and severity of the disease [5]. As with diarrhea, the strongest evidence for the role of zinc deficiency comes from zinc supplementation trials. Because of the lower incidence of pneumonia than of diarrhea, the data are more limited. Furthermore, the use of a range of illness definitions for acute respiratory infections (ALRI)/pneumonia makes summarizing these results more complicated than for diarrhea. The ten available trials of zinc supplementation were analyzed with regard to the outcome definitions used [12]. Zinc supplementation provided daily or weekly reduced the incidence of ALRI defined using specific clinical criteria by 35% (95% CI: 18–48%). There was no effect found using less-specific outcome case definitions based on caregiver report. A separate meta-analysis found an overall reduction of ALRI of 15% (95% CI: 3–25%), but a larger effect in children who had lower initial height-for-age (stunted) and in studies using more specific clinical criteria for the outcome [3].

Zinc has also been used in trials as an adjunctive treatment along with antibiotics for pneumonia. An initial trial in Bangladesh of zinc for treatment of severe pneumonia found a reduction in the episode duration of one day (hazard
ratio 0.75, 95% CI: 1–43) [13]. However, subsequent trials have not confirmed this result [14, 15], and the conclusion concerning a therapeutic effect in pneumonia will need to await several ongoing trials.

**Childhood Malaria**

Malaria is an important cause of child mortality, especially in sub-Saharan Africa [5], and zinc deficiency may be a risk factor for malaria infection or illness. Several randomized controlled trials have investigated the role of zinc supplementation for prevention of clinical malaria. Trials in The Gambia and Papua New Guinea found 32 and 38%, respectively, reductions in clinic visits for malaria, but a trial in Burkina Faso found no effect on the incidence of malaria diagnosed by home visits [3]. It is possible that the effect is on the severity of illness rather than the incidence, but the data are insufficient to determine if there is an important preventive effect. A multi-site trial of zinc for adjunctive treatment of malaria did not find any benefit [16].

**Child Mortality**

Two very large trials of daily zinc supplementation were done in Zanzibar and Nepal. These trials were designed to examine the effects on child mortality [17, 18]. In Zanzibar, children 1–36 months old were randomized to receive 10 mg of zinc (5 mg for infants) daily and followed for a total of 56,507 child-years [17]. Overall, there was a non-significant reduction of 7% in all-cause mortality in the zinc-supplemented group. The effect seemed to differ by age with a reduction of 18% (95% CI: 0–32%) in children 12–48 months and no effect in infants. There were non-significant trends for lower mortality due to malaria, diarrhea and other infections in the zinc group. In Nepal, children 1–35 months old were randomized using the same doses as Zanzibar and followed for total of 60,636 child-years [18]. Overall, there was a non-significant reduction of 8% in all-cause mortality. There was a non-significant reduction of 18% in mortality in children 12 months or older, but no effect in infants. There were non-significant trends for reductions in mortality due to diarrhea and ALRI in the zinc group.

In a meta-analysis of the effects of zinc supplementation on mortality, the study results were stratified by age and whether the zinc was given with iron and folic acid [3]. There was no effect of zinc supplementation in infants, but children 12 months old or more had an 18% reduction in all-cause mortality (95% CI: 4–30). When iron and folic acid were given with zinc, there was no benefit in either age group on mortality. Another meta-analysis of the effects of zinc supplementation on cause-specific mortality suggested effects on diarrhea and pneumonia of 15–18%, but these effects were non-significant [19].
Conclusions

Zinc deficiency is a prevalent condition that has important effects on childhood infectious diseases and mortality. It has been estimated that this deficiency results in more than 450,000 child deaths annually and nearly 4% of the disability-adjusted life years lost in children worldwide [1]. The preventive use of zinc supplements or fortified foods would be expected to reduce stunting and mortality, especially that due to diarrhea and pneumonia [20]. The benefits of zinc as adjunctive treatment for diarrhea are well demonstrated, and the World Health Organization and UNICEF since 2004 have recommended that zinc be used for treatment of all childhood diarrhea [21]. The usefulness of zinc for preventing malaria and treating pneumonia is unclear until the results of additional trials are available.

References


Discussion on Zinc in Maternal and Child Health

Zinc and Infection

There are ongoing studies in infants and young children of developing countries providing data to begin estimating prevalence of inadequate zinc intakes. Relatively good models now exist to examine the efficiency of absorption in various situations and the effect of phytate as the major factor affecting absorption. The issue was raised about the value of conducting more bioavailability studies, while the major contributing factor is the prevalence of diarrhea in the population (Dr. Allen). In response, the speaker (Dr. Hambidge) agreed with the point made, and indicated that the cornerstone of normal condition is required before expanded to cover infectious diseases. Eventually, both models are needed. Data have begun to emerge in relation to possible inflammation role in the gut, and further studies are required to elucidate whether part or whole of zinc deficiency problem is due to infection.

The issue of how acute infections including diarrhea and respiratory tract infections affect zinc requirements in children followed (Dr. Mobarak). Dr. Hambidge clarified that earlier works have shown that excessive zinc is lost in diarrheal fluid, so more zinc is needed to compensate for this loss. In addition, one may need additional zinc to correct for deficiency which is likely to be significant in this situation. The inquiry was then made of why given only zinc in diarrhea when other micronutrients could have been lost as well. The response was that while oral rehydration solution (ORS) is the core treatment, the benefit of zinc in diarrhea was clearly shown in earlier works by Bhutta et al. [1]. However, the same strength of evidence on other micronutrients and ORS for diarrhea treatment is lacking at the present time.

A question of whether zinc requirement in HIV population differs from normal population (Dr. Samburu) was addressed by Dr. Fawzi. So far, there are a few trials that investigated whether giving zinc supplements to HIV-infected individuals have a benefit. Earlier observational studies reported a sort of mixed
evidence, and some of the observational studies suggested that zinc was beneficial as low serum zinc was associated with harmful outcomes in terms of disease progression. Another observational study demonstrated that actually zinc intake was also harmful, so possibly reflecting a U-shaped relationship. These studies were all observational with certain limitations. As far as clinical trials are concerned, there are a few providing a sort of mixed evidence. In South Africa, zinc was beneficial in reducing diarrhea, while one study in Tanzania among pregnant women suggested no effect on CD4, CD8 or clinical outcomes. A third study more recently in the US among HIV drug users suggested that zinc supplements were actually beneficial in slowing disease progression particularly among individuals with advanced HIV. These were individuals with zinc deficiency, and the outcome was zinc supplements reduced the risk of failing on their antiretroviral therapy. So, the evidence is mixed and requires further studies (refer to the article by W. Fawzi in this book).

**Zinc and Infant Growth**

The issue concerns infants who are born growth retarded and whether zinc requirements differ between infants who are small for gestational age (SGA) and normal infants, and if there are any data available in terms of tracking of zinc from those pregnancies (Dr. Bhutta). Dr. Hambidge refers to the work of Dr. Nancy Krebs in this area with exchangeable zinc pools that shows lesser quantities of zinc in the zinc pools of SGA infants compared to those of controls. The follow-on comment (Dr. Solomons) pointed out that in animals with restricted growth, the nutrient requirements are reduced. Similarly, if the infant is primarily not growing and zinc in growing infants has to do with laying down of tissues, then zinc requirement will be reduced. On the other hand, when infant enters growth spurts, then requirements will go up because the amount of tissue being laid down is rapidly expanding.

Because zinc is one of those nutrients that dramatically falls in breast milk over a period of time, this phenomenon leads to the issue of zinc supplementation for both the growth-retarded and/or preterm babies, besides providing iron to prevent iron deficiency anemia (Dr. Mendoza). Dr. Hambidge indicated that it’s quite extraordinary in the term infant situation how rapidly the physiologic decline in milk zinc takes place, and levels are very low by 6 months in the term infant; similar situation could occur in the preterm infant with a lot of other issues and challenges happening at the same time.

The next discussion took the direction of iron and zinc absorption. The concern is that in preterm nutrition, the suggestion has been made to start giving iron quite early. Therefore, if iron affects zinc absorption, then zinc requirement should be increased in these preterm infants (Dr. Garg). Dr. Hambidge agreed that zinc should be given too.

A comment was also made that studies in infants and adults showed that when iron is given together with zinc in foods, there is no effect on zinc absorption.
Bioavailability
A question was raised concerning bioavailability of different zinc compounds, citing animal studies done in Beijing that reported zinc gluconate as the most bioavailable, followed by zinc sulfate and zinc lactate, respectively (Dr. Ludan). In response, Dr. Hambidge indicated that all water-soluble compounds are bioavailable. With respect to iron, calcium and phytate, the old data suggested that calcium phytate reduced zinc bioavailability. However, current modeling where iron and calcium data are available showed that absorption of zinc is increased, not decreased. Based on the assumption that the zinc and the iron were taking up some of the binding sites on the phytate, it increased the R^2 value from 0.81 to 0.87. Therefore, this issue needs to be revisited.

Stable Isotope Technique
The issue on the use of the stable isotope technique to measure zinc status and the usefulness of this technique to determine the exchangeable zinc pools was raised (Dr. Hurrell). The response is that there is no answer on this matter yet. However, at the time being, with the absorption figures as shown in the presentation as well as good dietary data that include zinc and phytate values and random sub-samples of serum zinc from diverse studies, these should result in useful estimates of population zinc status.

IOM and IZiNCG Estimates
Reconciliation of IOM [2] and IZiNCG [3] estimates is recommended. IZiNCG is planning a review on zinc requirement estimates to take into consideration new data from studies published after 2001. The model as presented on the inhibitory effects of phytate on zinc absorption should be taken into consideration (Dr. Wasantwisut).

Overall, the paper is well presented and raised valid points for consideration with regard to zinc requirements, including the role of infection, preterm infant growth, interaction of iron and zinc, bioavailability of zinc in relation to iron, calcium and phytate, as well as the usefulness of stable isotope technique and modeling for estimation of population zinc status.

Discussion on ‘The Role of Zinc in Child Health and Survival’ by R.E. Black and C.F. Walker

Zinc and Diarrhea
A question was raised regarding the reason why zinc given with ORS for the treatment of diarrhea is more effective in developing countries compared to developed countries (as demonstrated in ESPGAN study in Europe). Infants who are exclusively breastfed in the first 6 months are unlikely to be zinc deficient (Dr. Haschke). The response (Dr. Black) is that little information exists
on zinc in treatment of diarrhea in high-income countries; there is a potential
deficiency in the infant age group. In the first 6 months, this may not be the
issue, but the 6–11 months is certainly more equivocal, and zinc deficiency is
likely to occur. A comment followed that Bangladeshi children have subclinical
zinc deficiency due to low zinc in soil and foods being produced. Zinc therapy
for diarrhea is recommended; the question is whether 20 mg of zinc in young
children is too high and whether it could have an adverse effect (Dr. Alam). Dr.
Black clarified that often a lower dose (10 mg) is used in very young children. In
fact, where 20 mg has been used for a short time period, there are virtually no
side effects. In the meta-analysis of all of the trials, there is a few percent addi-
tional vomiting in the zinc versus placebo groups, but there is no interference
with oral rehydration therapy or clinical outcome. Similarly, an inquiry was
made on possible side effects in certain studies which gave as much as 45 mg
dose to the under-five children (Dr. Kalantari). In response, the 45-mg dose was
used in a couple of therapeutic trials, not in a daily preventive dose, and resulted
in more side effects. Such high dose apparently did not lead to any more benefit
than using a lower dose in the therapy of diarrhea. The WHO recommendation
is 20 mg except for younger children where the dose is lower [4].

With respect to the mechanism of diarrhea for the 20-mg zinc dose (Dr.
Hurrell), this may be due to potential immune mediation, probable physi-
ologic or pharmacologic reasons and anti-secretory effects observed in animal
models. Since many of the causes of diarrhea have very different pathogenesis
with respect to mucosa and secretory factors and so forth, an inquiry was made
whether zinc effect is more likely to be observed with certain kinds of diarrhea
such as bacterial versus viral (Dr. Rosenberg). The response is that most trials
did not conduct ideological investigation. There are two trials reporting benefits
of zinc in *Shigella* and cholera. It’s quite important in syndromes like cholera or
ideologic specific diarrhea like *Shigella* to show zinc does work because those
are the ones that would stand out. From a public health standpoint, the overall
effect such as that of ORS matters eventually, and implementation should not be
held back because of lack of ideological reasons.

For the benefits in diarrhea, this appears to be specific to zinc per se (Dr.
Manalaysay), while the probable usefulness of other micronutrients (Dr.
Mobarak) would require supportive evidence. Additional comment was made
(Dr. Bhattacharya) that in clinical practice, pediatric patients with bacterial
infection and bloody diarrhea received i.v. fluid, antibiotics and zinc supple-
ments upon discharge. The follow-up results were excellent with the trend of
improved appetite and faster weight gain.

Another inquiry was whether the existing knowledge on formulations of zinc
for the treatment of diarrhea is sufficient to indicate that the type of preparation
do not matter. In addition, there has been some suggestion that maybe the
effect seen in diarrhea is pharmacologically dependent upon the dosage, and if
the same amount is divided into 3 doses over a 24-hour period, one doesn’t see
the same effect (Dr. Bhutta). The response is that there are no data to respond to these issues, and further studies are required.

The consistent lack of zinc effect in the <12 months age group has been raised (Dr. Wasantwisut), and the reason could be the increased demand due to rapid growth spurt as well as the immune system which cannot be met through the zinc dose given. In addition, a good biomarker of zinc status is needed besides serum zinc, which increases during the period of supplementation. In a developing country, recommendation on zinc prevention or treatment (Dr. Hadad) should be based on dietary data or population level of serum zinc measurement.

Other Benefits of Zinc

An issue was raised with respect to routine provision of zinc to children beyond treatment of diarrhea (Dr. Fawzi). On the one hand there is clear benefit on prevention of diarrhea, on the other hand when zinc is given with iron there is some negative interaction. In the context of Sprinkles, the question is whether there is a certain ratio that should be avoided or promoted. In response, there is good evidence that zinc supplements have a benefit on infectious disease outcomes, and that the benefit on growth may be small. Some equivocal evidence exists of interference or reduction of that effect if zinc is given with iron. It's not yet known concerning the right balance of zinc versus iron in a supplement or a Sprinkle micronutrient powder. The suggestion to go for equal molar ratios lacks supportive evidence on functional outcomes or increases in serum zinc. The supplementation trials that gave zinc only, almost always demonstrated an increase in serum zinc. In this context, a comment was made (Dr. Bhutta) that so far, the three large Sprinkle studies have not been able to show any impact on zinc, using either a 7.5- or a 10-mg dosage.

Besides the treatment of diarrhea, an inquiry was made whether zinc may benefit pneumonia or urinary tract infection and other inflammatory disorders (Dr. Manalaysay). The response is that solid evidence in human trials is required prior to issuing any recommendation. The potential role of zinc in treating asthmatic symptoms with respect to pneumonia has been mentioned (Dr. Battacharya), and deserves further attention.

Supplements versus Foods

The people who have got sustained zinc supplementation as prevention for infectious morbidities would in fact over time meet and exceed the requirements. On the other hand, a question was raised whether the preventive aspect of the supplemental exposure would withhold if people were fortified or their diet diversified to meet their nutrient requirements within the range of recommendations (Dr. Solomons). In response, in zinc-replete populations, there would be no therapeutic benefit. With supplements, one could possibly achieve normal zinc status during the period of supplementation and a couple of months afterwards. In the case of fortification, the same scale of achievement has not
been shown. The lack of evidence on improved zinc status from fortified foods or Sprinkles versus supplements could be due to the fact that the absorption of zinc in supplements is much higher than from foods, so a higher fortified dose may be required to reach similar outcomes (Dr. Hurrell). Another factor could be that there are no sensitive methodologies or biomarkers to measure the impact of zinc in foods. More studies are on the way to unravel the issue surrounding the formulation and whether or not adding Sprinkles or adding zinc to Sprinkles per se or zinc to Sprinkles without iron is a means of assessing their bioavailability (Dr. Bhutta).

An inquiry was made regarding multiple interventions, in this case zinc in Sprinkles and zinc supplements for diarrhea, of whether there is a risk of toxicity (Dr. Penny). The response was that there has to be evidence on the impact of zinc in Sprinkles on improving zinc status before judging potential excess level of intake. In addition, the safety margin with zinc is incredibly high. Additional comment was made that the efficacy of supplements is different from Sprinkles or micronutrient powder in food due to phytic acid. A study has just been completed where micronutrient powder that contains endogenous phytase actually reduces the phytic acid content of the complementary food resulting in a significant increase in serum zinc (Dr. Zimmermann). In addition, a randomized control trial using different dosages of zinc against the background of Sprinkles is underway and should provide the results by the end of the year (Dr. Bhutta).

Overall, the paper was well presented and generated a lot of discussion, primarily on the benefits of zinc in diarrhea and other infectious diseases and the mechanism involved, the ideological spectrum of pathogen-specific environment, the differences in efficacy trials of zinc supplements compared to food-based strategies, and the concern of excess level of intake with multiple interventions.

Emorn Wasantwisut

References


Global Burden and Significance of Multiple Micronutrient Deficiencies in Pregnancy

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Abstract

Maternal mortality, low birthweight infants and childhood stunting continue to be major global public health problems, part of a recurring cycle of disadvantage. Maternal undernutrition in particular is one of the most neglected aspects of nutrition in public health. One possible low-cost public health intervention that might help address these problems is the antenatal provision of multiple micronutrient supplements. If the evidence base could be established, cost-effectiveness found to be acceptable and safety ensured, supplementation could ameliorate the impact of poor nutrition and diets, high disease burdens and the sociocultural factors contributing to these problems. There have been good studies in over a dozen countries addressing some of these issues but with conflicting results. Consequently, at least three meta-analyses have been undertaken to establish significant findings that could help guide policies and programs. They concluded that multimicronutrient supplementation improves birthweight and likely reduces the number of infants born low birthweight. Supplementation with iron-folic acid or multimicronutrients also appears to have positive longer-term impacts on the health and development of the offspring. There remain concerns about possible increased infant mortality in some populations. Given the results of the meta-analyses, cautious scaling-up of country effectiveness trials appears justified with careful monitoring and evaluation.

Introduction

High levels of maternal mortality, infants of low birthweight (LBW) and childhood stunting remain major public health problems, especially in resource-
poor settings. They are also problems that have remained largely intractable, although there has been some recent progress. Maternal undernutrition in particular is one of the most neglected aspects of nutrition in public health globally [1]. Consequently, low-cost public health interventions that might help ameliorate the impact of poor nutrition and diets, high disease burdens and the sociocultural factors contributing to the high levels of these problems are needed. One of these may be the antenatal provision of multiple micronutrients. This paper looks at the evidence for micronutrient deficiencies and the likely impact of these, especially in women of low- and middle-income countries, the global burden of such deficiencies, their significance, and recent evidence that supplements of multimicronutrients may help to address poor health and nutrition of women and improve subsequent reproductive outcomes, including even longer-term benefits to the children of women who received the supplementation. The paper by Bhutta [2] discusses intervention strategies to address such multiple micronutrient deficiencies in pregnancy.

**Micronutrient Deficiencies in Women of Reproductive Age**

Concurrent deficiencies of micronutrients are well documented among young pregnant women (and young children), especially in low- and moderate-income countries [3–5]. The commonest deficiency is iron, but often there is at least one other deficiency [4]. These deficiencies in maternal micronutrient status are a result of poor-quality diets, high fertility rates, repeated pregnancies, and short inter-pregnancy intervals, increased physiological needs, as well as inadequate health systems with poor capacity, poverty and inequities, and sociocultural factors such as early marriage and adolescent pregnancies and some traditional dietary practices [6]. A systematic review identifying all studies that had been published between 1988 and 2008 reporting on micronutrient intakes in women living in resource-poor environments showed that, except for vitamin A (29%), vitamin C (34%), and niacin (34%), the reported mean/median intakes in over 50% of the studies were below the estimated average requirements, demonstrating that inadequate intakes of multiple micronutrients are common amongst women living in resource-poor settings [7], including urban settings [8].

Data on vitamin and mineral metabolism and requirements during pregnancy are surprisingly imprecise, largely because of the complexity of maternal metabolism during pregnancy. Requirements for many, but not all, micronutrients increase during pregnancy. Studies of micronutrient status in adolescents, including when pregnant, have found poor micronutrient intakes and status [9], including in the UK [10], and increased risk of small for gestational age (SGA) and LBW infants at birth [6].
Global Burden

Information on the burden of diseases can help countries assess their comparative importance in causing premature death, loss of health and disability, and so assist countries in deciding health policies and programs [11]. Although the numbers of pregnant women affected by micronutrient deficiencies, especially iron deficiency and anemia, are considerable, the actual global burden of multiple micronutrient deficiencies during pregnancy has not been estimated [3, 11]. It would be difficult to estimate as the burden rests not only on women’s mortality, morbidity and reproductive health outcomes [1], but also inter-generational effects that affect both the mother and her neonate’s immediate burden and that of her children, especially in terms of intellectual and physical development and the later incidence of noncommunicable diseases [5, 12–16].

The micronutrients, for which the global burden is reported upon, are iodine deficiency, vitamin A deficiency, and iron deficiency anemia, and an estimate for zinc has also been made [3, 11]. However, while the numbers of those affected may be great, e.g. up to two billion for iron deficiency or those living in areas of poor iodine availability, the burden (as measured by disability-adjusted life years – DALYs) on women of child-bearing age (15–59 years) is limited. Except for anemia, most of the impact is on the infants and children, e.g. the number of child deaths attributed to zinc, vitamin A and iron deficiencies was estimated in 2002 to be 19% of all child deaths or just over two billion children [3]. In total, micronutrient deficiencies were estimated in the 2002 World Health Report to cause about 6% of global DALYs [3]. Since then, progress has continued to be made in the prevention and control of iodine deficiency and vitamin A deficiency, where the burden of disease for females caused by iodine and vitamin A deficiency is only 0.2% and 0.0% of the global total of DALYs, respectively [3, 11]. By contrast, little progress has been made with iron deficiency, and estimates attribute it causing a fifth of early neonatal mortality and a tenth of maternal mortality, along with the additional morbidity of reduced cognitive development in children and work performance in adolescents and adults. About 800,000 deaths and 2.4% of global DALYs were attributed to iron deficiency in 2002 [3] with the global figures, based on 2004 estimates, in table 1, with updated figures due this year (2011) [11].

Although the global burden for reproductive-aged women from deficiencies of a range of vitamins and minerals is not estimated as such, WHO does give estimates of the three most prevalent micronutrient deficiencies by gender and by WHO region, and which show marked differences. The impact on women of poor micronutrient status is double that of males, and is far greater in low-income countries, so that the burden (as measured by DALYs) of iron deficiency anemia in low-income countries e.g. is 12.5 times that of high-income countries [11]. In the eastern Mediterranean, South Asia (SEAR) and Sub-Saharan Africa (AFR), 3 out of every 10 deaths are due to ‘communicable, reproductive or nutritional
conditions’ [11]. Most of the deaths from iron deficiency anemia in women (15–59 years) occur in low-income countries (about 70,000 poorer women annually to virtually none in high-income countries) contributing an estimated 5,792,000 DALYs (or about 1.6%) [11]. By region, SEAR bears 4.1% of the global burden for iron deficiency anemia, 3.8% in the eastern Mediterranean and 3.0% in AFR. The burden of maternal conditions in AFR and SEAR is responsible for 8% of the total global burden in women aged 15–59 years [11]. Almost all of this loss of healthy years of life is avoidable. In equivalent years of healthy life lost for women from anemia, the number in low- and middle-income countries for women is 2.4% or 7.4 million women [11]. There is no information on the probably fairly small contribution to the global burden of disease from other micronutrients of public health importance such as folate, vitamin B12 and other B vitamins and selenium, although the total number of women deficient in two or more of these is considerable, e.g. 46% of rural Ethiopian women are said to have folate deficiency. Another way of looking at this is the global burden of prematurity and LBW (to which maternal nutrition and health contribute importantly), which is 2.9% of total DALYs, and which again rises to 3.9% in low-income countries [11].

### Significance

The significance of this likely relatively large burden (in numbers if not percentages of total) due to multiple micronutrient deficiencies is that it is heavily weighted against women and especially those in low-income countries. In resource-poor settings where diagnosis and treatment may be difficult to access, solutions are likely to need a large public health component approach [6]. Because LBW is associated with higher infant mortality rates, stunting and impaired intellectual

<table>
<thead>
<tr>
<th>Nutritional cause</th>
<th>Deaths as % of total (n)</th>
<th>Burden by DALYs as % of total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>both sexes¹</td>
<td>females²</td>
</tr>
<tr>
<td>Protein-energy malnutrition</td>
<td>0.4 (251)</td>
<td>0.4 (16)</td>
</tr>
<tr>
<td>Iodine deficiency</td>
<td>0.0 (5)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>Vitamin A deficiency</td>
<td>0.0 (17)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>Iron deficiency anemia</td>
<td>0.3 (153)</td>
<td>0.4 (55)</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate total numbers in thousands.

¹ Includes children.
² For numbers only – age range for females is 15–59 years.
development, there is a need to shift, at a population level, the whole birthweight curve towards adequate weight in low and middle-income countries [6]. The risks in doing this are likely to have been overemphasized, but it still means that health service improvements and sufficient monitoring and evaluation need to be strengthened, along with maternal antenatal nutritional interventions [17].

**Multimicronutrient Supplementation**

Over the last decade, there have been studies in at least 12 countries testing the impact of antenatal micronutrient supplementation [6], and at least four meta-analyses [12, 14, 15, 18]. However, the many confounding factors, different epidemiological environments and diverse methodological approaches mean that it remains difficult to have a consistent body of evidence in order to come to conclusions and make concrete recommendations, although there are some clear directions emerging. While most of the studies and meta-analyses have appropriately guarded conclusions, it has been noted that in public health, action can rarely wait for perfect knowledge [6]. The prevalence of HIV in communities and mothers has provoked a lot of the multiple micronutrient research, using composition of supplements ranging from single micronutrients, iron-folic acid supplements (IFAs) and multiple vitamins and minerals of varying composition and multiples of RNIs/RDAs. Women's stature probably leads to different study results – from the slight, undernourished women of rural Nepal, to the somewhat better nourished women of China, or the underweight but taller women of parts of Africa and so on [6]. Further confusing issues are the measured outcomes e.g. miscarriage rates, fetal deaths, neonatal deaths, size at birth, micronutrient status of the newborn, development of the young child and so on.

**Maternal Impact**

Clinically speaking, iron deficiency and anemia in pregnancy are associated with higher maternal mortality and morbidity, premature delivery, LBW, and iron-deficiency and anemia in infants [19]. Supplementation with iron and folic acid during the antenatal period has long been WHO policy and the policy of most countries. Surprisingly few of the antenatal supplementation studies have looked at the micronutrient status of mothers themselves, but where it has been examined for, there has generally been an improvement in the levels of other micronutrients as well, although not always. Long-term intermittent multiple micronutrient supplementation enhanced hemoglobin and micronutrient status more than IFA in Bangladeshi adolescent girls with nutritional anemia [9]. In the SUMMIT study, there was also a 33% decrease in the number of anemic mothers [20]. One meta-analysis that was done of ten randomized controlled efficacy and effectiveness trials covering 12 countries showed, among other things, supplementation can improve outcomes beyond anemia, including deficiencies.
of other micronutrients and birthweight and that the multiple micronutrients worked as well, if not better, than IFA in terms of reducing anemia [6]. Multiple micronutrient supplementation increased hemoglobin synthesis to the same extent as supplementation with iron or iron-folic acid supplements, although they often contained lower amounts of iron (30 mg as opposed to 60 mg) [4]. Nevertheless, a high prevalence of various micronutrient deficiencies persisted in those receiving the multiple micronutrient supplements [4].

Supplementation with multivitamins has also been shown to reduce morbidity and mortality in non-pregnant HIV-infected women [21]. Another finding in many of the studies was that adolescent girls and women in real-life situations can have very adequate compliance/adherence rates when there are adequate supplies and some support [4, 6, 9, 17].

**Neonatal Impact**

In an early study in low-income urban US women, supplement use was associated with an approximately twofold reduction in risk of preterm delivery and in overall risk of LBW [22]. However, a Cochrane review in 2005 found that taking vitamin supplements prior to pregnancy or in early pregnancy did not prevent women from experiencing miscarriage or stillbirth, but they were less likely to develop pre-eclampsia, but also more likely to have a multiple pregnancy [23]. In a study of 4,752 US women (of whom 95% self-reported vitamin supplement use in early pregnancy), it was found that any use of vitamins during pregnancy was associated with decreased odds of miscarriage in comparison with no exposure [24]. On the other hand, Alwan et al. [25] in a prospective birth cohort study of UK women in Leeds found that regular multimicronutrient supplement use during pregnancy in a developed country setting, while not associated with size at birth was associated with reduced preterm birth if taken daily in the third trimester. In the context of HIV, a placebo-controlled trial in Tanzania showed that HIV-infected pregnant women who took multivitamins of multiples of RDAs gave birth to fewer low-weight and preterm infants [21]. Interestingly, a recent paper demonstrated that multivitamins at multiple and single doses of the RDA had similar effects on the risk of LBW [21]. An Indian study found that early neonatal morbidity was decreased by 58% [26].

Using three sets of DHS data, an Australian group found that IFA during pregnancy in Indonesia significantly reduced the risk of early neonatal death [27]. In a double-blind cluster-randomized trial in Lombok, where Indonesian women were given multimicronutrient supplements by midwives to be taken daily, their infants had an 18% reduction in early infant mortality compared with those of women given IFAs, and infants of those mothers who were undernourished or anemic at enrollment had a reduction in early infant mortality of 25 and 38%, respectively [20].

However, some important questions remain. Findings from two separate, and different, studies in Nepal showed an increase, not statistically
significant, in the risk of early neonatal mortality associated with the use of multiple micronutrient supplements compared with IFA and vitamin A, but which was significant when the studies were combined [28, 29]. The findings of an increased neonatal mortality have not been supported by at least three of the systematic reviews, as well as by data from other studies in different populations [2, 6, 12]. The meta-analysis of Ronsmans et al. [15] concluded that there was no overall difference in deaths, although when the SUMMIT results were excluded from the analysis because of significant heterogeneity (p < 0.10), there was a statistically higher odds ratio of early neonatal mortality [6]. Nevertheless, overall the meta-analysis reported by the United Nations System Standing Committee on Nutrition (SCN) found no statistically significant differences between infants of mothers supplemented with IFC and those supplemented with multimicronutrients, and noted that increased birthweight has consistently been found to be associated with reduced risk of dying in infancy in other situations [6]. A recent review by Bhutta’s group concluded that there was no significant increase in the risk of neonatal mortality where skilled birth care is available and the majority of births take place in facilities [2], a not common situation in many populations with the greatest prevalence of multiple micronutrient deficiencies.

Effects on Birthweight

The meta-analysis reported on by the SCN and others observed a mean increase in birthweight of 22 g (4.9–75.5 across studies), and a reduction in the prevalence of LBW of about 10%, with a larger impact on birthweight in infants of heavier women [6, 14]. There was a positive shift in the entire birthweight distribution, with decreases in the numbers of LBW babies and SGA babies with no differences in birth length or head circumference [6]. It was noted that the small significant increase in mean birthweight among infants of supplemented mothers is of similar magnitude to that produced by food supplementation during pregnancy [6]. Haider and Bhutta in their meta-analysis for the Cochrane review, found a relative risk of 0.84 reduction in LBW compared with iron and folic acid [12]. There was a 14% reduction in LBW with a 33% decrease in anemic mothers in the large Lombok study [20].

Another meta-analysis and systematic review of 13 published trials that included HIV-positive women also found that prenatal multimicronutrient supplementation in resource-poor settings was associated with a significantly reduced risk of LBW by 17–19% and with an improved mean birthweight of 54 g when compared with IFAs [18]. They found no significant effect of the supplementation on the risk of preterm birth or SGA infants [18]. They concluded that: ‘With the possibility of reducing the incidence of low birth weight by 17%. . .providing pregnant women with multimicronutrient supplementation offers the highest possible return for the investment. . .and could avert 1.5 million births of LBW infants globally each year’ [18].
Increases in birthweight with antenatal multiple micronutrient supplementation have now also been found in studies reported since two of the meta-analyses were published but included in that of Shah and Ohlsson [18]. One was with well-nourished French women in a hospital setting where there was an increase in the average birthweight by 251 g as compared with a placebo [Hininger et al., cited in 6]. The other was an Indian study, also hospital-based, of thin women (BMI <18.5), and where the infants of the micronutrient-supplemented mothers group were heavier by a mean 98 g and measured 0.8 cm longer and 0.2 cm larger in mid-arm circumference compared with the placebo group [26]. Incidence of LBW declined from 43.1 to 16.2% with multimicronutrient supplementation (a 70% decrease).

Effects on Infant and Child Development of Maternal Micronutrient Supplementation

Besides the health and well-being of the mother herself, and successful immediate reproductive outcomes, the subsequent health and development of the infant and older child can be clearly affected by the intrauterine environment. Early iron deficiency is known to alter the neuroanatomy, biochemistry, and metabolism in the infant leading to changes in the neurophysiologic processes that support cognitive and motor development [5]. In a long-term study of Finns, there was a direct positive association between Hb levels of the pregnant women and the educational achievement of their children later in life (at 14 and 16 years of age) with also increased odds of having a higher level of education at the age of 31 years [30]. In a rural area of Nepal where iron deficiency is prevalent, aspects of intellectual functioning including working memory, inhibitory control and fine motor functioning among offspring were positively associated with prenatal IFA supplementation [5]. Positive functional and developmental milestones of the Nepalese children whose mothers were supplemented in pregnancy with multiple micronutrients showed small improvements in weight and a decrease in peripheral adiposity after 2 years [5]. Although in several of the studies in Nepal, the addition of other micronutrients or zinc attenuated or negated the impact of the IFA, this is the opposite to a study in China where multimicronutrient prenatal supplementation found modest improved changes in cognitive outcomes compared with IFA [31]. Similarly, in Bangladesh there were small but significant improvements in motor scores and activity ratings compared with mothers receiving iron/folic acid [32]. This was also found for psychomotor but not cognitive development indices of children born to multimicronutrient-supplemented HIV-positive Tanzanian mothers (compared to placebo) [33]. Reductions in subsequent stunting, e.g. in Vietnam, have also been reported [6, 17]. The public health significance of such findings is not yet known, but may be setting these children on a different developmental trajectory that mitigates the risk of chronic disease in adult life [6], a problem that is now greater
in low- and moderate-income countries, and in rural populations as well as urban ones [8, 16].

**Significance for Interventions**

Public health antenatal and obstetric interventions on a larger scale, as the above evidence seems to suggest, would also require monitoring for effectiveness, sustainability and impact. WHO already has recommendations regarding supplementation to improve iron stores in adolescent girls and women before they enter pregnancy in areas where anemia rates are greater than 40%, and more recently a position statement suggested intermittent IFA where the prevalence of anemia in women of reproductive age is greater than 20% [19]. It is also policy to provide HIV-infected pregnant women multiple micronutrients (of a single RDA), and multimicronutrients for pregnant and lactating women in emergency situations have been encouraged in a WHO/WFP/UNICEF statement. Nevertheless, cost-effectiveness has not been established, and neither has there been clear proof that there is an advantage over IFA, although it does seem likely that a lower dose of iron is effective in a multimicronutrient supplement.

There are some other positive aspects. Most of the research appears to be heading in largely the same direction, at least in terms of much improved antenatal maternal care, including supplementation where relatively high adherence rates have been shown to be achievable in field settings where it is emphasized [6, 9, 17]. There is increased policy and donor attention to maternal and early child nutrition and health, including through a wish to achieve the relevant MDGs by 2015. From a public health perspective, the maternal and child nutrition *Lancet* series concluded that effective micronutrient interventions for pregnant women should include supplementation with iron-folic acid, and noted that multiple micronutrients reduced the risk of LBW at term by 16% [34]. There is general agreement that supplementation programs should be part of a larger improvement in antenatal care programs including increased contact, improved nutrition, deworming and access to clinic care and delivery and so on. A very recent review by Haider et al. [35] concluded that there is good evidence ‘of a significant benefit of MMN supplementation during pregnancy on reducing SGA births as compared to iron-folate, with no significant increase in the risk of neonatal mortality in populations where skilled birth care is available and majority of births take place in facilities’. Nevertheless, some outstanding issues remain: the possible increased risk of neonatal mortality in some populations; the appropriate levels of the multiple vitamins and minerals in supplements [6], and issues of cost-effectiveness [13] as interventions are needed that are both efficacious and effective [1]. Nevertheless, taking all the studies together, and with experience in countries such as Indonesia,
Mexico, Vietnam and elsewhere, it is likely that effective distribution and promotion systems can be developed for different target groups and settings [36] ‘in the context of available services in health systems and [with] birth outcomes monitored’ [35].

**Conclusions**

There are substantial inequalities in maternal and newborn health, and in access to health care. In an ideal world, young women would be born with adequate birthweight, develop appropriately without stunting, and go into their first pregnancy no earlier than 18 years, and be adequately nourished. Their health, sanitation and social support should also be optimal, not least because it seems likely the significantly better survival and other results found in Lombok may well have been due to the attention paid to antenatal services and maternal care in general. In the absence of this happening, all evidence-based, simple and cost-effective measures must be adequately tried in low-resource settings. It has been frequently noted that antenatal supplement with multiple vitamins and minerals is the norm in many affluent countries and often for the affluent in poorer countries. More programmatic information will be coming from the limited number of countries using multiple micronutrient interventions to pregnant women, but these need to be closely monitored. WHO is currently using its new NUGAG process to develop guidance and then policy for recommendations.

Building on the commentaries written around the different meta-analyses, some conclusions might be drawn.

- Deficiencies in micronutrients affect many women of reproductive age, and are associated with adverse maternal and perinatal outcomes and have even likely longer-term impacts into adulthood
- Micronutrients likely to be important for maternal, infant and child outcomes include iron, vitamin B₁₂, folate, vitamin D and selenium and probably zinc and maybe others (along with appropriate dietary energy intakes)
- In addition to multimicronutrient supplementation, optimal interventions to improve maternal nutrition need to address household food insecurity, reduce the burden of maternal infections such as HIV and malaria, and actively address gender and social disadvantage
- In the meantime, if proven effective and safe in representative health care systems, supplementation with multimicronutrients should replace supplementation with iron and folic acid in vulnerable populations as a way of breaking the intergenerational reality of LBW infants growing up disadvantaged and stunted to repeat the cycle.
References


Multiple Micronutrient Deficiencies in Pregnancy


Intervention Strategies to Address Multiple Micronutrient Deficiencies in Pregnancy and Early Childhood

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Abstract
Deficiencies of multiple micronutrients are prevalent among women of reproductive age and young children, and represent a risk factor for increased morbidity and mortality in these women and children. The role of multiple micronutrient supplementation during pregnancy and early childhood has been evaluated in randomized trials. Multiple micronutrient supplementation during pregnancy has a positive effect on birthweight and reduces prevalence of low birthweight and small for gestational age babies. It had comparable effects on prevalence of anemia regarding iron-folate supplementation. Multiple micronutrient supplementations in children have been shown to improve linear growth, weight, hemoglobin, serum zinc, serum retinol levels and motor development. Some of the most commonly used strategies to deliver multiple micronutrients include powders (e.g. Sprinkles®), crushable tablets (e.g. Foodlets), etc. Multiple micronutrient supplementation during pregnancy and early childhood seems to be an effective way of prevention of micronutrient deficiencies and has a significant protective effect against adverse outcomes related to their deficiencies. Their use on a larger scale should be considered to improve the survival and decrease morbidity and mortality in children and women.

Micronutrient deficiencies are widespread, and are a major global health problem worldwide [1]. The World Health Organization (WHO) estimates that more than 2 billion people are deficient in key vitamins and minerals, particularly vitamin A, iodine, iron and zinc [2]. Most of these people live in low-income countries and are typically deficient in more than one micronutrient. The groups most vulnerable to micronutrient deficiencies are pregnant women, lactating women and young children [3, 4]. Pregnancy represents a state of
increased metabolic requirements, and intake of key micronutrients by pregnant women especially in developing countries is usually inadequate. This inadequate intake and increased requirement during pregnancy further exacerbates the preexisting maternal deficiency [5]. The resulting micronutrient deficiencies may lead to potentially adverse effects on the mother such as anemia, hypertension, complications of labor and even death [6] and adverse perinatal outcomes such as low birthweight, small for gestational age and preterm birth [3]. The time from conception until the age of 2 years is the most critical period for any child. During pregnancy, maternal malnutrition (macro-/micronutrient) can adversely affect the birth outcomes. After birth, if exclusive breastfeeding is not practiced during the first 6 months of life or if the solid foods introduced after that period are nutrient poor, young children are likely to suffer vitamin and mineral deficiencies [4]. Table 1 shows daily requirement of some of the micronutrients according to different age groups. Figure 1 shows the consequence of mineral and vitamin deficiencies during the life cycle [7].

Globally, about 1.62 billion people are anemic, with the highest prevalence in preschool age children (47%) and the second highest in pregnant women (42%). For pregnant women, over 80% of the countries have a moderate or severe public health problem [8]. According to the latest report of the WHO, globally about 190 million preschool age children and 19.1 million pregnant women are vitamin A deficient (i.e. serum retinol <0.70 μmol/l) [9]. Approximately 100 million

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>RNI 0–3 months and 4–6 months</th>
<th>RNI 7–9 months or 10–12 months</th>
<th>RNI 1–3 years</th>
<th>Tolerable upper intakes</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, mg</td>
<td>350</td>
<td>350</td>
<td>400</td>
<td>800 mg/day (1–3 years)</td>
</tr>
<tr>
<td>D, μg</td>
<td>8.5</td>
<td>7</td>
<td>7</td>
<td>25 μg/day (0–24 months)</td>
</tr>
<tr>
<td>E, mg</td>
<td>0.4 mg/g PUFA</td>
<td>0.4 mg/g PUFA</td>
<td>0.4 mg/g PUFA</td>
<td>10 mg/100 kcal of formula</td>
</tr>
<tr>
<td>K, μg</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>not given</td>
</tr>
<tr>
<td>B₁ (thiamine), mg</td>
<td>0.2</td>
<td>0.2/0.3</td>
<td>0.5</td>
<td>not given</td>
</tr>
<tr>
<td>B₂ (riboflavin), mg</td>
<td>0.4</td>
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<td>0.6</td>
<td>not given</td>
</tr>
<tr>
<td>Niacin (equivalents), mg</td>
<td>3</td>
<td>4/5</td>
<td>8</td>
<td>2 mg/day (1–3 years)</td>
</tr>
<tr>
<td>B₆ (pyridoxine), mg</td>
<td>0.2</td>
<td>0.3/0.4</td>
<td>0.7</td>
<td>5 mg/day (1–3 years)</td>
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<tr>
<td>B₁₂, μg</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>not given</td>
</tr>
<tr>
<td>Biotin, μg</td>
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<td>not given</td>
<td>not given</td>
<td>7.5/100 kcal</td>
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<tr>
<td>Pantothenate, mg</td>
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<td>1.2/100 kcal</td>
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<tr>
<td>Folic acid, μg</td>
<td>50</td>
<td>50</td>
<td>70</td>
<td>200 μg/day (1–3 years)</td>
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<tr>
<td>C, mg</td>
<td>25</td>
<td>25</td>
<td>30</td>
<td>30 mg/100 kcal</td>
</tr>
</tbody>
</table>

PUFA = Polyunsaturated fatty acids; RNI = reference nutrient intake. Vitamin A expressed as retinol equivalent (RE): 1 μg RE = 3.33 IU. Vitamin D (calciferol): 1 μg = 40 IU. Vitamin E, α-tocopherol equivalent: 1 mg = 1 IU. Vitamin C expressed as ascorbic acid.
Intervention Strategies to Address Multiple Micronutrient Deficiencies

women of reproductive age suffer from iodine deficiency [10]. An estimated 82% of pregnant women worldwide have inadequate intakes of zinc to meet the normative needs of pregnancy [11]. Suboptimal vitamin B6 and B12 status has been observed in many developing countries [12].

Deficiency of micronutrients is a risk factor for increased incidence and severity of infectious illness and of dying from diarrhea, measles, malaria and pneumonia [13]. Data from intervention studies have shown that unsupplemented vitamin A-deficient children have increased risk of diarrhea mortality (RR 1.47, 95% CI: 1.25–1.75) and measles mortality (RR 1.35, 95% CI: 0.96–1.89) [4]. Similarly, risk associated with zinc deficiency for children below 5 years is RR 1.27 (95% CI: 0.96–1.63) for diarrhea, 1.18 (95% CI: 0.90–1.54) for pneumonia and 1.11 (95% CI: 0.94–1.30) for malaria [4]. Anemia during pregnancy is a risk factor for maternal mortality [14]; however, anemia in childhood has not been shown to be associated with an increased risk of mortality [13]. However, it is well established that anemia during childhood can adversely affect the developmental outcomes [15]. Evidence suggested that iron deficiency anemia accounted for a loss of 1–2 intelligence quotient points [16, 17]. Iodine deficiency has adverse effects on both pregnancy outcome and child development, and it has been shown that chronic iodine deficiency can lead to a 13.5
point reduction in intelligence of the affected population [4]. Table 2 presents the number of deaths and disability-adjusted life years lost to micronutrient deficiencies.

Given the significant impact of deficiencies of key micronutrients during pregnancy and early childhood [4], there has been an increased interest in supplementation with multiple micronutrients, and supplementation with multiple micronutrients during pregnancy may be a feasible public health strategy [18]. The purpose of this chapter was to summarize the current evidence on strategies of multiple micronutrient supplementations during pregnancy and early childhood. A literature search was conducted on PubMed, Cochrane library and WHO/UNICEF databases.

### Maternal Micronutrient Supplementation during Pregnancy

Many workers had attempted augmentation of iron-folate supplementation in pregnancy with additional micronutrients, but the first systematic efforts to undertake this were almost a decade ago [5]. In 1999, the UNICEF/WHO/UN University proposed a prenatal supplement UNIMAPP containing fifteen micronutrients, including iron and folic acid which could provide one recommended daily allowance of each and potentially replace standard iron-folate supplements for pregnant women in low- and middle-income countries [19]. A Cochrane review on the subject [20] indicated that iron-folate and multiple micronutrient supplementation had a comparable effect on maternal anemia and a significant effect on incidence of low birthweight babies and small for gestational age babies (fig. 2, 3). Another systematic review was undertaken by a team commissioned by UNICEF/WHO/SCN which analyzed data from 12 trials using the UNIMAPP formulation and evaluated effects on maternal and pregnancy outcomes [21]. The pooled results had shown that multiple micronutrient supplementation was associated with an increase in mean birthweight (mean difference 22.4 g, 95% CI: 8.3–36.4 g), a reduction in the prevalence of low birthweight (odds ratio, OR = 0.89, 95% CI: 0.81–0.97) and small for gestational

### Table 2. Global deaths and disease burden measured in disability-adjusted years (DALYs) in children <5 years due to micronutrient deficiency in 2004 [4]

<table>
<thead>
<tr>
<th>Measure</th>
<th>Deaths</th>
<th>Death in children &lt;5 years, %</th>
<th>Disease burden 1,000 DALYs</th>
<th>DALYs in children &lt;5 years, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A deficiency</td>
<td>667,771</td>
<td>6.5</td>
<td>22,668</td>
<td>5.3</td>
</tr>
<tr>
<td>Zinc deficiency</td>
<td>453,207</td>
<td>4.4</td>
<td>16,342</td>
<td>3.8</td>
</tr>
<tr>
<td>Iron deficiency</td>
<td>20,854</td>
<td>0.2</td>
<td>2,156</td>
<td>0.5</td>
</tr>
<tr>
<td>Iodine deficiency</td>
<td>3,619</td>
<td>0.03</td>
<td>2,614</td>
<td>0.6</td>
</tr>
</tbody>
</table>
### Fig. 2. Multiple micronutrient supplements during pregnancy: effect on incidence of low-birthweight babies [20].

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>log[ ]</th>
<th>SE</th>
<th>Weight</th>
<th>IV, random, 95% CI</th>
<th>IV, random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bhutta 2009</td>
<td>0.1838</td>
<td>0.1906</td>
<td>3.0%</td>
<td>1.20 [0.82, 1.76]</td>
<td></td>
</tr>
<tr>
<td>Christian 2003</td>
<td>-0.1625</td>
<td>0.0674</td>
<td>20.6%</td>
<td>0.85 [0.74, 0.97]</td>
<td></td>
</tr>
<tr>
<td>Fawzi 2007</td>
<td>-0.1863</td>
<td>0.07255</td>
<td>18.3%</td>
<td>0.83 [0.72, 0.96]</td>
<td></td>
</tr>
<tr>
<td>Friis 2004</td>
<td>-0.3011</td>
<td>0.2537</td>
<td>1.8%</td>
<td>0.74 [0.45, 1.22]</td>
<td></td>
</tr>
<tr>
<td>Gupta 2007</td>
<td>-0.9675</td>
<td>0.3026</td>
<td>1.3%</td>
<td>0.38 [0.21, 0.69]</td>
<td></td>
</tr>
<tr>
<td>Kaestel 2005</td>
<td>-0.1278</td>
<td>0.1954</td>
<td>3.1%</td>
<td>0.88 [0.60, 1.29]</td>
<td></td>
</tr>
<tr>
<td>Osrin 2005</td>
<td>-0.2876</td>
<td>0.1138</td>
<td>8.5%</td>
<td>0.75 [0.60, 0.94]</td>
<td></td>
</tr>
<tr>
<td>Ramakrishnan 2003</td>
<td>-0.04082</td>
<td>0.2571</td>
<td>1.8%</td>
<td>0.96 [0.58, 1.59]</td>
<td></td>
</tr>
<tr>
<td>Roberfroid 2008</td>
<td>-0.09431</td>
<td>0.17166</td>
<td>3.9%</td>
<td>0.91 [0.65, 1.27]</td>
<td></td>
</tr>
<tr>
<td>SUMMIT 2008</td>
<td>-0.1508</td>
<td>0.0819</td>
<td>15.0%</td>
<td>0.86 [0.73, 1.01]</td>
<td></td>
</tr>
<tr>
<td>Sunawang 2009</td>
<td>0.1988</td>
<td>0.2027</td>
<td>2.8%</td>
<td>1.22 [0.82, 1.81]</td>
<td></td>
</tr>
<tr>
<td>Tofail 2008</td>
<td>-0.1508</td>
<td>0.09778</td>
<td>11.1%</td>
<td>0.86 [0.71, 1.04]</td>
<td></td>
</tr>
<tr>
<td>Zagre 2007</td>
<td>-0.1392</td>
<td>0.1487</td>
<td>5.2%</td>
<td>0.87 [0.65, 1.16]</td>
<td></td>
</tr>
<tr>
<td>Zeng 2008</td>
<td>-0.1054</td>
<td>0.1797</td>
<td>3.6%</td>
<td>0.90 [0.63, 1.28]</td>
<td></td>
</tr>
<tr>
<td><strong>Subtotal (95% CI)</strong></td>
<td>100.0%</td>
<td>0.86 [0.79, 0.92]</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 15.55$, df = 13 ($P = 0.27$); $I^2 = 16$

Test for overall effect: $Z = 4.14$ ($P < 0.0001$)

### Fig. 3. Multiple micronutrient supplements during pregnancy: effect on incidence of small for gestational age babies [20].

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>log [risk ratio]</th>
<th>SE</th>
<th>Weight</th>
<th>Risk ratio IV, random, 95% CI</th>
<th>Risk ratio IV, random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bhutta 2009</td>
<td>-0.3147</td>
<td>0.3223</td>
<td>0.7%</td>
<td>0.73 [0.39, 1.37]</td>
<td></td>
</tr>
<tr>
<td>Christian 2003</td>
<td>-0.0726</td>
<td>0.0471</td>
<td>33.7%</td>
<td>0.93 [0.85, 1.02]</td>
<td></td>
</tr>
<tr>
<td>Fawzi 2007</td>
<td>-0.2357</td>
<td>0.0617</td>
<td>19.6%</td>
<td>0.79 [0.70, 0.89]</td>
<td></td>
</tr>
<tr>
<td>Friis 2004</td>
<td>-0.2107</td>
<td>0.2347</td>
<td>1.4%</td>
<td>0.81 [0.51, 1.28]</td>
<td></td>
</tr>
<tr>
<td>Gupta 2007</td>
<td>-0.51082</td>
<td>0.2068</td>
<td>1.7%</td>
<td>0.60 [0.40, 0.90]</td>
<td></td>
</tr>
<tr>
<td>Kaestel 2005</td>
<td>-0.2744</td>
<td>0.255</td>
<td>1.1%</td>
<td>0.76 [0.46, 1.25]</td>
<td></td>
</tr>
<tr>
<td>Osrin 2005</td>
<td>-0.2357</td>
<td>0.1928</td>
<td>2.0%</td>
<td>0.79 [0.54, 1.15]</td>
<td></td>
</tr>
<tr>
<td>Ramakrishnan 2003</td>
<td>-0.1508</td>
<td>0.22806</td>
<td>1.4%</td>
<td>0.86 [0.55, 1.34]</td>
<td></td>
</tr>
<tr>
<td>Roberfroid 2008</td>
<td>-0.1863</td>
<td>0.1247</td>
<td>4.8%</td>
<td>0.83 [0.65, 1.06]</td>
<td></td>
</tr>
<tr>
<td>SUMMIT 2008</td>
<td>-0.0305</td>
<td>0.0787</td>
<td>12.1%</td>
<td>0.97 [0.83, 1.13]</td>
<td></td>
</tr>
<tr>
<td>Sunawang 2009</td>
<td>-0.1392</td>
<td>0.16468</td>
<td>2.8%</td>
<td>0.87 [0.63, 1.20]</td>
<td></td>
</tr>
<tr>
<td>Tofail 2008</td>
<td>-0.1053</td>
<td>0.1355</td>
<td>4.1%</td>
<td>0.90 [0.69, 1.17]</td>
<td></td>
</tr>
<tr>
<td>Zagre 2007</td>
<td>-0.1984</td>
<td>0.1344</td>
<td>4.1%</td>
<td>0.82 [0.63, 1.07]</td>
<td></td>
</tr>
<tr>
<td>Zeng 2008</td>
<td>-0.1165</td>
<td>0.0843</td>
<td>10.5%</td>
<td>0.89 [0.75, 1.05]</td>
<td></td>
</tr>
<tr>
<td><strong>Subtotal (95% CI)</strong></td>
<td>100.0%</td>
<td>0.87 [0.83, 0.92]</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 10.96$, df = 13 ($P = 0.61$); $I^2 = 0$

Test for overall effect: $Z = 4.97$ ($P < 0.00001$)
age birth (OR = 0.90, 95% CI: 0.82–0.99), and an increase in the prevalence of large for gestational age birth (OR = 1.13, 95% CI: 1.00–1.28). There were no significant effects of multiple micronutrient supplementation on birth length, head circumference or the duration of gestation [22].

Recently, there has been interest in prenatal food supplementation with both macro- and multiple micronutrients. A randomized trial from Burkina Faso has shown that combined supplementation with balanced protein energy macronutrient and multiple micronutrient has more pronounced effect on birth length compared to multiple micronutrients alone [23]. An effort should be made to reproduce results of this study in other parts of the developing world as deficiencies of macro- and micronutrients coexist.

Prenatal Multiple Micronutrient Supplementation in HIV-Affected Populations

Observational studies have demonstrated an association with low biochemical and dietary levels of micronutrients and increased risk of mother to child transmission (MTCT) in HIV-infected mothers [24–26]. However, a review of randomized trials by Mills et al. [27] showed conflicting evidence without strong evidence of benefit or harm on micronutrients. The review included three trials on vitamin A, of whom two suggested no difference in MTCT, while the third and largest trial suggested an increased risk of MTCT (RR 1.35, 95% CI: 1.11–1.66, p = 0.009). Two of the vitamin A trials addressed the impact of supplementation on preterm delivery; one suggested a benefit (RR 0.65, 95% CI: 0.44–0.94) and the other no difference. Two of the included trials looked at multivitamin use, and one reported data on MTCT with a non-significant relative risk of 1.04 (95% CI: 0.82–1.32). There was no significant effect on preterm delivery or child mortality at 1 year [27].

Multiple Micronutrient Supplementation among Children at Risk of Deficiencies

As deficiencies of important micronutrients like iron, zinc vitamin A, etc. are prevalent in children in developing countries, efforts have been made to supplement infants and children with multiple micronutrients [28]. A review by Ramakrishnan et al. [29] based on 20 randomized trials has shown that multiple micronutrient interventions improved linear growth (effect size 0.09; 95% CI: 0.008–0.17). Another review by Allen et al. [28] has shown that in children, multiple micronutrient interventions resulted in small but significantly greater improvements in length or height (effect size 0.13; 95% CI: 0.055–0.21) and weight (effect size 0.14; 95% CI: 0.029–0.25), hemoglobin (effect size 0.39; 95% CI: 0.25–0.53), serum zinc (effect size 0.23; 95% CI: 0.18–0.43), serum retinol
(effect size 0.33; 95% CI: 0.050–0.61) and motor development. In addition to these benefits, multiple micronutrients have a beneficial effect on mental development of children. A review by Eilander et al. [30] has shown that multiple micronutrient supplementation during childhood has a significant effect on academic performance (effect size 0.30 SD, 95% CI: 0.01–0.58). There was however no significant effect on fluid intelligence (effect size 0.14 SD, 95% CI: –0.02 to 0.29) or crystallized intelligence (effect size –0.03 SD, 95% CI: –0.21 to 0.15).

**Delivery of Multiple Micronutrients to Pregnant Women and Children**

Several strategies have been applied to deliver multiple micronutrients to pregnant women and children [31, 32]. Table 3 gives composition of some of the products delivering multiple micronutrients [33]. Sprinkles® are the most extensively studies product among these, and consist of sachets containing micronutrients in a powdered form, to be sprinkled onto a portion of food just before it is consumed [34, 35]. Crushable or chewable tablets (Foodlets) are multiple micronutrient tablets that can be easily dissolved in a small amount of liquid, or crushed and added to foods [36]. The other micronutrients packages in table 3 have been used at different location; however, the efficacy of some of them needs to be tested in randomized trials.

In pregnant women, different approaches have been used for increasing maternal intake of multiple micronutrients [37]. One of them is to improve dietary quality, which in many situations might require increasing consumption of animal source foods, fruits and vegetables. In some situations, well-designed nutrition education programs can improve dietary quality and pregnancy outcome [38]. Other approach is to provide multiple micronutrient supplements (described above) to women during pregnancy. In the Cochrane review on the topic that included 17 trials, 7 trials enrolled participants in the first trimester of pregnancy, one when participants were <28 weeks of gestation, 3 trials enrolled in the second trimester, 2 trials enrolled in both second and third trimester, whereas 3 trials enrolled pregnant women who were less than 37 weeks of gestation [20]. In all the included studies, micronutrients were given in the form of supplements, except one study that used fortification for the delivery of micronutrients.

In children, the most commonly used approach is the home fortification of complementary foods. Dewey et al. [31] has reviewed home fortification strategies of complementary foods. Home fortification was highly effective at reducing iron deficiency and decreased the prevalence of anemia by half. There was no effect on growth with micronutrients only; however, there was a significant effect when micronutrients were combined with a small amount of energy (including fat and protein). These results were based on the pooled data from two efficacy trials in Africa [39, 40]. Home fortification also had a beneficial
impact on morbidity in high-risk populations in some studies; however, there was no overall significant impact. Acceptability of home fortification by caregivers and young children was high, and side effects were rare [31].

### Side Effects

There is considerable debate on the potential adverse effects of providing multiple micronutrient supplements during pregnancy with a concern about an increase in neonatal mortality in less developed health systems that have suboptimal maternal care [41, 42]. There are a few studies that reported mortality beyond the neonatal period with maternal multiple micronutrient supplementation. Christian et al. [43] reported infant deaths (0–3 months) in the multiple

<table>
<thead>
<tr>
<th>Plumpy’Nut micronutrients</th>
<th>amount</th>
<th>B5¹ micronutrients</th>
<th>amount</th>
<th>Sprinkles micronutrients</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>910 μg</td>
<td>Vitamin B₁</td>
<td>0.52 mg</td>
<td>Vitamin A</td>
<td>300 μg</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>16 μg</td>
<td>Vitamin B₂</td>
<td>0.52 mg</td>
<td>Vitamin D</td>
<td>5 μg</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>20 mg</td>
<td>Niacin</td>
<td>6.5 mg</td>
<td>Vitamin E</td>
<td>6 IU</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>53 mg</td>
<td>Vitamin B₅</td>
<td>0.87 mg</td>
<td>Vitamin B₁</td>
<td>0.5 mg</td>
</tr>
<tr>
<td>Vitamin B₁</td>
<td>0.6 mg</td>
<td>Vitamin B₁₂</td>
<td>1.3 μg</td>
<td>Vitamin B₂</td>
<td>0.5 mg</td>
</tr>
<tr>
<td>Vitamin B₂</td>
<td>1.8 mg</td>
<td>Vitamin C</td>
<td>40 mg</td>
<td>Niacin</td>
<td>6 mg</td>
</tr>
<tr>
<td>Vitamin B₆</td>
<td>0.6 mg</td>
<td>Vitamin A</td>
<td>1567 IU</td>
<td>Vitamin B₆</td>
<td>0.5 mg</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>1.8 μg</td>
<td>Vitamin D</td>
<td>172 IU</td>
<td>Vitamin B₁₂</td>
<td>0.9 μg</td>
</tr>
<tr>
<td>Vitamin K</td>
<td>21 μg</td>
<td>Vitamin E</td>
<td>3.5 mg</td>
<td>Vitamin C</td>
<td>30 mg</td>
</tr>
<tr>
<td>Biotin</td>
<td>65 μg</td>
<td>Folic acid</td>
<td>130 μg</td>
<td>Folic acid</td>
<td>160 μg</td>
</tr>
<tr>
<td>Folic acid</td>
<td>210 μg</td>
<td>Pantothenic acid</td>
<td>2.2 mg</td>
<td>Iron</td>
<td>12.5 μg</td>
</tr>
<tr>
<td>Pantothenic acid</td>
<td>3.1 mg</td>
<td>Biotin</td>
<td>62.5 μg</td>
<td>Zinc</td>
<td>5 mg</td>
</tr>
<tr>
<td>Niacin</td>
<td>5.3 mg</td>
<td>Calcium</td>
<td>600 mg</td>
<td>Copper</td>
<td>0.3 mg</td>
</tr>
<tr>
<td>Calcium</td>
<td>320 mg</td>
<td>Magnesium</td>
<td>60 mg</td>
<td>Iodine</td>
<td>90 μg</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>394 mg</td>
<td>Iron</td>
<td>10 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium</td>
<td>1,111 mg</td>
<td>Phosphorus</td>
<td>600 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnesium</td>
<td>92 mg</td>
<td>Zinc</td>
<td>10 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>14 mg</td>
<td>Iodine</td>
<td>100 μg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td>1.78 mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>11.53 mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iodine</td>
<td>110 μg</td>
<td></td>
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</tr>
<tr>
<td>Sodium</td>
<td>&lt; 290 mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selenium</td>
<td>30 μg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Efficacy not proven in trials.
micronutrient recipients versus controls (receiving vitamin A only) with a non-significant higher risk in the micronutrient group (RR = 1.07; 95% CI: 0.75–1.58). In contrast, Shankar et al. [44] in the considerably larger SUMMIT trial, reported a statistically significant 18% reduction in early infant mortality (0–3 months) in the multiple micronutrient group compared to iron-folate (RR = 0.82; 95% CI: 0.70–0.95; p = 0.010), with comparable results for postneonatal mortality – from 29 to 90 days after birth (RR = 0.70; 95% CI: 0.55–0.89). A pooled analysis in a review for Live Saved Tool [45] showed a non-significant overall effect on neonatal mortality (RR = 1.17; 95% CI: 0.95–1.44). However, a subgroup analysis based on provision of skilled attendance at birth showed that there was an increased risk of neonatal mortality in places where most of the deliveries were conducted at home (fig. 4). There was no increased risk in settings where the majority of births took place at facilities. These findings suggest that the use of

<table>
<thead>
<tr>
<th>Foodlet</th>
<th>micronutrients</th>
<th>amount</th>
<th>micronutrients</th>
<th>amount</th>
<th>micronutrients</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vitamin A</td>
<td>375 μg</td>
<td>Vitamin B_1</td>
<td>2.8 mg</td>
<td>Vitamin A</td>
<td>300 IU</td>
</tr>
<tr>
<td></td>
<td>Vitamin D</td>
<td>5 μg</td>
<td>Vitamin B_2</td>
<td>8.2 mg</td>
<td>Vitamin C</td>
<td>10 mg</td>
</tr>
<tr>
<td></td>
<td>Vitamin E</td>
<td>6 mg</td>
<td>Niacin</td>
<td>50 mg</td>
<td>Iron</td>
<td>12 mg</td>
</tr>
<tr>
<td></td>
<td>Vitamin B_1</td>
<td>0.5 mg</td>
<td>Vitamin B_6</td>
<td>1.65 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vitamin B_2</td>
<td>0.5 mg</td>
<td>Pantothenic acid</td>
<td>28 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Niacin</td>
<td>6 mg</td>
<td>folic acid</td>
<td>2 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vitamin B_6</td>
<td>0.5 mg</td>
<td>Vitamin B_12</td>
<td>13 μg</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vitamin B_12</td>
<td>0.9 μg</td>
<td>Vitamin C</td>
<td>600 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vitamin C</td>
<td>35 mg</td>
<td>Vitamin A</td>
<td>23,000 IU</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Folic acid</td>
<td>150 μg</td>
<td>Vitamin D</td>
<td>2,000 IU</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Iron</td>
<td>10 mg</td>
<td>Vitamin E</td>
<td>75 IU</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zinc</td>
<td>10 mg</td>
<td>Calcium</td>
<td>2,600 mg</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Copper</td>
<td>0.6 mg</td>
<td>Zinc</td>
<td>120 mg</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Iodine</td>
<td>59 μg</td>
<td>Iron</td>
<td>80 mg</td>
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</table>
multiple micronutrient supplements in populations to address maternal anemia and reduce the incidence of small for gestational age must be accompanied by the provision of skilled care at delivery and facility births to offset any potential increase in the risk of obstructed labor and birth asphyxia [46, 47].

As different strategies are being evaluated to supplement children with iron to prevent anemia, there are concerns about increased risk of severity of infectious disease in the presence of malaria and/or undernutrition [48]. Given the reported increase in malaria-associated morbidity following the use of large-scale iron supplementation in Zanzibar, the entire rationale for blanket iron supplements in children has been questioned [49]. Nutrient interactions, especially in complex formulations, are another area for study as they may hinder absorption of some nutrients [50].

**Conclusions**

Multiple micronutrient supplementation during pregnancy and early childhood in populations at risk is an effective way of prevention of micronutrient deficiencies. Although preparations and modes of administration differ and there are uncertainties around interactions, such strategies have protective effects against adverse outcomes associated with micronutrient deficiencies.
Further studies are needed on cost-effectiveness and delivery strategies in health system settings.

References


41 Bhutta ZA, Haider BA: Prenatal micronutrient supplementation: are we there yet? CMAJ 2009;180:1188–1189.


Summary on Micronutrient Requirements and Deficiencies in Maternal and Child Nutrition

Following on the fabulous summary from Wafaei Fawzi, the important thing to take home is that some of these interventions or potential strategies are really quite interconnected. I don’t know how many of you might have seen a recent analysis of the factors that determined how countries have been able to reduce their child mortality in the last 3–4 decades. This analysis by Prof. Chris Murray’s team at the Institute for Health Metrics and Evaluation evaluated a range of factors influencing child mortality trends over time and determined that the strongest determinant amongst all affecting child mortality change was maternal education. It actually contributed to 54% of the total reduction that was seen across a huge range of data sets in countries. So I can’t underscore more the importance of maternal education and supporting something as fundamental as this not just for improving health and nutrition but from a development perspective.

This has been an exceptionally informative day. This was just the opening innings of the entire micronutrient workshop, and I am sure there will be much more. Today, we focused principally on setting the scene and addressing some of the key issues around micronutrients; what is it that we do and don’t know? We began by looking at the landscape in terms of what exists in relation to burden data of deficiencies, key micronutrients and then moved on to very specific nutrients and then combinations thereof.

I am not going to repeat the points that have already been made, but one of the key messages from the first session and the latter part of the afternoon was the importance of getting good representative data. It is probably a fair summation that on the interface of maternal newborn and child nutrition as well as adolescent nutritional status, our information and data bases are very skimpy, and therefore a concerted effort needs to be made across the public health community to try and strengthen this database. It is also a responsibility of the nutrition industry working in areas of global nutrition to try and see
how they can help plug some of those data gaps, because these are clearly not only of public health importance; they also have importance in terms of their own R&D for products that may be of value to public health and nutrition outcomes.

A large proportion of the discussion this morning focused on the relationship of zinc to growth and outcomes, and I am going to just very briefly outline the things that I have picked up in areas that you may consider in your deliberations of the next couple of days, and these are not necessarily in the order of the sessions or the presentations. The first important issue was the need for much better information around zinc requirements, and we were really pleased to hear from Michael Hambidge that there has been progress in terms of reconciling the desperate recommendations between two large technical groups looking at this issue, one that I sit on as well, and the other being the Institute of Medicine and some of the WHO people. And it’s great to see this coming together. We also heard how uncertain we currently are in trying to match the science of stable isotope methodology with some of the fundamental data gaps around requirements and around the whole issue of status. Several amongst the audience pointed out to the difficulty that we have in assessing zinc at the population level given the poor association of plasma or serum zinc with status measurements. This being such an important issue, I would make the case that we should focus our efforts on trying to come up with measures of assessing zinc status and requirements thorough easily applicable protocols that can be implemented in developing country settings, and I know that there are some that are currently being planned. We had quite a lot of discussion in various presentations on the heterogeneity of the data around zinc and growth, particularly around preventive zinc supplementation, and these data are probably amenable to further analysis using some of the approaches that we had in the multiple micronutrient field. You have also seen how several groups working independently have been able to bring the science together, and approaching the issue completely independent of each other have come up with very comparable conclusions. It is also gratifying to see this because it tells you exactly what needs to be done around zinc too. So, rather than just do pure meta-analysis and come up with perfunctory forest plots, we need robust meta-regressions across studies to try and understand some of the contextual differences. I believe we need to control analysis across studies for factors such as intrauterine growth retardation and the proportion of those children who start off with deficits that may already be too late to change. Today’s presentations and discussions have raised a number of ideas as to how we can try and shed more light on the zinc and growth literature both in terms of understanding it and also importantly determine future studies. One issue that came up in the discussion around zinc was that we do not necessarily have a good vehicle for administration, that there are serious concerns that Sprinkles or micronutrient powders may not be an appropriate strategy for administering zinc because we have so limited data on
bioavailability of zinc therefrom. So, I certainly think a lot of attention needs to be focused on getting those information gaps resolved.

Robert Black presented findings from the zinc mortality studies on potential interactions of zinc and iron. This is a hugely important issue that needs to be resolved perhaps employing stable isotope methodology and studies that also look at some of the potential mechanisms that may exist for such interaction. There were some uncertainties which were also highlighted around the information which should be priorities areas for research. One of those is the impact of zinc on both the treatment of diarrhea and other biological outcomes in the prevention mode in relatively zinc-replete populations. This is an issue which is increasingly highlighted because of the negative studies in Europe recently and some of the previous studies from Australia which have failed to show an effect. I do think we have to address this, and perhaps best through well-designed prospective trials to answer the question if there are populations which will not benefit from zinc supplementation. Secondly, given the burden of morbidity and mortality therein, the whole issue of younger infants under 6 months is important. I think the data do point out that zinc therapy for diarrhea is not effective in this population, but our understanding of the reasons why is inexact. This information gap does cast a shadow on the entire zinc and diarrhea treatment literature, so we need studies that also intercalate some mechanistic information.

There were lots of questions raised on the lack of information on diarrhea etiology in relation to response to zinc: Should we be giving zinc for treatment of diarrhea in a background of preventive zinc dosing? Does fortification work as a strategy, so if you have got fortified foods coming in, do you really need additional zinc supplementation? I don't have the answers to those questions, but they are all very important and need to be addressed in a robust manner. In particular, the role of Sprinkles as a vehicle for supplying zinc is an issue, not just because my group works on them, but because there is now a global rollout of micronutrient powders and Sprinkles. If there is a question mark in terms of availability of zinc from Sprinkles, we need to resolve that very quickly. I was pleased to learn that there is a study going on in Kenya using methodologies to address whether Sprinkles with zinc and Sprinkles with zinc and iron are comparably effective. These are high-priority studies, and they probably need to be done with diverse dietary background so that we know how much of the issue is diet and how much is actually the bioavailability from Sprinkles.

The other issue that did come up this morning and is not really partitioned in the context of zinc or others is this whole issue of the confusion that we currently have of what to do for acute versus chronic malnutrition. When we did the *Lancet* series, a lot of emphasis was put on using stunting as a measure of undernutrition because we felt from robust analysis that it was a much better marker of undernutrition than the currently available MDG1 target of underweight. We also recognized at the time when we did the analysis that if you
looked at the overlap of severe acute malnutrition and stunting, the overlap was relatively minimal, and therefore conceptually as a public health indicator stunting and severe acute malnutrition would allow us to target those populations at risk appropriately, one being a much more chronic process, the other being an acute process that you targeted with therapeutic feeding. I think the confusion that has arisen has been largely around now putting up the moderate acute malnutrition category which overlaps to a fairly large extent with stunting, and therefore countries and practitioners are getting very confused whether moderate acute malnutrition also represents acute malnutrition or there are subgroups within it with chronic malnutrition. I would make a plea to the nutritionists who are also on a public health nutrition platform to try and harmonize this because it’s causing enormous confusion at scale. I just got a message from a colleague in Pakistan who is involved in this controversy right now, and it’s causing huge policy confusion whether we should be addressing moderate acute malnutrition in populations with therapeutic feeding at scale. This is not a trivial issue, and I would underscore that as something that came up today, and we should definitely keep it on the table.

Lastly, I think the whole multiple micronutrient session despite the obvious overlap between Ian Darnton-Hill’s and my presentation was an extremely illuminating one for me personally because we have got a lot of feedback from the group. I would like to underscore a few things. We don’t need more small-scale studies in this area. I think what we need is larger effectiveness kind of evaluation of this intervention layered on top of fairly robust in-depth scientific evaluation of what multiple micronutrients mean, what kind of deficiencies we are tackling and what kind of outcomes we should be preparing for. If the malaria community can raise 30–40 million USD to address malaria issues and response to treatment with some of the best genomics platforms that you can find, I can’t understand why we, as a nutrition community, cannot address this huge issue that is associated with 18–20 million low birthweight births every year. There was also an understandable note of caution of moving carefully before we make this a programmatic priority because of potential questions that we haven’t answered, and I entirely agree with this. I think if large-scale effectiveness evaluations around some of the multiple micronutrient questions can be designed, the safety elements can be easily answered.

Zulfiqar Bhutta
Vitamin A Supplementation, Infectious Disease and Child Mortality: A Summary of the Evidence

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Departments of ①Nutrition, ②Epidemiology and ③Global Health and Population, Harvard School of Public Health, Boston, MA, USA

Abstract

This manuscript reviews the evidence related to the effects of vitamin A (VA) supplementation of women and children on child health and mortality. VA supplementation of children aged 6–59 months has been well studied, and meta-analyses have consistently demonstrated effects on all-cause mortality, yet its mechanisms and the reasons for heterogeneous effects on mortality across trials continue to be debated. Recent meta-analysis of cause-specific mortality suggests beneficial effects on diarrheal mortality, with null but potentially beneficial effects also present for mortality from measles, lower respiratory infection, and meningitis. Some evidence suggests that pneumonia severity may increase with VA supplementation in this age group, particularly among well-nourished children. Maternal supplementation with VA during pregnancy has not shown benefits on neonatal mortality in large trials. A recent meta-analysis suggested that high-dose supplementation of lactating women immediately following delivery did not affect child survival. There is still uncertainty around the benefits of neonatal VA supplementation that should be resolved once the findings of ongoing trials are reported.

Introduction

Next year will mark the 100th anniversary of the isolation of ‘fat soluble A’ by McCollum and Davis [1]. Although interest in vitamin A (VA) waned over much of the subsequent century, a renewal of interest in the 1980s led to numerous randomized trials testing the efficacy of VA supplementation on adverse outcomes among preschool age children, infants and neonates, and pregnant and lactating women. In this chapter, we provide an overview of the epidemiological
evidence on routine preventive VA supplementation of neonates, infants, and children and child health outcomes. It is important to note that VA supplementation has long been demonstrated as efficacious in the treatment of measles and both treatment and prevention of xerophthalmia, and in the interest of brevity we have not extensively reviewed that evidence here [2].

Epidemiology and Causes of Vitamin A Deficiency during Childhood

VA deficiency is a problem of greatest public health concern among children less than 5 years of age and pregnant and lactating women. It has been estimated that 190 million preschool children and 19.1 million pregnant women have low serum retinol concentrations (<0.70 μmol/l) in countries at risk [3]. Night blindness, the first stage of clinical VA deficiency, affects 5.2 million preschool age children and 9.75 million pregnant women in countries at risk. Among the WHO regions, South East Asia and Africa have the highest prevalence of preschool subclinical VA deficiency (at 49 and 44%, respectively), although the absolute number of children at risk is much higher in South East Asia (91 vs. 56 million children) [3].

The mean requirement for VA during early childhood ranges from 180 μg retinol equivalents (RE)/day for neonates and infants to 200 μg RE/day up to age 6 years, with recommended safe intake set at 375 and 450 μg RE/day, respectively [4]. In children, clinical symptoms of VA deficiency include night blindness as the first physical manifestation, proceeding to conjunctival and corneal involvement which then leads to corneal ulceration and scarring resulting in blindness if uncorrected [2]. It is well accepted that the immune-compromising effects of VA deficiency manifest long before the physical appearance of deficiency [2].

VA deficiency during infancy and early childhood is caused by multiple factors including maternal VA deficiency (and low concentrations of retinol in breast milk), suboptimal breastfeeding, complementary foods low in VA, and severe or repeated episodes of infectious disease [5]. Sources of performed VA include liver, eggs, milk, and fortified foods, all of which are consumed infrequently in developing countries. Generally, foods containing provitamin A carotenoids such as green leafy vegetables and yellow-orange fruits such as mango and papaya are thought to provide the majority of dietary VA in such countries. Following the downward adjustment of conversion factors for provitamin A carotenoids for estimating intake of VA from plant foods, it has been generally assumed that such foods contribute relatively little towards meeting VA requirements [6–8]. While plant sources of food are unlikely by themselves to fully meet requirements when consumed in the quantities typical of many developing country diets, there is some evidence that their consumption may still offer protection against xerophthalmia, mortality, and growth faltering, particularly among children not supplemented with VA [9–12].
Supplementation during Pregnancy

Early work by Mellanby and Green reported in 1929 suggested the potential for VA to reduce puerperal septicemia, although little follow-up research on the potential for VA to reduce mortality or morbidity among pregnant women occurred over the next 60 years [13]. More recently, a large randomized controlled trial in Nepal reported a 44% reduction in maternal mortality associated with daily supplementation with VA or β-carotene throughout pregnancy [14]. Based on these promising findings, large confirmatory trials were launched in Bangladesh and Ghana, neither of which reported significant decreases in pregnancy-related mortality up to 6 weeks [15, 16]. Pooled analysis of these three trials suggests no overall effect of supplementation on maternal mortality, though heterogeneous findings have spurred debate over whether the differences are attributable to chance or context [17]. One consistent observation across all of these large studies was the lack of an effect on neonatal mortality, effectively putting to rest the suggestion that VA supplementation during pregnancy could be an effective child survival initiative although other potential benefits are being explored [17].

Postpartum Vitamin A Supplementation

Postpartum VA supplementation was originally devised as a strategy to improve VA status of the mother/infant dyad, and was once implemented as a program in many countries. A recent meta-analysis examined the effect of maternal postpartum VA supplementation on infant mortality and morbidity [18]. The analysis reported no significant effect on mortality (RR = 1.05, 95% CI: 0.92–1.20) or risk of diarrhea or acute respiratory infection [18]. Based on a review of the evidence, WHO recently issued guidelines stating that VA supplementation of postpartum women is not recommended for the prevention of maternal and infant morbidity or mortality (table 1) [19].

Maternal Vitamin A Supplementation and HIV Transmission

Observational studies in the 1990s suggested that low maternal VA status was associated with greater risk of HIV transmission from mother to child as well as higher rates of infant mortality [20–22]. Accordingly, four randomized placebo-controlled trials exploring various combinations of VA and/or β-carotene supplementation to women and infants were launched in Tanzania, Malawi, South Africa, and Zimbabwe [23–26]. The design and results of these trials are presented in table 2. In a pooled analysis of these trials, antenatal or postpartum maternal supplementation with VA or VA/BC was found to have no effect on HIV transmission (RR = 1.04, 95% CI: 0.87–1.24) [27].
It is important to note heterogeneity in the content of supplements and timing of supplementation across these trials. Findings from two of the trials suggest that VA supplementation may increase the risk of HIV transmission. The Tanzania trial, which tested the effects of supplementation with VA/BC during pregnancy and postpartum reported a 38% increase in HIV transmission or death compared to the control group (RR = 1.38, 95% CI: 1.09–1.76, p = 0.009) [23]. In the Zimbabwe trial, risk of either HIV transmission or mortality was higher among infants whose mothers received a one-time 400,000 IU dose or who directly received a 50,000 IU dose as neonates [26]. Paradoxically, the group that had received both maternal and child supplementation showed no difference in risk compared with the control group. Yet, among those infants who were PCR negative at 6 weeks, all three groups including either maternal postnatal and/or neonatal VA supplementation had approximately double the mortality risk compared with the placebo group (table 2).

<table>
<thead>
<tr>
<th>Population subgroup</th>
<th>Recommendations</th>
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<tbody>
<tr>
<td>Pregnant women [53]</td>
<td>Not recommended for the prevention of maternal and infant morbidity and mortality, but in areas where VA deficiency is a severe public health problem, VA supplementation is recommended for prevention of night blindness. Quality of evidence: high for maternal mortality, moderate for other outcomes.</td>
</tr>
<tr>
<td>Early infants (1–5 months) [54]</td>
<td>Not recommended as a public health intervention for the reduction of morbidity and mortality. Quality of evidence: moderate for infant mortality, low for other outcomes.</td>
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<tr>
<td>Older infants and young children 6–59 months of age (including HIV+) [49]</td>
<td>Infants 6–11 months of age: 100,000 IU once. Children 12-59 months of age: 200,000 IU every 4–6 months. Quality of evidence: high for all-cause mortality, moderate to very low for other outcomes.</td>
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</table>

There is some evidence from in vitro and other epidemiological studies supporting the hypothesis that VA could increase transmission [28]. An in vitro study suggested that VA could increase the expression of CCR5 receptors leading to increased replication of HIV-1 and increased susceptibility of monocytes and macrophages to HIV [29]. Evidence from a prospective cohort study in the
Table 2. Randomized controlled trials exploring the effect of VA or VA/BC supplementation on HIV transmission from mother to child

<table>
<thead>
<tr>
<th>First author and year</th>
<th>Setting</th>
<th>Design and participants</th>
<th>Findings</th>
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<tbody>
<tr>
<td>Coutoudis, 1999 [25]</td>
<td>South Africa</td>
<td>Randomized controlled trial of 728 HIV-infected women recruited at 17–39 weeks’ gestation. Women received daily oral dose of (1) VA supplement containing 5,000 IU retinyl palmitate and 30 mg β-carotene or (2) placebo through delivery. Women in the VA group also received 200,000 IU VA.</td>
<td>No difference in risk of HIV infection by 3 months of age was observed between the VA group (20.3% HIV+, 95% CI: 15.7–24.9) and the placebo group (22.3% HIV+, 95% CI: 17.5–27.1), and no significant differences in fetal or infant mortality.</td>
</tr>
<tr>
<td>Fawzi, 2002 [23]</td>
<td>Tanzania</td>
<td>Randomized 2 × 2 factorial trial of 800 HIV-1 infected pregnant women enrolled from 12–27 weeks gestation. Women received: (1) VA + βC (30 mg β-carotene plus 5,000 IU preformed VA), (2) multivitamins excluding VA + βC, (3) MV including VA + βC or (4) two tablets of placebo from enrollment onwards. Women in VA groups also received 200,000 IU VA at delivery.</td>
<td>The total risk of infection was significantly higher in the VA group compared with the non-VA group (RR = 1.38, 95% CI: 1.09–1.76, p = 0.009). No significant effect on mortality by 24 months (RR = 1.00, 95% CI: 0.80–1.24), or on composite HIV infection/mortality end point (RR = 1.13, 95% CI: 0.94–1.36).</td>
</tr>
<tr>
<td>Kumwenda, 2002 [24]</td>
<td>Malawi</td>
<td>Randomized placebo-controlled trial among 697 HIV-infected pregnant women enrolled at 18–28 weeks’ gestation. Women received daily oral dose of either (1) VA (10,000 IU; n = 340) or (2) placebo (n = 357). Women in both groups received 200,000 IU VA at delivery.</td>
<td>No significant differences between groups in the proportion of HIV-infected infants at 6 weeks (VA: 26.6% vs. placebo: 27.8%), 12 months (VA: 27.3% vs. placebo: 32.0%), and 24 months (VA: 27.7% vs. placebo: 32.8%), all p values &gt;0.20.</td>
</tr>
<tr>
<td>Humphrey, 2006 [26]</td>
<td>Zimbabwe</td>
<td>Randomized factorial trial among 14,110 mother-infant pairs including (1) maternal supplementation with 400,000 IU VA administered postpartum and 50,000 IU VA administered to the neonate, (2) maternal VA as above with placebo administered to neonate, (3) maternal placebo and 50,000 IU VA administered to the neonate, (4) maternal placebo and neonatal placebo.</td>
<td>Neither postnatal maternal nor neonatal VA supplementation significantly affected postnatal HIV transmission or overall mortality at 24 months, although both group 2 and 3 had increased risk of the infection or death outcome at 12 months (40.3 and 40.0%, respectively) compared with placebo (35.4%). Among PCR-negative infants at baseline, all three VA groups appeared to have heightened risk of mortality vs. placebo, though one group narrowly missed statistical significance (group 1: HR = 2.05, 95% CI: 1.14–3.67, p = 0.02; group 2: HR = 1.82, 95% CI: 0.99–3.31, p = 0.05; group 3: HR = 1.89, 95% CI: 1.05–3.40, p = 0.03)</td>
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United States suggested a U-shaped relationship between dietary intake of VA and HIV disease progression and mortality [30, 31]. Further analysis of breast milk samples from the Tanzanian trial revealed that VA/β-carotene supplementation was associated with increased risk of subclinical mastitis as well as increased HIV viral load in breast milk, suggesting a potential mechanism for increased transmission [32, 33].

**Neonatal Vitamin A Supplementation**

Two recent meta-analyses have consolidated the findings of studies of VA supplementation within the first 30 days of life, but reached different conclusions [34, 35]. The first reported no significant effect on mortality risk during a 12-month follow-up period (6 studies, RR = 0.92, 95% CI: 0.75–1.12) [36]. The second study presented findings disaggregated by time to follow-up, and found a significant reduction of mortality risk among all infants at 6 months (5 studies, RR = 0.86; 95% CI: 0.77–0.97) but not at 12 months (4 studies, RR = 1.02; 95% CI: 0.87–1.20) [35]. The latter paper argued that routine programmatic VA supplementation reached many children above 6 months in most of the included study countries, potentially leading to an attenuation of findings when using a follow-up period exceeding the first 6 months of life. A WHO technical consultation on neonatal VA supplementation also noted disparate findings in effects of supplementation by region; studies from Asian contexts appeared more suggestive of benefit than studies in Africa, although such differences were not statistically significant in meta-regression [37]. A separate meta-regression has also suggested that a protective effect appears to be more evident among neonates supplemented in the first 48 h of life living in areas of prevalent VA deficiency [38].

At this time, the WHO does not recommend neonatal VA supplementation [39]. In response to recommendations from a WHO technical consultation on neonatal supplementation research priorities, trials are currently underway in Tanzania, Ghana, India, and Pakistan to better understand the potential contextual differences noted above and improve estimates of the efficacy of neonatal supplementation [37]. More work is also needed to understand potential mechanisms through which neonatal supplementation might affect mortality risk; it has been hypothesized that it may do so through effects on the immune system or organ maturation [37].

**Vitamin A Supplementation among Infants 1–6 Months of Age**

In contrast to older (or younger) age groups, there is little evidence that VA supplementation of infants 1–6 months of age is protective against mortality.
Several studies in Asia, one in Africa, and a multicenter study have been undertaken, and none showed statistically significant benefit; a recent pooled estimate was also null (5 studies, RR = 1.05, 95% CI: 0.88–1.26) [40].

**Vitamin A Supplementation for 6- to 59-Month-Olds**

*All-Cause Mortality*

The most recent and comprehensive meta-analysis of published studies reported that VA supplementation of children 6–59 months of age led to a 24% reduction in all-cause mortality (RR = 0.76, 95% CI: 0.69–0.83), an estimate that remained largely the same as previous analyses [41, 42]. Heterogeneity in the findings of included studies was also apparent (I² = 48%, p = 0.02). Many explanations have been proposed for heterogeneity across trials including coexisting micronutrient deficiencies or differences in dietary fat intake (which may influence absorption or utilization of VA), differences in dosing frequency or amount, and differences in the incidence/prevalence of infectious disease. In the most recent review, significant beneficial effects were apparent in both Africa and Asia, among both boys and girls, and among multiple age strata (6–12 and 12–60 months) [41].

An interesting nuance is that more frequent dosing than the currently recommended WHO frequency of 4–6 months was associated with a 54% reduction in all-cause mortality (compared with a 19% reduction among those supplemented every 4–6 months). This suggests the possibility that at least in some settings, a more frequent dosing schedule than is currently implemented could increase the efficacy of the intervention (provided that it could be implemented with similar coverage rates). Paradoxically, the analysis also found that those who received less frequent dosing appeared to have greater mortality benefit than those who received supplementation every 4–6 months, an observation that cannot be easily explained [41].

Another important recent development in the evidence base related to supplementation of this age group was the presentation of preliminary findings of the DEVTA trial, the largest randomized controlled trial of VA supplementation to date (conducted in India and involving over 1 million participants), a trial that remains unpublished at the time of this publication (2012). Findings available online suggested that supplementation every 6 months with 200,000 IU had only a small (RR = 0.96, 99% CI: 0.88–1.05) statistically non-significant benefit on all-cause mortality [8]. In sensitivity analysis of all trials conducted to date including these findings, the overall effect estimate of the meta-analysis remained statistically significant, but the benefit was cut in half (from 24 to 12%) [41]. Some have used these findings to support arguments questioning the efficacy of VA [43]. Others have argued that the continued presence of VA deficiency in the VA arm of the trial (2.2% had Bitot’s spots and 11% had plasma retinol <0.35 μmol/l) is evidence that the dose may not have been adequate to achieve maximal efficacy [8, 44].
VA has a number of important roles in immunity including maintenance of epithelial integrity, regulating differentiation and function of monocytes, and influencing differential Th1/Th2 responses [45]. Early research in the 1930s generated interest around the 'anti-infective' properties of VA, and an early trial by Ellison demonstrated that VA dramatically lowered mortality rates of children with measles [1, 46, 47]. Since that time, a number of hospital-based trials have firmly established the efficacy of VA in the treatment of measles [45, 48].

In the most recent meta-analysis of epidemiological studies, VA supplementation significantly reduced the risk of mortality due to diarrhea by 28% (RR = 0.72, 95% CI: 0.57–0.91) as well as incidence of diarrhea by 15% (13 studies, RR = 0.85 (95% CI: 0.82–0.87), but no effect on prevalence of diarrhea was observed (2 studies, RR = 1.08, 95% CI: 1.05–1.12). Based on the relationship between supplementation and mortality, it has been posited that VA may help to protect against severe diarrhea, but might not affect more mild forms, although recent meta-analyses have not disaggregated by severity of the outcome [2]. The mechanism through which VA affects incidence or severity of diarrhea is uncertain, although the vitamin plays an important role in maintenance of epithelial integrity and in mucus secretion, both of which may help to protect against diarrhea [48].

It is well documented that measles is a precipitating factor of a great deal of child xerophthalmia and that supplementation with VA as treatment during a measles episode strongly reduces the risk of mortality [2]. Recent meta-analysis of trials of routine preventive supplementation also suggests reductions in measles-related mortality, although this finding was not statistically significant (5 trials, RR = 0.80, 95% CI: 0.51–1.24).

In contrast to diarrhea and measles, evidence for an effect of VA supplementation on lower respiratory tract infections including pneumonia has been greatly inconsistent. The most recent review of the effects of supplementation on cause-specific morality attributable to lower respiratory tract infections indicated a non-significant trend toward benefit (7 studies, RR = 0.78, 95% CI: 0.54–1.14), while finding a non-significant increase in incidence of lower respiratory tract infections (7 studies, RR = 1.14, 95% CI: 0.95–1.37). Another recent meta-analysis of the effects of VA on mortality specifically attributable to pneumonia also reported null effects, though the risk estimate was significantly attenuated compared with the above analysis, and some of the included studies differed (7 studies, RR = 0.94, 95% CI: 0.67–1.30) [40].

There is evidence from both hospital-based studies and community-based trials that VA supplements may increase the risk of acute lower respiratory tract infections among well-nourished children [48]. It has been speculated that high-dose VA might cause dysregulation of the immune system or a proinflammatory immune response, although this is uncertain [45, 48].
Programmatic Considerations

WHO recently issued guidance recommending high-dose VA supplementation for infants and children 6–59 months of age in settings where VA deficiency is a public health problem (table 1) [49]. Economic analyses have repeatedly ranked VA supplementation of children 12–59 months of age as one of the most cost-effective health interventions available, it was recently ranked at the top of the list along with zinc supplementation of interventions chosen by the Copenhagen consensus. Biannual distribution of capsules to children 6–59 months containing 200,000 IU VA is a relatively inexpensive intervention to implement and requires little additional human resource mobilization. Initially coupled in many countries with National Immunization Days for polio (for at least one round per year), in recent years efforts have been made to distribute VA through child health days along as part of a package often including insecticide-treated bednets, anti-helminth medication, and other core child health interventions. Critics of VA capsule supplementation programs have expressed concern that capsule supplementation, originally considered to be a short-term solution to controlling the deficiency has been implemented in many countries for decades, and that it might be holding back funding of other approaches to controlling VA deficiency [43].

There are also programmatic uncertainties and issues requiring further research. Two-round coverage rates are often quite high, nearing 80% in many countries, but children who are missed by one or both rounds are often socioeconomically worse off than those reached, and could therefore be at greatest risk of deficiency [50]. It is difficult to quantify how many lives are saved each year from such programs, as few effectiveness evaluations have been undertaken. It is possible that more frequent supplementation could be more efficacious, particularly since it is known that serum retinol declines 4 months after supplementation [2], yet more frequent supplementation also requires more resources and risks donor and community fatigue. There is some evidence suggesting that the effect of VA supplementation on risk of all-cause mortality may differ by vaccination status of children [51, 52]. These findings are of potential concern to programs given that vaccination is a convenient potential health contact for the administration of VA. Lastly, the rapid expansion of other strategies for addressing VA deficiency including home fortification with micronutrient powders, fortification, and homestead food production, suggests the need for more work to develop strategies to balance or target different approaches including consideration of the potential adverse effects of VA fortification in areas of high HIV prevalence.

Conclusions

The basis for VA supplementation of 6- to 59-month-old infants and young children is well established through many trials. More work is needed to...
understand the mechanisms through which VA reduces all-cause and cause-specific mortality and to more firmly establish the evidence related to supplementation of other groups, particularly neonates.

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Issues and Controversies with Vitamin A in Childhood

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Abstract

Vitamin A deficiency is common in the developing world. Vitamin A supplementation (VAS) has been used to prevent or treat vitamin A deficiency and to decrease mortality and morbidity in children. However, there are still controversial issues in relation to the role of universal VAS in different populations. Thus, studies that look at mortality outcomes reveal that VAS decreases mortality in children >6 months of age; however, there is still controversy on the extent to which reduction in morbidity from diarrhea and respiratory infection, other than measles, decreases mortality. Studies in infants 1–5 months old show no protective effect of VAS on mortality; whether this is secondary to environmental influences (breastfeeding), or interactions with DTP vaccine, needs to be further investigated. Studies with VAS in newborns have resulted in contrasting results in countries in Africa and Asia; trials are underway to better understand this. VAS does not have a universal protective effect on lower respiratory tract infection in children; some studies reveal an increase in respiratory morbidity associated with VAS, especially in well-nourished children; in contrast, VAS may confer some protection to malnourished children. The interaction of VAS with different vaccines is under current debate; some discussions are presented.

Introduction

Vitamin A deficiency (VAD) is common in the developing world. About 190 million children under 5 years and 19.1 million pregnant women are vitamin A deficient (i.e. serum retinol <0.70 μmol/l), representing about 33% of children under 5 years in populations at risk of VAD. Xerophthalmia, a condition associated with VAD, is the world's leading preventable cause of blindness [1].

Vitamin A has been described as an anti-infectious vitamin because of its role in regulating human immune function and epithelial integrity; studies in
animals and humans have reported an association between VAD and increased susceptibility to infections [2].

In addition to its preventive and therapeutic effect against xerophthalmia, vitamin A supplementation (VAS) has been used to prevent or treat VAD and to decrease mortality and morbidity in children. However, there are still controversial issues, especially in relation to VAS’s role in deaths or morbidity not related to measles in the pediatric population.

The WHO recommends VAS: 100,000 IU for infants 6–12 months of age and 200,000 IU for children over 12 months of age, every 4–6 months [3]. The international community recommends VAS for all children between the ages of 6 months and 5 years in all countries where over 70 in 1,000 children die before the age of 5 years, ‘as this is the internationally accepted proxy to indicate that VAD is a public health problem’ [4]. However, worldwide, a large proportion of children receiving it are not vitamin A deficient, stunted or wasted. The effect of such a public health policy on this population needs to be carefully assessed.

**Mortality Outcomes: Vitamin A Supplementation in >6-Month-Old Children**

In the 1930s, Ellison [5] first documented the protective effect of vitamin A on measles mortality. The first evidence of the role of prophylactic VAS in preventing death was demonstrated the 1980s in Indonesia; it concluded that children who received massive-dose vitamin A supplements had a 34% lower mortality from all causes than those not receiving the supplement [6]. These results were dramatic; however, no causes of death were reported. Also, the control children had more clinical signs of VAD and poorer growth than the children receiving VAS. During the following years, several other trials were performed. A meta-analysis of these initial studies concluded that VAS to patients hospitalized due to measles was highly protective against mortality. In addition, when given periodically to children at the community level, it decreased overall mortality (0.70; 0.56–0.879) [7]. It is important to emphasize that these studies were performed mostly in Asia, in countries with a high incidence of VAD and also in population with not universal measles immunization. Could it be that the significant reduction in mortality rates in children receiving vitamin A supplements in some studies was due to a reduction in measles deaths? This question has never been specifically addressed. Latham [8] suggested that the statistical difference in deaths might disappear if measles mortality were excluded from some studies of VAS. “The ‘causes’ of death in such studies were established by ‘verbal autopsies’. It appeared entirely feasible, based on many years’ experience in the field in Africa, that many deaths recorded as due to respiratory infections, diarrhea
or fever, might in fact be measles deaths. Measles can cause all of these symptoms”. Whether VAS might still have a positive effect in countries with high measles immunization coverage remains to be established, and is an area of controversy.

In a recent meta-analysis by Imdad et al. [9], including 43 trials, involving 215,633 >6-month-old children, there was an observed reduction in the risk of all-cause mortality (24%) and a 28% overall reduction in diarrheal mortality. Of interest, there was no effect on the mortality associated with lower respiratory tract infection (LRTI). Again, the authors stated that ‘the causes of morbidity and mortality were characterized by uncertainty’. So, the controversy of measles’ cofounding action remains on the scene. Moreover, one of the largest studies exploring whether massive-dose vitamin A administration is associated with a reduction in childhood mortality was taken up in India between 1999 and 2004. In that study, children were given 6-monthly massive doses of vitamin A, 6-monthly deworming, or both, or neither. Approximately 1 million children were followed, and mortality rates in children 1–6 years of age were recorded. There was no significant difference in death rates between children who received the massive dose of vitamin A and those who did not [10]. Unfortunately, this trial has not been published to date, and details that might explain the difference between this study and previous meta-analyses are not available so far.

Mortality Outcomes: Children 1–5 Months of Age

Studies where children were supplemented with vitamin A between 1 and 5 months of age did not show any positive effect on overall mortality [11–14] (see tables 1 and 2). Plausible explanations for these findings have focused on differences in environmental influences on this age group in relation to older infants (e.g. the protection of breastfeeding against malnutrition and infection) or on differences among the populations studied (e.g. different prevalence rates of infectious diseases among study sites may affect underlying mortality rates). Whether this could be explained by the interactions of vaccines given at this age, such as inactivated diphtheria-tetanus-pertussis (DTP) vaccine, with vitamin A, needs to be further investigated (see below).

Vitamin A in Newborns

In developing countries, infants are born with low stores of vitamin A and depend on external sources. Sometimes breastfeeding is not enough and newborns and infants need VAS; however, results obtained with this strategy have been contradictory. Studies from Bangladesh [15], India [16] and Indonesia [17] have shown reductions in all-cause mortality (15, 22 and 63%, respectively)
in infants who received VAS relative to controls. Also, VAS has shown to reduce diarrhea case-fatality rates and the incidence of fever [18]. One study performed in Nepal showed no overall effect on early infant mortality, and there was a tendency for the relative risk of mortality among vitamin A recipients to rise with improved nutritional status [19]. Trials in Africa, in countries like Guinea-Bissau [20] and Zimbabwe [21] suggest a lack of benefit from VAS. A meta-analysis by Gogia and Sachdev [22] proposes that there is insufficient evidence to support neonatal supplementation with vitamin A, and there is an increased risk of acute respiratory infection or respiratory difficulty (1.11; 1.02–1.21). Although the study is appropriate, its application of general inclusion criteria may limit its ability to ascertain the role of the prevalence of VAD in infant mortality. Many speculations have so far been formulated in order to understand these controversial results. A recent meta-regression analysis performed by Rotondia and Khobzia [23] suggests that VAS to neonates within the first

### Table 1. Main controversies related to periodical VAS in children (mortality outcomes)

<table>
<thead>
<tr>
<th>Age at intervention</th>
<th>Results</th>
<th>Controversial issues</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;6 months [9, 10]</td>
<td>Decreased all-cause and diarrhea-specific mortality No effect</td>
<td>To what extent is this related to measles?</td>
</tr>
<tr>
<td>1–5 months [11–14]</td>
<td>No effect on mortality</td>
<td>Are these results explained by breastfeeding practices? To what extent may vaccination at this age interfere with a positive response?</td>
</tr>
<tr>
<td>Newborns</td>
<td>African trials: no effect on mortality</td>
<td>Are these results secondary to regional or biological factors?</td>
</tr>
<tr>
<td>African trials [19–21]</td>
<td>Asian trials (most): decreased mortality</td>
<td>Could a difference in the prevalence of VAD in pregnant women explain differences?</td>
</tr>
<tr>
<td>Asian trials [15–17]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Effect of VAS on mortality in infants 1–5 months of age

<table>
<thead>
<tr>
<th>First author</th>
<th>Recipients</th>
<th>Vitamin A IU</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daularie [14]</td>
<td>1,058</td>
<td>50,000</td>
<td>0.99 (0.41–2.41)</td>
</tr>
<tr>
<td>West [11]</td>
<td>11,127</td>
<td>100,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,020 (1–3 months)</td>
<td>50,000</td>
<td>1.26 (0.88–1.80)</td>
</tr>
<tr>
<td></td>
<td>4,021 (4–5 months)</td>
<td>50,000</td>
<td>0.84 (0.49–1.45)</td>
</tr>
<tr>
<td>Rahman [12]</td>
<td>199</td>
<td>25,000 × 3</td>
<td>0.97 (0.29–3.25)</td>
</tr>
<tr>
<td>WHO [13]</td>
<td>9,424</td>
<td>25,000 × 3</td>
<td>0.96 (0.73–1.27)</td>
</tr>
</tbody>
</table>
2 days of life may confer benefit, especially in regions where the prevalence of vitamin A deficiency is 22% or more among pregnant women. This is an important finding given the current debate as to whether giving neonates vitamin A supplements helps reduce infant mortality in populations where endemic VAD and high infant mortality exist. However, not all neonatal VAS trials support a strong association between vitamin A status and baseline infant mortality, and the VAS effect. Thus, in the Indonesian trial mentioned above, mothers had a good vitamin A status, and infants a very good effect of VAS [17]. Biological and regional differences among populations need to be further studied to better understand contrasting results.

And finally, there has also been a debate in relation to a possible difference to neonatal VAS by sex. However, results from a recent meta-analysis performed by Kirkwood at al. [24] indicated that there is no differential effect of the intervention in boys and girls. Overall, there was no benefit from VAS.

From these results, it is clear more research is needed in this area before sound recommendations can be presented. It is possible that we will have better idea of the impact of this intervention by the year 2013 when information from ongoing trials in Ghana, India and Tanzania become available.

**Morbidity Outcomes: Diarrhea, Lower Respiratory Tract Infection and Measles**

In relation to morbidity, another controversy arises: to what extent is the decrease in mortality due to reduction in morbidity from diarrhea and respiratory infection (other than measles). One study in India, with measles-vaccinated and not severely malnourished children, concluded that VAS did not reduce respiratory and diarrheal morbidity as compared to the placebo controls [25].

The meta-analysis by Imdad et al. [9] revealed a reduced incidence of diarrhea (0.85; 0.82–0.87) and measles (0.50; 0.37–0.67) for VAS compared with control. But again, it included studies where it was difficult to control for measles comorbidity. Of interest, there was no effect on the incidence of respiratory disease or the incidence of hospitalizations due to diarrhea or respiratory disease. All of these studies were performed in developing countries, cointerventions were not analyzed, and there was statistical heterogeneity. Again, the controversy remains because of this and the uncertainty of morbidity conditions.

Even more controversial, VAS has been shown to be detrimental in pediatric population. In a blinded, randomized controlled trial (RCT) performed in Mexico [26], where measles vaccination coverage is very high and mortality in children <5 years has been estimated at 17.4/1,000 live births (Information from DGIS, SS Mexico, 2009), VAS to infants 6–15 months old was associated with a
27% increase in diarrheal disease and a 23% increase in cough with fever (RR: 1.23; 1.02–1.47). The authors postulated that the increase in diarrhea in this study might reflect the effect of VAS to downregulate the Th1 response, which protects against rotavirus. Similarly, they speculated that negative respiratory outcomes might be explained by the adverse effect of VAS in respiratory syncytial virus infections [27, 28].

A meta-analysis by Chen et al. [29] concluded that VAS does not have a universal protective effect as prophylaxis for LRTI in children; indeed, three studies showed an increase in respiratory morbidity associated with VAS (table 3). There was also post-hoc evidence to show that VAS in children with poor nutritional status may decrease the incidence of LRTI while the opposite was true for well-nourished children.

Another systematic review by Mathew [30], which included 11 prophylactic and 9 therapeutic trials concluded that VAS has no benefit for childhood community-acquired pneumonia.

Wu et al. [31] published a meta-analysis involving six trials with 1,740 children, some of them <6 months of age. They concluded that disease severity after supplementary high-dose vitamin A was significantly worse compared with placebo (one study). However, low-dose vitamin A (10,000 IU twice daily for the first 6 days, followed by 1,500 IU/day administered for the next 20 days) significantly reduced the recurrence rate of bronchopneumonia (OR 0.12; 0.03–0.46). Moderate vitamin A doses (100,000 IU of vitamin A to children aged one year and older, and 50,000 IU to infants under the age of one year) significantly reduced the time to remission of signs in children with normal serum retinol (>200 μg/l).

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**Table 3. Controversies surrounding VAS in children (diarrheal and respiratory morbidity)**

<table>
<thead>
<tr>
<th>Morbidity</th>
<th>Results</th>
<th>Controversial issues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence of diarrhea [9, 25, 26]</td>
<td>Decrease/increase/no change</td>
<td>Are these differences secondary to pathogen-specific responses to vitamin A?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>To what extent is this related to measles?</td>
</tr>
<tr>
<td>Incidence of respiratory infections [9, 26, 29]</td>
<td>No change/increase/decrease</td>
<td>Better results with lower nutritional status?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pathogen-specific responses?</td>
</tr>
<tr>
<td>Respiratory severity [29]</td>
<td>Therapeutic: no effect/increase</td>
<td>Are these differences pathogen-specific?</td>
</tr>
<tr>
<td></td>
<td>Prophylactic: no effect/increase</td>
<td>Are these results related to different doses? Lower doses better results?</td>
</tr>
</tbody>
</table>
Further RCTs, possibly with measured vitamin A levels and varying vitamin A doses, may provide sufficient evidence to clarify the role of vitamin A in non-measles pneumonia and other conditions.

**Measles and Vitamin A Supplementation**
A recent review which included eight trials (2,574 participants) revealed that there was no significant reduction in the risk of mortality in the vitamin A group when all the studies were pooled (0.70; 0.42, 1.15), and the pooled estimate from four studies suggested the risk of pneumonia-specific mortality (0.57; 0.24–1.37) was not significantly reduced in supplemented children. However, there was evidence of benefit with two mega-doses of VAS on consecutive days. This approach reduced the mortality in children aged <2 years (0.21; 0.07–0.66) and the risk of pneumonia-specific mortality (0.33; 0.08–0.92); it also reduced the incidence of croup by 41% [32, 33]. It is important to recognize that benefit was demonstrated in hospitalized children aged <2 years, given two doses of VA and in areas where the case fatality rate was greater than 10%. Controversy still remains as to whether these findings could be generalized to populations with high rates of measles immunization (low exposure), with low case fatality rates and in nonhospitalized children.

Apart from its role in decreasing mortality associated with measles pneumonia in children <2 years, the best target population for VAS in respiratory conditions, the best dosage, and the best timing are not clear yet.

**Effect of Vitamin A Supplementation on Vaccine’s Response: Good, Bad, Indifferent?**

The interaction of VAS with different vaccines may vary depending on the immunogen, the host, vitamin A dosage and many other factors. Benn et al. [34] have suggested that VAS could interact with the vaccines by amplifying their nonspecific effects. According to this thesis, VAS would have beneficial effects when administered with live vaccines such as BCG at birth or measles after 6 months of age, but no effect or even negative effects when administered with inactivated DTP vaccine between the age of 1 and 5 months. This hypothesis remains to be tested.

Some relevant findings related to VAS and immunizations are summarized in the following paragraphs.

**Bacille Calmette-Guerin Vaccine**
The effect of VA administered at birth along with this vaccine has been studied in a large (n = 1,894 and 1,426 in final analyses) randomized, placebo-controlled trial in Guinea-Bissau. The percentage of purified protein derivative of
Mycobacterium tuberculosis responders was significantly lower in 2-month-old boys who received VAS than in placebo recipients [35].

**Measles Vaccine**

There is some evidence that the effect of VAS is highly dependent on age at vaccination. One RCT study in Indonesia in which infants received the Schwarz measles vaccine (MV) and either the supplement (30 mg retinol equivalent) or a placebo at 6 months of age reported that supplemented infants had lower seroconversion rates at age 12 months, but only in infants with high levels of specific antibodies before vaccination (titers >8). It was suggested that the combined immune effects of VA and circulating maternal antibodies could neutralize the vaccine antigens [36, 37].

In children >6 months, there have been controversial results regarding the effect of VAS on MV rate of seroconversion or serologic titers. Some RCTs have shown higher seroconversion rates, some higher serum titers and other no changes associated with VAS [38–42]. Contradictory results from these studies may be explained by various factors, including preintervention VA status, age at vaccination and supplementation and hence presence/absence of maternal antibodies, vaccine strain, and antibody serological assays. There is some evidence to suggest that the most important factor responsible for the effect of VAS on MV response is the baseline-specific antibody titers. From a meta-analysis based on 3 studies in which this information was available [36, 42, 43], it was found that VAS was associated with reduced seroconversion rates in children having high baseline antibody levels [OR 0.57; 0.35–0.94]. In contrast, VAS did not affect seroconversion rates in children with low baseline antibody titers [44].

**Diphtheria-Tetanus-Pertussis Vaccine**

Most studies have not shown an improved tetanus-specific antibody response associated with VAS, as compared to controls [45, 46]. However, in an Indonesian study, the oral administration of 200,000 IU of vitamin A to tetanus-naive children 3–6 years of age resulted in significantly higher titers of anti-tetanus toxoid after immunization compared to those in the placebo group. In this study, vitamin A was given 2 weeks before immunization [47].

These controversial results may be related to differences in the VAS timing, basal vitamin A status or other factors that need to be further studied.

A group of researchers in Denmark have raised questions regarding the safety of VA administration with DTP vaccines. Thus, reanalyzing data from a study performed in Ghana, VAS had a negative effect in measles-vaccinated girls who were missing one or more doses of DTP at enrollment, a group who often received DTP during follow-up (mortality RR: 2.60; 1.41–4.80) [48]. The controversy regarding a potential detrimental effect of VA administered with DTP vaccines has led to considerable discussion. The possible negative
interaction is biologically plausible; however, the evidence produced so far is not abundant.

Polio Vaccine
VA has no effect on seroconversion to any of the 3 serotypes of poliomyelitis vaccine, although one study has shown a positive effect on seroprotection against poliovirus type 1 [49].

Antagonism with Vitamin D
Animal studies suggest that vitamin A is an antagonist of vitamin D action. Massive doses of vitamin A have been shown to intensify the severity of bone demineralization and to inhibit the ability of vitamin D to prevent such demineralization [50]. The effect of supplementing with mega-doses of vitamin A vitamin D-deficient children born to malnourished or not sun-exposed women with a low calcium intake needs to be seriously addressed.

Conclusions
There is evidence that VAS in children >6 months of age decreases childhood mortality; however, there is controversy on the relation of the cause-specific protective effect. Could the difference in deaths possibly disappear if measles mortality were excluded from some studies of VAS? Might those in fact be measles deaths? If this is the case, could VAS still have a positive effect in countries with high measles immunization coverage? VAS in newborns and infants <6 months seems not to have a protective effect on mortality; however, there is controversy if newborn VAS could be useful in regions where pregnant women have a high prevalence of VAD.

There is evidence to suggest VAS might be beneficial for diarrheal morbidity; however, this is controversial when considering measles comorbidity. Even though there are theoretical reasons that VAS might be effective for acute LRTI, most studies have not supported this concept or even have revealed the opposite. The reasons for these findings are not clear. The modulatory effects of vitamin A in the immune system, its potential interaction with the specific immune responses to different viral/bacterial pathogens, its role in several pathways of disease and the dose effect on disease development/protection remain to be elucidated to understand these complex results. VAS reduces measles mortality if mega-doses are given to children <2 years of age in regions with high case fatality rates. VAS improves immune response to MVs if circulating maternal antibodies are low or absent. There is controversy regarding the interaction between DTP and VAS; a single research group has reported possible adverse interaction; however, there is no conclusive evidence.
References


Discussion on Vitamin A Supplementation in Childhood

Vitamin A deficiency (VAD) is a very common public health problem in more than half of the world, especially in Africa and Southeast Asia, with young children and pregnant women in low-income countries being hardest hit. The WHO estimates that 250 million preschool children have VAD and that a substantial proportion of pregnant women in VAD geographic areas is vitamin A deficient. Globally, between 1995 and 2005, ~5.2 million preschool children and ~9.7 million pregnant women were affected by night blindness. If retinol concentrations <70 μmol/l are reviewed, 190 million children have low levels of retinol compared to 19 million pregnant women. For children, VAD significantly increases the risk of severe illness, and even death from common illnesses such as diarrhea and measles. For pregnant women, VAD occurs during the last trimester and the mother presents with night blindness. The relationship or association of VAD and vertical transmission of HIV remains to be clarified.

Dietary deficiency can begin in the neonatal period due to lack of breastfeeding and continue into adulthood due to diets deficient in vitamin A-containing foods such as liver, cheese, milk, eggs, fruits and vegetables and fortified foods. Randomized controlled studies of vitamin A supplementation in women before, during or after pregnancy have yielded conflicting results. West et al. [1] in a double-blind, placebo controlled study in Nepal, demonstrated that weekly 7,000 retinol equivalents as vitamin A or provitamin A β-carotene were associated with a 44% reduction in maternal mortality. In the SUMMIT trial conducted in Indonesia, early infant mortality was reduced in infants born to mothers receiving micronutrient supplementation including 800 μg of retinol (35.5/1,000 vs. 43/1,000, RR 0.82, 95% CI: 0.70–0.95), but there was no effect on maternal mortality [2, 3]. More recently, Kirkwood et al. [3], found no significant effect on pregnancy-related mortality or on all-cause mortality with vitamin A supplementation. There were no effects on the rate of still birth, perinatal, neonatal or infant death.

The effect of VAD on all cause and diarrheal mortality remains controversial. In one study of over 200,000 children, vitamin A supplementation decreased
mortality, whereas in the DEVTA study from India, no differences in mortality were observed [4]. Further, a question raised was whether vitamin A supplementation would still have a beneficial effect on mortality in countries with a high measles immunization rate. In 1995, Ramakrishnan and colleagues found that respiratory and diarrheal morbidity were similar in children less than 3 years of age who were vaccinated against measles but were not severely malnourished, suggesting that overall nutrition is a very important confounding factor. There appears to be an inverse relationship between vitamin A supplementation and all-cause mortality with declining mortality with increased number of doses per child per year. On the other hand, vitamin A supplementation in doses ranging from 1,000 to 9,000 IU/kg per day for 28 days increased markers of oxidative stress in animal models.

In children, protection from VAD comes from breastfeeding in the neonatal period, and later providing a periodic supply of high-dose vitamin A has demonstrated a reduction in mortality. Since breast milk is a good source of vitamin A, and its level can be influenced by supplementation of the lactating mother, promoting breastfeeding is a good strategy to prevent VAD. For deficient children, a periodic supply of high doses of vitamin A has been demonstrated to reduce mortality by 23% and up to 50% in those with measles. The exact number of doses and the dose itself is still not clear. Overall, in an analysis by Kirkwood et al. [3], there was a favorable trend towards reduced mortality in boys, but not in girls. However, of the 6 studies used in the analyses for boys, the confidence interval crossed 1 in 5 of the 6, whereas they crossed 1 in all 6 studies for girls. There were no significant differences in outcomes that included infant mortality, newborn mortality, respiratory or diarrheal morbidity [5]. Vitamin A supplementation during infancy does not appear to adversely interact with common immunizations with no differences in mortality in the first year in supplementation vs. placebo and DPT/polio vaccination [6].

Control of VAD can be done by different approaches: improving the availability and intake of vitamin A through dietary intake of vitamin A-rich foods; increasing the dietary intake through fortification of foods as has been done in Central and South America; periodic delivery of 200,000 IU of vitamin A in preschool children with half the dose given between 6 and 11 months of age appears very successful [7, 8]. The periodic approach to supplementation has been shown to reduce the risks of xerophthalmia by ~90% and mortality by up to 23% in young children [9]. Many high-risk countries have also adopted the WHO policy of supplementing mothers within 6 weeks after delivery to increase vitamin A content of breast milk.

There appear, however, to be geographic differences with trials in Africa of vitamin A supplementation in infants that did not alter mortality, and those in Asia that did. In infants between 1 and 5 months of age, there was no effect on mortality. In infants >6 months of age, vitamin A supplementation decreased all-cause and diarrhea-specific mortality in some studies, but not others.
Thus, for the continuum of vitamin A sufficiency, one would include breastfeeding for 6 months, appropriate timely vitamin A supplementation between 6 months and 6 years of age, and food fortification after that (Guatemala, for example, where sugar is fortified). For specific vulnerable populations, growing fruits and vegetables complements dietary diversity and food fortification.

These three approaches combined with other public health initiatives to promote breastfeeding, oral rehydration therapy, vaccination and birth spacing can all lead to significant reductions in VAD and related morbidities.

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References


Influence of Inflammatory Disorders and Infection on Iron Absorption and Efficacy of Iron-Fortified Foods

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Abstract

The provision of iron-fortified foods is a common strategy to prevent iron deficiency; however, ensuring adequate iron absorption is a challenge. Iron bioavailability depends on the choice of iron compound, the presence enhancers and inhibitors of absorption in the food matrix, and the physiological state of the consumer, including iron status, other nutritional deficiencies and inflammatory disorders. Inflammation associated with infections and inflammatory disorders would be expected to decrease iron absorption and reduce the efficacy of iron-fortified foods. The decreased absorption is due to an increase in circulating hepcidin in response to inflammatory cytokines. Hepcidin degrades ferroportin and blocks the passage of iron from the intestinal cell to the plasma. This is the innate immune response to infections and aims to restrict pathogen growth by restricting iron supply. Stable isotope studies have reported women and children with chronic malaria parasitemia or febrile malaria to have increased inflammatory cytokines, increased hepcidin and much decreased iron absorption. No studies have specifically investigated the efficacy of iron-fortified foods in the absence and presence of infections. In contrast, inflammation and increased hepcidin associated with adiposity in overweight have been linked to both lower iron absorption and the decreased efficacy of iron-fortified foods.

Introduction

Approximately 90% of daily iron needs can be met by the reutilization of red cell iron after the breakdown of circulating red cells at the end of their natural life span. Although there is no physiological mechanism for active iron excretion from the body, there are obligatory iron losses from skin, intestine and urinary
tract and additional losses during menstruation in women of child-bearing age. To maintain iron balance, obligatory and menstrual iron losses, plus the iron required for growth in infants, children, and adolescents must come from the diet. This represents about 1–2 mg of absorbed iron per day. Many young women, infants and children fail to meet their iron needs from diet, and these population groups make up the majority of the 2 billion people worldwide estimated to be iron deficient [1]. The situation is worse in the developing world due to low iron bioavailability from plant-based diets, and iron deficiency has more serious negative health and economic consequences including poor pregnancy outcome, poor cognitive development in children, and decreased physical performance and productivity [2].

Four strategies are currently being used to target iron deficiency. These are dietary diversification, supplementation, food fortification and biofortification, although fortification and supplementation are often the only practical options. Improving iron bioavailability by including more animal tissue foods, fruits and vegetables in the diet is usually limited by cost and supply, especially in the developing world, and biofortification of plant staples by breeding or genetic engineering is not yet ready for introduction into the food supply. Iron supplementation can be both therapeutic and preventive and involves the provision of relatively large amounts of medicinal iron as tablets or syrups to population known or expected to be iron deficient. Supplements are recommended to be taken in the absence of foods as food per se decreases iron absorption [3]. Iron-fortified foods contain lower doses of iron than supplements, and are regarded as the best long-term approach for the prevention of iron deficiency. Iron-fortified staple foods and condiments are usually planned and mandated by government, and will reach all population groups including women and older children. Iron-fortified manufactured weaning foods are targeted specifically at infants and young children and, for the lower socioeconomic groups, micronutrient powders and micronutrient-fortified fat-containing pastes have been introduced in recent years. These products, usually in the form of food aid, are designed to be added at time of consumption to the cereal gruels commonly eaten by these infants and young children. The food industry additionally markets a range of iron-fortified foods (milk products, breakfast cereals, chocolate drinks) targeted at infants, children, adolescents and young women.

For the many infants, children and women at risk of iron deficiency, iron-fortified foods can fill the gap between iron intake from the regular diet and iron needs. However, ensuring adequate absorption of the fortification iron is far from easy and iron bioavailability from fortified foods depends on the choice of iron compound [1], the food matrix and the physiological state of the consumer. Iron-fortified foods often contain inhibitors of iron absorption such as phytic acid and polyphenols in cereal and legume foods [4, 5] and calcium in milk products [6]. In order to overcome the negative effects of these inhibitors,
the food manufacture usually adds ascorbic acid, although NaFeEDTA and phytase can also be added [7].

However, even if the food matrix is optimized, iron absorption ultimately depends on the physiological state of the consumer. There is an inverse correlation between iron status and iron absorption [8] with a 50% decrease in iron stores doubling of iron absorption, although the magnitude of the inhibitory or enhancing effects are independent of iron status. Genetic disorders, nutritional deficiencies, infection and inflammation can also influence iron absorption [9]. Homozygotes for thalassemia and hemochromatosis may have increased iron absorption and must be under medical surveillance, although dietary iron absorption by single gene carriers of these traits is little affected. Vitamin A deficiency can influence several stages of iron metabolism, and riboflavin deficiencies may decrease iron incorporation into hemoglobin. Chronic inflammation [10] and obesity [11] increase hepcidin expression and would be expected to decrease iron absorption [12]. This review evaluates the potential influence of inflammatory disorders and infection on iron absorption and efficacy of iron-fortified foods.

Iron Metabolism during Infection and Inflammation

Iron needed for new red cell synthesis and for replenishing iron enzymes and myoglobin is transported to the bone marrow or body tissues via transferrin in the plasma. Most of the iron supply enters the plasma via macrophages after the destruction of senescent red cells. The remainder enters as absorbed iron via the mucosal cells and, in times of need, from the iron stored as ferritin mainly in hepatocytes. The passage into the plasma is mediated via the transport protein ferroportin situated on the cell membrane [13], and its entry is strictly controlled by the regulatory hormone, hepcidin. This protein is secreted by the liver when iron status is adequate and inhibits the transport of iron into the plasma from both the macrophages and the intestinal cells [14]. Hepcidin binds ferroportin at the cell membrane, causing internalization and degradation [15], and when iron status is low, hepcidin release from the liver is decreased and iron absorption is maximized.

The innate immune response to microbial infection is to increase hepcidin via an inflammatory response and to restrict microbial growth by restricting the entry of iron into the plasma [16]. The anemia of infection results from an interruption in the recycling of red cell iron. Macrophage iron is not released resulting in insufficient iron for erythropoiesis. The outcome of many infectious diseases depends on preventing the invading pathogen from obtaining its iron supply, and provision of high iron doses to an infected patient can worsen the infection. In addition to preventing iron release from the macrophage, the inflammatory response would also be expected to prevent iron release from the mucosal cell and thus restrict iron absorption. Recent evidence indicates that the inflammatory response to malarial parasitemia decreased iron absorption from an iron-fortified sorghum gruel [12],
and that the inflammation associated with obesity and overweight decreases iron absorption and reduces the efficacy of iron-fortified foods [17].

### Influence of Infection on Iron Absorption and on the Efficacy of Iron-Fortified Foods

Recent studies provide good evidence that the acute-phase response to malarial infection [18] increases cytokines such as IL-6 which upregulate hepcidin synthesis [19] and decrease iron absorption. Doherty et al. [20] investigated iron absorption from an iron-fortified orange juice in young Gambian children 1 day and 15 days after the treatment of malaria. The acute-phase response to the infection, as indicated by a raised serum ferritin in anemic children, was still present on day 1 after treatment but was much decreased at day 15 (table 1). In this study, thirty-seven 8- to 36-month-old, anemic (Hb <110 g/l) children with uncomplicated malaria followed a 3-day treatment with chloroquine and fansidar. On day 1 after the treatment, they consumed an orange juice fortified with iron and ascorbic acid and labeled with 57 iron stable isotope as ferrous sulfate. Iron absorption was quantified by measuring the incorporation of the stable isotope into erythrocytes at 14 days. On day 15 after treatment, a second iron absorption measurement was made on the same children who again consumed an iron- and ascorbic acid-fortified orange juice, but this time labeled with 58 ferrous sulfate. Fourteen days later, erythrocyte incorporation of the isotope was similarly quantified. The control group consisted of anemic children in the same age range but with no

| Table 1. Iron absorption and iron status following malaria treatment of anemic 8- to 36-month-old Gambian children and subsequent iron supplementation [20] |
|---|---|---|
| | Percent iron absorption | Hb, g/l | Serum ferritin, μg/l |
| **Children with malaria** | | | |
| Day 1 after treatment | 8.7 | 84 | 74 |
| Day 15 after treatment | 15.5 | 100 | 22 |
| Day 30 after treatment | – | 111 | 20 |
| **Children free of malaria, no treatment** | | | |
| Day 1 | 26.6 | 91 | 6 |
| Day 15 | 24.0 | 97 | 13 |
| Day 30 | – | 103 | 16 |

Children were supplemented with 2 mg Fe/kg bodyweight per day from day 1 to day 30.
malaria and no malarial treatment. From day 1 to 30, all children received iron supplementation with liquid iron glycine sulfate at 2 mg Fe/kg bodyweight, and the hemoglobin and serum ferritin response of the postmalarial and nonmalarial anemic children to iron supplementation was compared.

Selected results from this study are summarized in table 1. The serum ferritin values have been recalculated from the published graphics. Iron absorption in the children who had been treated for malaria increased from 8.7% on day 1 after treatment to 15.5% on day 15, but on both days iron absorption was still significantly lower than in the noninfected control children, whose absorption was ca. 25% (p < 0.001). Serum ferritin in the children treated for malaria decreased from 74 μg/l on day 1 to 22 μg/l on day 15 after treatment, even though the children received iron supplements over this period. Thus, the malarial treatment appeared to have decreased the acute-phase response, although not completely. Providing iron supplements to the control children over the same time period increased their serum ferritin from 6 to 16 μg/l. Although the children treated for malaria absorbed less iron than the controls over the supplementation period, they showed a much greater increase in hemoglobin concentration between days 1–15 (p < 0.001) and between days 16–30 (p < 0.001). This can be explained by an increased store of macrophage iron from the destruction of senescent red cells being prevented by hepcidin from entering the serum during the malarial infection, but being released following malarial treatment.

In contrast to the Gambian study which investigated iron absorption in children with uncomplicated febrile malaria, a more recent study in Benin investigated iron absorption in women with malarial parasitemia but no fever or other symptoms. While acute febrile malaria affects individuals only a few days a year, in areas of perennial transmission, asymptomatic parasitemia affects much of the population for most of the year giving rise to protracted low-level inflammation [21]. In the Benin study [12], women with relatively high loads of *Plasmodium falciparum* (>500 parasites/µl blood) but no fever or other infections, consumed a sorghum porridge labeled with 57 ferrous sulfate before and after a 10-day treatment with Malarone™. Iron absorption was measured as incorporation of the stable isotopes into hemoglobin 14 days after consumption of the test meals. Hepcidin and other inflammation indices were measured at baseline and on day 25 after clearance of the infection and immediately prior to the second test meal. The results are shown in table 2. Treatment of the malarial parasitemia increased mean iron absorption from 10.2 to 17.6% (p < 0.01), which could be explained by a decrease in inflammation as demonstrated by a decrease in IL-6, -8 and -10 and an almost 50% fall in plasma hepcidin.

From these studies, it would be expected that inflammatory disorders and infections that lead to an inflammatory response would lead to a hepcidin-modulated decrease in iron absorption. Any long-term or chronic condition would then be expected to blunt the absorption of iron-fortified foods and the efficacy of iron fortification programs. To date, the only infection investigated
in relation to iron absorption is malaria, although other common infections such as tuberculosis [22] and *Schistosoma haematobium* [23] also lead to an inflammatory response and increase hepcidin concentrations. There is no direct evidence however that the efficacy of iron fortification programs is blunted in malaria-endemic areas or regions of widespread infections. Lack of efficacy of electrolytic iron-fortified cereal products in Ivory Coast [24] and Kenya [25] has been blamed on widespread infections, but lack of gastric acid in malnourished populations could be an alternative explanation. Electrolytic iron-fortified wheat flour products were efficacious in China and Thailand [26]. While no studies have directly compared efficacy in infected and noninfected subjects from the same populations, there are efficacy studies with similarly fortified salt or cereals which have been made in populations with little or no infection or with widespread infections. The salt studies with ferric pyrophosphate in Morocco (no infections) [27] and Ivory Coast [28] reported similar improvements in serum ferritin and transferrin receptor as did the NaFeEDTA-fortified cereal studies in India (no infections) [Muthayya et al., unpubl.] and Kenya [25]. This failure to demonstrate reduced efficacy in the presence of infection is probably due to differences in baseline iron status and other physiological differences in the subjects, as well as differences in food vehicle, diet, and fortification levels.

**Obesity and Overweight in Relation to Iron Absorption and the Efficacy of Iron-Fortified Foods**

American national surveys have consistently shown that overweight toddlers, children, adolescents and adults are more likely to be iron deficient than their
normal weight counterparts, and recent evidence suggests that adiposity-related inflammation may play a central role through its regulation of hepcidin [11]. In a recent Swiss study, overweight children were reported to consume similar amounts of bioavailable iron as normal weight children but have a lower iron status, higher serum hepcidin and higher subclinical inflammation as measured by IL-6 and C-reactive protein [29]. The adipokine leptin was also upregulated, and both IL-6 and leptin can increase hepcidin expression [11].

We have earlier reported that adiposity in women and children in transition countries predicts decreased iron absorption, iron deficiency and a decreased response to iron fortification [17]. The stable isotope iron absorption studies were made in 92 premenopausal Thai women of which some 20% were iron deficient and 22% overweight. They all consumed a reference meal of vegetable soup and white rice labeled with $\approx 4$ mg of $^{57}$Fe/$^{58}$Fe-ferrous sulfate. Iron absorption was quantified from erythrocyte incorporation of the isotope after 14 days. Independent of iron status, a higher BMI was associated with a lower iron absorption ($p < 0.03$; fig. 1). After adjustment for the differences in iron status (serum ferritin), fractional iron absorption was negatively correlated with C-reactive protein ($p < 0.001$) and BMI ($p < 0.05$).

In order to evaluate the influence of adiposity on the efficacy of iron fortification programs, we analyzed baseline ($n = 1,688$) and intervention ($n = 727$) data from previously published efficacy studies in schoolchildren in Morocco and India to look for associations between BMI z scores and iron status measures. In this cohort of children, 42% were iron deficient but only 6.3% overweight;
however, a lower BMI z score predicted poorer iron status at baseline ($p < 0.001$) and less improvement in iron status on consumption of the iron-fortified food (fig. 2). Iron status was measured as the serum ferritin-to-transferrin receptor ratio. This influence of adiposity on iron intervention has been confirmed in a recent Italian study in 15 obese children which reported that weight loss was associated with a significant decrease in circulating hepcidin and an increased response to iron supplements [30]. Overweight is increasing in some low-income countries at 2–4 times the rate in the developing world, and the high levels of overweight in women and school children in transition countries such as Thailand and India [11] could reduce the impact of iron interventions.

**Conclusions**

Inflammatory disorders and inflammation associated with infections would be expected to decrease iron absorption and reduce the efficacy of iron-fortified foods. The likely explanation is that the passage of iron from the intestinal cell to the plasma is prevented by the degradation of the transport protein ferroportin by higher hepcidin levels produced as a response to the inflammatory cytokines. While iron absorption has been reported to be much reduced in response to

![Fig. 2. BMI z score and the change in body iron stores (calculated from the ratio of SF/TfR) in iron fortification intervention studies in Moroccan and Indian children (n = 727) [17].](image-url)
chronic malarial parasitemia and acute malarial infection, there is no evidence to confirm a reduced efficacy of iron-fortified foods in areas of widespread malaria and other infections. In contrast, increased inflammation and circulating hepcidin levels as a result of adiposity have been linked to both decreased iron absorption and a reduced efficacy of iron-fortified foods. While this might be due to the additional influence of the adipokine leptin in further increasing hepcidin levels in overweight individuals, it seems likely that inflammation associated with infections will decrease the impact of iron-fortified foods, but well-planned studies have yet to be made.

References


Safety of Iron Fortification and Supplementation in Malaria-Endemic Areas

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Abstract

This review considers the safety of iron supplementation and fortification for the prevention and correction of iron deficiency in malaria-endemic areas, with a focus on potential means whereby provision of additional iron might heighten the risks of malaria and other infections. Iron deficiency itself may increase the risk of morbidity and mortality from malaria and other infections. The available evidence indicates that iron interventions are safe in settings without endemic malaria, and, with adequate health care, in regions with high transmission of malaria and other infections. Without regular surveillance and treatment of malaria and other infections, iron supplementation of individuals who are iron deficient seems safe, but individuals who are iron replete may have an increased risk of adverse outcomes. The mechanisms responsible for harmful effects with iron supplementation have not been established. These are likely to include the effects of (a) increased amounts of absorbed iron, with the production of plasma non-transferrin-bound iron, (b) increased amounts of iron in the gastrointestinal tract, with effects on gastrointestinal structural integrity and on gut microflora, and (c) the complex immune effects of iron interventions. Iron fortification appears to be generally safe, although more data from malaria-endemic areas are needed.
malaria, studies have generally found no indication of iron-induced increases in infectious illness [1–5]. After summarizing the available evidence from prospective trials of iron interventions in malarial areas, proposed mechanisms of adverse interactions of iron interventions with malaria and other infections will be considered.

Iron supplements are iron preparations given orally to population groups for prevention and treatment of iron deficiency [6], typically without food in doses of 1–2 mg/kg bodyweight. Iron fortification usually has the primary goal of preventing iron deficiency and includes both (a) the addition of iron to staple foods or condiments during processing and (b) the use of iron-containing powders, crushable tablets or spreads that are intended to be added to complementary foods prepared at home [6]. Iron-fortified processed foods and condiments provide lower levels of iron than supplements and are generally for adults, older children and adolescents. Iron preparations for home fortification supply intermediate levels of iron and are mainly for infants and young children. Iron fortification has generally been considered to be safe, but has not been specifically evaluated in malaria-endemic areas.

**Iron Interventions, Malaria and Other Infections**

Nearly half the world’s population now lives in regions endemic for malaria in Africa, Asia and South America [7, 8]. In these same regions, iron deficiency, the most common form of malnutrition, further increases the risks of disability and death [9]. Lack of iron causes anemia, impairs cognitive and behavioral development, decreases work capacity, and when severe, increases mortality during pregnancy, infancy and childhood [10]. Iron deficiency and malaria usually are found together with other nutritional deficiencies and infectious pathogens, as well as with inherited red blood cell abnormalities, such as the thalassemias and sickle cell disorders. In these settings, iron nutrition can be improved by modifying the diet, by fortifying foods, treating helminth infections and by other public health interventions. Along with these measures, supplemental iron is needed for prevention and correction of iron deficiency, especially among infants, children and pregnant women [11).

Concerns about the safety of programs to provide additional iron have been long standing [1, 12]. Earlier studies had generally suggested that the benefits of iron supplementation exceeded the risks [2, 13]. In 2006, publication of the results of a large, randomized, controlled trial in Pemba, Zanzibar, Tanzania (hereafter, the Pemba trial) [14], was followed by a sustained reexamination of the risks of iron supplementation and fortification. Because of the importance of these results, the design and major findings of the Pemba trial will be outlined, together with subsequent appraisals of the safety of iron interventions in malaria-endemic regions by a World Health Organization (WHO)
Consultation, by a systematic review from the Cochrane Collaboration, and by a Technical Working Group (TWG) convened by the Eunice Kennedy Shriver National Institute of Child Health and Human Development of the US National Institutes of Health and the Bill and Melinda Gates Foundation.

**Pemba Main Study**

The Pemba trial was a randomized, placebo-controlled trial of children aged 1–35 months living in Pemba, Zanzibar, Tanzania [14], in a setting where suboptimum care seeking and treatment in the routine public health system was associated with high mortality from infectious diseases. Children were assigned to daily oral supplementation with iron (12.5 mg) and folic acid (50 μg), iron, folic acid, and zinc, or placebo; children aged 1–11 months received half the dose. Most children were given supplements between meals. Based on available weights, the mean (SD) iron dose in mg/kg per day for infants 0–5 months old was 0.92 (0.23), for infants 6–11 months old was 0.80 (0.14), and for children aged 12 months or older was 1.15 (0.23). On the recommendation of the data and safety monitoring board, the iron and folic acid-containing groups of the trial were stopped early. At that time, 24,076 children contributed a follow-up of 25,524 child-years. Those who received iron and folic acid with or without zinc were 12% (95% CI: 2–23%, p = 0.02) more likely to die or need treatment in hospital for an adverse event and 11% (1–23%, p = 0.03) more likely to be admitted to hospital; there were also 15% (–7 to 41%) more deaths in these groups, although this difference was not statistically significant (p = 0.19). Overall, the analyses of cause-specific admissions to hospital and deaths in the main study indicated that malaria and other infectious diseases were significantly increased in children given iron and folic acid.

**Pemba Substudy**

The Pemba substudy included 2,413 children in whom baseline iron status and anthropometry were assessed. Although not an explicit aim, the children in the substudy were also provided with active surveillance for malaria and free treatment for infection in an attempt to balance the benefits and burdens of study participation [4, 15]. In the children enrolled in the substudy, iron status at baseline seemed to modify the risk of adverse events. An elevated erythrocyte zinc protoporphyrin (≥80 μmol/mol heme) was used as the criterion for iron deficiency. As shown in figure 1, iron deficiency greatly increased the risk of severe illness and death. The rate of adverse events (hospitalizations and deaths) in iron-deficient children treated with placebo (9.76 events/100 child-years) was increased by 202% compared to that in iron-replete children (4.83 events/100 child-years; p = 0.04). Iron and folate supplementation benefited iron-deficient children, reducing their rate of adverse events by 38% (to 6.00 events/100 child-years; p = 0.02). By contrast, iron and folate supplementation seemed to harm iron-replete children, increasing their risk of severe illness and death by 63% (to
7.86 events/100 child-years), although this result was not statistically significant (p = 0.24). Unlike the increase in adverse events observed in the main study, the overall effect of supplementation with iron and folic acid in the substudy was a non-significant reduction in adverse events. The different results between the main study and the substudy were interpreted as the result of more intensive diagnosis and management of children with malaria and other infections in the substudy. Although the increase in adverse events in iron-replete children given iron and folate in the substudy was not statistically significant, the investigators used this result to suggest that the increase in hospitalizations and deaths in the main study was the consequence of giving iron and folic acid supplementation to iron-replete children [14].

**WHO Consultation on Prevention and Control of Iron Deficiency in Infants and Young Children in Malaria-Endemic Areas**

After publication of the results of the Pemba trial, the WHO and the United Nations Children’s Fund (UNICEF) issued a joint statement [16], and convened a WHO Consultation to examine the results of the Pemba trial. The WHO Consultation then made a series of recommendations:

‘Universal iron supplementation for children under the age of 2 years is not recommended in malaria-endemic areas. However, iron therapy may have an important positive impact on child survival if it is directed to iron-deficient children in the setting of appropriate treatment of malaria and the complicating bacterial infections. Prior screening to identify iron-deficient children should be a necessary component of any such intervention’ [6].

‘The safety of iron preparations administered through home fortification of complementary foods for infants and young children, i.e. powders, crushable tablets, and fat-based spreads, is uncertain in malaria-endemic areas. Although there is reason
to believe that those preparations may be safer than iron supplements, they cannot be recommended until this has been demonstrated’ [6].

The WHO Consultation also noted that the safety of the use of processed foods fortified with iron in malarial areas had not been documented. Overall, because of the difficulties of diagnosing iron deficiency in resource-limited settings, the practical consequence of following these recommendations would be to halt programs of iron supplementation and fortification in malaria-endemic areas.

Cochrane Review: Oral Iron Supplementation for Preventing or Treating Anemia among Children in Malaria-Endemic Areas
A subsequent systematic review, published in 2009, examined additional data and information that were not included in the WHO Consultation [5]. The meta-analysis found that (a) the risk of clinical malaria was not increased by iron supplementation (RR: 1.00, 95% CI: 0.88–1.13; 22,724 children, 14 trials) and (b) the risk was similar among children who were non-anemic (and thus less likely to be iron deficient) at baseline (RR: 0.96, 95% CI: 0.85–1.09). Nonetheless, in trials that did not provide malaria surveillance and treatment, an increased risk of malaria with iron was observed (RR: 1.16, 95% CI: 1.03–1.31). Growth and other infections were generally not affected by iron supplementation. The conclusions of the review were that:

‘Iron does not increase the risk of clinical malaria or death, when regular malaria surveillance and treatment services are provided. There is no need to screen for anemia prior to iron supplementation’ [5].

‘Based on our review, routine iron supplementation should not be withheld for children living in malaria-endemic areas. Iron supplementation in malaria-endemic areas need not be based on baseline iron assessment; rather, emphasis should be on appropriate malaria prevention and surveillance’ [5].

National Institute of Child Health and Human Development /Gates Foundation Technical Working Group Review
For regions with a high prevalence of malaria and other infections, the TWG evaluated potential mechanisms that might be responsible for adverse effects of iron, assessed the usefulness of biomarkers for assessing iron status, and reviewed the available evidence for the safety and effectiveness of iron supplementation and fortification [17], taking account of the results of the WHO Consultation and Cochrane Review. The TWG Iron and Malaria Technical Report [17] identified an overall consistency between the findings of the Pemba trial and the conclusions of previous reviews, the WHO Consultation, and the Cochrane review. All agreed that when iron supplementation is given in settings with inadequate health care services, the risk of malaria and other infections is increased. Iron supplementation with adequate medical surveillance and prompt treatment of malaria and other infections appeared to be safe. Altogether, the TWG noted the practical difficulties of determining the adequacy of health care and
emphasized the need for further research to provide an improved understanding of the mechanisms underlying the adverse effects of iron interventions.

**Potential Mechanisms for Adverse Effects of Iron in Settings with a High Prevalence of Malaria and Other Infections and Inadequate Health Care**

The adverse effects of iron observed in trials of iron supplementation almost certainly involve a complex interaction between iron status, the specific iron intervention, and malaria and other infections. Broadly, underlying potential mechanisms for adverse effects with iron supplementation or fortification may be considered as those resulting from (a) the increased amounts of iron absorbed, (b) the increased amounts of iron in the gastrointestinal tract, and (c) the immune effects of iron interventions. While a single mechanism might underlie the harmful effects of iron administration reported in the Pemba and other trials, a variety of mechanisms acting in concert is more likely to be responsible.

**Mechanisms for Adverse Effects of Iron Interventions Involving Increased Iron Absorption**

The WHO Consultation [6] and the TWG report [17] identified plasma non-transferrin-bound iron as a probable cause of the adverse events in the Pemba trial:

‘When bolus doses of iron are administered parenterally or orally, especially without food, they may increase plasma iron concentrations and transferrin saturation and exceed the binding capacity of transferrin, leading to the appearance of non-transferrin-bound iron (NTBI). NTBI is potentially toxic because it may promote free radical formation and be more readily available to pathogens’ [6].

While direct toxic effects mediated by harmful free radical reactions [18, 19] could be involved, analyses of cause-specific admissions to hospital and deaths in the Pemba main study identified malaria and other infectious diseases as the most common causes of hospitalization and death in children given iron and folic acid [14].

For nonmalarial infections, the most likely explanation for an increased risk would seem to be plasma non-transferrin-bound iron being available to pathogens reaching the blood stream and enhancing their growth [20]. Concomitant bacteremia is common in patients with malaria infection. Bacterial virulence is greatly enhanced by freely available iron and the appearance of plasma non-transferrin-bound iron could transform bacteremia into a serious or fatal infection.

With respect to the potential adverse effects of plasma non-transferrin-bound iron on malarial infection, direct donation of iron to *Plasmodium falciparum* seems unlikely. The available evidence indicates that the growth of *P. falciparum* in infected red blood cells is independent of host iron status [19]. Extracellular iron does not seem to be required for intra-erythrocytic parasite growth. Iron
used by the parasite seems to be transported directly across the parasite plasma membrane from the infected red blood cell pool of cytosolic iron rather than from the digestive vacuole or from heme [21].

Plasma non-transferrin-bound iron has been reported to increase the expression of vascular endothelial adhesion molecules involved in sequestration of \textit{P. falciparum} [22], providing a potential explanation for the more severe clinical course in children in the Pemba trial [14] given iron and folic acid. Increased sequestration of infected erythrocytes would increase the risk of more severe forms of malaria, especially of cerebral malaria, by worsening microvascular obstruction. Another potential mechanism would involve the hepatic phase of malarial infection [21]. Plasma non-transferrin-bound iron is rapidly removed from the portal circulation by hepatocytes. Increased iron availability within hepatocytes might enhance merozoite production, resulting in higher parasitemia during the erythrocytic phase of the infection.

To date, measurements of plasma non-transferrin-bound iron have not been reported in patients with malaria. In noninfected subjects, oral administration of doses of iron of about 1 mg/kg bodyweight, similar to those used in the Pemba trial, resulted in the appearance of plasma non-transferrin-bound iron in iron-deficient, anemic women [23] and in volunteers with normal iron stores [24]. Our own studies [25] in women with low iron stores (serum ferritin <25 μg/l), summarized graphically in figure 2, have confirmed the appearance of plasma non-transferrin-bound iron after oral administration of ferrous sulfate, 60 mg iron, or about 1 mg iron/kg, similar to the dose of iron used in the Pemba trial. Giving the same dose of iron with a meal (rice with a vegetable sauce) greatly reduced the amounts of plasma non-transferrin-bound iron formed. Giving a dose of iron of 6 mg/kg, an amount comparable to that used in iron fortification, with the same meal failed to produce a significant increase in plasma non-transferrin-bound iron. Similar studies are needed in subjects in malaria-endemic areas, but these preliminary results suggest that oral iron supplements can produce plasma non-transferrin-bound iron, that administration of iron supplements with food can decrease the amounts formed, and that lower doses, like those used in iron fortification, given with meals can further reduce or eliminate the production of plasma non-transferrin-bound iron.

\textit{Mechanisms for Adverse Effects of Iron Interventions Involving Increased Amounts of Iron in the Gastrointestinal Tract}

Because only a fraction of supplementation and fortification iron is absorbed, most of the dose given passes into the lower small intestine and colon. Iron and iron status affect the structural and immunological integrity of the gastrointestinal tract as well as the gastrointestinal microflora, potentially promoting invasion by pathogenic enteric bacteria. Because \textit{P. falciparum} sequesters within the gastrointestinal microvasculature, the inflammatory effects of malaria could
interact with oxidant effects of iron on the gut wall, facilitating systemic bacte-
rial invasion, bacteremia, and septicemia. Increased intestinal mucosal perme-
ability has been reported in iron-supplemented Zambian children [26].

The normal gastrointestinal tract is populated by beneficial barrier bacte-
ria that help protect against infection by preventing pathogenic colonization.
Increases in gastrointestinal iron could alter this balance by enhancing the
colonization and virulence of pathogenic enterobacteria [27]. Some studies have
reported an increase in diarrhea in groups receiving iron supplements [2]. In a
recent trial in African children, administration of iron-fortified biscuits resulted
in a decrease in protective lactobacilli and an increase in fecal enterobacteria,
together with an increase in mean fecal calprotectin, an indicator of gut inflam-
mation [28]. In this population, iron fortification seemed to result in a more
pathogenic gastrointestinal microbiota in association with evidence of increased
gastrointestinal inflammation.

Fig. 2. Plasma non-transferrin-bound iron (NTBI) depends on the quantity of iron con-
sumed and the presence of food [25]. In brief, 16 women with low iron stores (serum fer-
ritin <25 µg/l) were administered 60 mg iron as a ferrous sulphate solution, with and
without a meal of rice and vegetable sauce, or the same meal without a supplement but
containing 6 mg iron as ferrous sulphate added as fortification iron to the meal. On com-
bining results from the three test administrations, there was a strong correlation between
increase in serum iron and the appearance of NTBI ($R^2 = 0.58$).
Mechanisms for Adverse Effects of Iron Interventions Involving Immune Effects of Iron Interventions

Both iron and malaria modify host immune responses in complex and still poorly characterized fashions. An extensive cross-regulatory network interconnects iron metabolism and immune function. Iron alters cytokine secretion and transcription factors involved in the immune response, while both cellular and systemic iron homeostasis are modulated by acute-phase proteins and mediators derived from immune cells [29]. The withholding of iron from pathogens is a central component of host defense. Supplemental and fortification iron, by compromising iron withholding and interfering with the regulation and coordination of innate and adaptive immune defenses both systemically and within the gastrointestinal tract, might impair defenses against a variety of pathogens. In addition, malaria-induced dysregulation of innate and adaptive immune responses also interferes with protection against a variety of microorganisms [30].

Conclusion

Iron deficiency itself may increase the risk of morbidity and mortality from malaria and other infections. The available evidence indicates that efforts to prevent and control iron deficiency by iron supplementation and fortification are safe in settings without endemic malaria, and, with adequate health care, in regions with high transmission of malaria and other infections. Without regular surveillance and treatment of malaria and other infections, iron supplementation of individuals who are iron deficient seems safe, but individuals who are iron replete may have an increased risk of adverse outcomes. The mechanisms responsible for adverse effects with iron supplementation have not been established. These are likely to include the results of (a) increased amounts of iron absorbed, with the production of plasma non-transferrin-bound iron, (b) increased amounts of iron in the gastrointestinal tract, with effects on gastrointestinal structural integrity and on gut microflora, and (c) the complex immune effects of iron interventions. Iron fortification appears to be generally safe, although more data from malaria-endemic areas are needed. Further research is needed to provide an improved understanding of the mechanisms underlying the adverse effects of iron interventions.

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Discussion on Iron

Discussion of Session V was focused on two important aspects of iron nutrition that have a significant impact on current efforts to reduce the worldwide prevalence of iron deficiency. The first presentation by Dr. Hurrell dealt with the effects of inflammation on iron absorption, and the second by Dr. Brittenham with safety issues and the putative risks of supplementation in regions where malaria is endemic.

The currently available strategies for alleviating nutritional iron deficiency are dietary diversification, supplementation, food fortification and biofortification. Fortification of staple foods and/or condiments is currently regarded as the best long-term approach to reaching all segments of the population. However, there are several obstacles to ensuring adequate absorption. The choice of iron compound, the food matrix and interactions with other nutrients are all important, but physiological control is the major determinant of absorption [1]. Hepcidin is the systemic regulator of iron absorption. Plasma levels rise in concert with increasing iron stores. Hepcidin binds to ferroportin, the only known cellular iron exporter on the surface membrane of intestinal mucosal cells and macrophages, causing it to be internalized and degraded, thereby inhibiting iron absorption and its release from stores.

Hepcidin is also a type II acute-phase reactant. Iron absorption is therefore decreased in infectious and inflammatory disorders adding another level of complexity to the correction of iron deficiency in regions where malaria and other infectious disorders are prevalent. Dr. Hurrell presented experimental evidence from studies employing stable isotopes demonstrating increased concentrations of inflammatory cytokines, C-reactive protein and hepcidin, and significantly decreased iron absorption in children with febrile malaria and women with afebrile malarial parasitemia. Dr. Sall asked whether minor infections such as skin infections and rhinitis that are common in Senegal have the same effect. In reply, Dr. Hurrell indicated that there is as yet no information on iron absorption in the presence of other infections, but that his group’s studies suggest that there is some degree of inhibition of absorption in regions with widespread infection. In response to a question from Dr. Rosenberg, Dr. Hurrell said that there is,
however, no direct evidence of reduced efficacy of fortification in regions where malaria is endemic. Salt fortified with ferric pyrophosphate was equally efficacious in Morocco (no malaria) and Ivory Coast (prevalent malaria). However, the studies compared different groups of children. More research is needed to clarify this issue. Efficacy should be compared in children drawn from the same population who are either healthy or infected. Dr. Hurrell also mentioned that based on their observations, the incorporation of iron into hemoglobin is not affected by infection.

The optimal approach to nutritional iron deficiency in the presence of infection was discussed briefly. Clinicians have traditionally withheld iron supplements until the infectious process has been controlled because the iron is unlikely to be absorbed and could be harmful. It may increase the virulence of pathogens. Iron requirements are reduced during episodes of infection because erythropoiesis is suppressed. Iron is redistributed from the functional compartment to stores. This iron is readily mobilized once normal erythropoiesis is restored. The effect of recurrent periods of infection on the iron status of iron-sufficient individuals may consequently be relatively limited. The impact is likely to be greater in those who are iron deficient because their ability to restore optimal iron balance is limited by infection. Individualized supplementation in the intervals between episodes of infection is unlikely to be feasible. Moreover, the intake of staple foods or condiments fortified with iron cannot easily be adjusted for recurrent infections. The best approach may be the use of highly bioavailable iron compounds that allow rapid upregulation of iron absorption during the intervals between episodes of infection.

Dr. Hurrell’s presentation describing the putative relationship between obesity and iron absorption generated a lot of discussion. Dr. Bhatia pointed out that the correlation between body mass index and iron absorption in the Thai women reported by Zimmermann et al. [2] is not very strong. This was, however, a retrospective analysis using data from an earlier study. The women were only mildly overweight with an average body mass index of 27.

The postulated etiology of reduced iron absorption in obese individuals is increased hepcidin production caused by proinflammatory cytokines secreted by visceral fat. While accepting the evidence of poor iron status in obese children, Dr. Haschke questioned the pathogenetic relationship between obesity and impaired iron absorption at this age. He stated that the distribution of fat in children is different from that in adults. Children have less central obesity and visceral fat which is the site of cytokine production. However, the evidence presented by Dr. Hurrell strongly supports an inflammatory process as the link between overweight and reduced iron absorption. Iron intake did not seem to be an important factor. It was the same in overweight and normal-weight children in their Swiss study. Dr. Zimmermann pointed out that children as young as 6 years of age have increased IL-6 and C-reactive protein associated with obesity. This relationship has been observed in healthy children in Switzerland,
Italy and the United States. He went on to mention a soon to be published prospective study in South Africa which includes obese, normal-weight and stunted children. The observed odds ratio of remaining iron deficient after a period of supplementation among obese children is twice that of the other two groups.

Childhood obesity and its potential long-term consequences are the subject of intensive research at the present time. To some extent, the disagreement between the discussants may have been their failure to specify the age groups of the children that they were commenting on. There is a considerable body of evidence supporting an association between obesity and low-grade inflammation in children over the age of 3–6 years, the age group that Dr. Zimmermann was referring to. The situation may be more complex in younger children [3]. Finally, Dr. Zhou asked about the practical implications of the reduced iron absorption associated with obesity. In Dr. Hurrell’s opinion, it is too early to make specific recommendations. More research is needed to define the magnitude of the effect as well as the long-term consequences.

One aspect of the relationship between inflammatory and infectious disorders and iron absorption was not discussed. Some conditions affect the duodenal mucosa directly. Chronic *Helicobacter* infections may lead to achlorhydria and reduce iron absorption in older men and women [4]. Gluten enteropathy (celiac disease) may cause significant impairment of iron absorption. It is prevalent in several western societies. Finally, the possible role of tropical enteropathy and recurrent diarrhea deserves further study.

In reply to a question from Dr. Bhatia, Dr. Hurrell stated that it is difficult to document the impact of mass fortification in western countries. The practice was introduced without any prior efficacy or effectiveness studies. The evidence for an impact of fortification of infant foods on the prevalence of early childhood anemia is more convincing. Mass fortification is nevertheless generally considered to be a contributor to the very low prevalence of iron deficiency in the United States. At least 20% of the iron consumed is derived from fortification. On the other hand, the impact of current policies addressing mass fortification in the developing world may be considerably smaller. Dr. Hurrell described briefly an analysis carried out a few years ago to evaluate the potential impact of wheat flour fortification in 78 countries that have established national programs to fortify wheat flour with iron as well as other micronutrients [5]. The conclusion drawn from the analysis was that an impact on iron nutrition could be expected in only 6 countries. The primary reason for the anticipated lack of effectiveness is the widespread use of iron compounds that are not bioavailable, particularly hydrogen-reduced iron powders. They are favored by millers because of their low cost and lack of chemical reactivity. The workshop participants recommended that efforts be made to convince millers to use electrolytic iron or other more absorbable iron compounds instead of hydrogen-reduced iron powders.
There were two questions related to the risk of fortification. Dr. Ganguly asked about the effect on potentially pathogenic bacteria in the gastrointestinal tract. Dr. Hurrell replied that in their study in the Ivory Coast there was a fivefold increase in pathogenic enterobacteria and a decrease in lactobacilli among the children receiving biscuits fortified with electrolytic iron [6]. They also reported evidence of gut inflammation. However, more research is needed to establish the practical implications of this finding. Dr. Bhatia expressed his concern about iron overload in individuals who are iron sufficient and consuming a fortified diet. Dr. Hurrell replied that the risk for the general population is low. Individuals who are single trait carriers of the common form of hemochromatosis (HFE hemochromatosis) or the thalassemia syndromes do not accumulate excess iron. The problem is limited to the minority of individuals who are hemochromatosis homozygotes and those who have clinically evident thalassemia. The risk is further reduced in HFE hemochromatosis because of the low penetrance of the disorder.

Dr. Brittenham introduced his presentation by describing the dilemma faced by nutritionists because of the observations reported from the iron, folic acid and zinc supplementation trial carried out in Pemba, Tanzania [7]. The results indicate that the risk for severe morbidity and mortality from malaria is increased in young children who are iron deficient. On the other hand, universal iron and folic acid supplementation raised the risk for severe morbidity and mortality in the population of children under the age of 3 years as a whole. The results of this trial were discussed in some depth. It was pointed out that earlier smaller studies raised concerns about the risk of iron supplementation in regions where malaria is endemic. However this very large, well-controlled trial is the first to provide a more definitive answer. It would be valuable to know whether we are dealing with a relatively minor although important factor that affects the whole population or a more powerful contributor in an as yet unidentified subgroup. Dr. Black stated that the data from the Pemba study do not provide the information that would be needed to address this issue. A redesigned trial with the statistical power to answer the question does not seem feasible at the present time. Dr. Brittenham said that alternative approaches that are focused on developing a better understanding of the underlying pathophysiological mechanisms are more likely to be fruitful. Such studies should include pregnant women.

Dr. Rosenberg suggested that folic acid supplementation could have played a role since it was always given with the iron, and the antimalarial drug being used at the time is an antifolate agent. Dr. Black discounted this possibility because responses in community and hospital-treated children did not differ. Furthermore, earlier studies tend to incriminate iron.

The putative risk of iron supplementation in the setting of other infectious diseases was discussed briefly. Dr. Brittenham stated that there is evidence implicating iron in HIV disease and tuberculosis, and that the observations made by Zimmermann et al. [6] suggest that it may well play a role in diarrheal
diseases. They reported that iron fortification favors the growth of potentially pathogenic gut bacteria associated with inflammation of the gastrointestinal tract in African children.

The postulated mechanisms for the adverse effects of iron supplements were discussed at some length. A pivotal role for non-transferrin-bound iron (NTBI) is favored at the present time. It could promote microbial virulence. Dr. Brittenham described recent unpublished observations that he and his colleagues have made that support this contention. When blood that has been stored for some time is transfused, the red cells are rapidly sequestered and processed in the spleen resulting in the release of NTBI. They transfused stored blood into healthy human volunteers. Blood samples from these volunteers contained NTBI. They allowed bacteria that cause infections in human beings to grow more readily, suggesting that NTBI may well increase the risk of severe morbidity and mortality from bacteremia.

The mechanisms that lead to increased morbidity in malaria may be different. Dr. Brittenham listed several possibilities in his presentation. One involves increased expression of endothelial adhesion molecules, which might lead to the sequestration of Plasmodium falciparum-infected erythrocytes in the microvasculature causing obstruction and severe clinical manifestations such as cerebral malaria. Dr. Brittenham pointed out that if this postulate proves to be correct, supplemental iron may not be a risk factor in vivax malaria since vascular adhesion does not occur. It might also provide an explanation for potentially disastrous consequences of P. falciparum malaria in pregnancy.

The concentration of NTBI is proportional to the serum iron level. The serum iron rises rapidly after a dose of supplemental iron. The height of the rise is determined by dose, the presence of absence of food and the iron status of the individual. The highest levels occur when supplemental iron is consumed in the fasting state. When taken with food, the increment is smaller. There is no measurable increase with fortification iron. It seems paradoxical that the highest peak serum iron levels are encountered in iron-deficient individuals who seem to benefit from iron supplements. One would anticipate the generation of more NTBI and increased risk in them. However, Dr. Solomons said that they have unpublished observations demonstrating that the areas under the curves for NTBI concentrations after a dose of supplemental iron are similar in iron-sufficient and iron-deficient volunteers. In addition, NTBI clearance may be accelerated in iron deficiency because of the increased demand for iron.

Several questions related to the best pragmatic approach to iron deficiency in individuals suffering from malaria or other infections. Dr. Brittenham recommended that the infection be treated before giving iron since there is usually no immediate urgency to correct iron deficiency. Dr. Solomons pointed out that although the correction of iron deficiency is not regarded as urgent in the clinical sense, periods of iron deficiency in early childhood may have long-term consequences for physical, cognitive and emotional development. Iron
deficiency should therefore be corrected as soon as possible. Dr. Brittenham concluded by saying that there are no simple, universally applicable answers to these questions. The guiding principle should be avoidance of the production of NTBI by using lower doses of iron over longer time periods. He also emphasized the importance of prevention in infancy and childhood. Enhancement of the mother's iron status during pregnancy and the correction of iron deficiency in women before pregnancy are essential.

Sean Lynch

References

Summary on Vitamin A and Iron

I think it was really an excellent discussion both in the vitamin A section and in the iron section. I have made three points here with the vitamin A, and I really didn’t know so much about the vitamin A when I came to this meeting. It seems to me that vitamin A supplementation historically has been targeted to reduce morbidity and mortality. As Noel Solomons said, the paradigm of death reduction is not to reduce vitamin A deficiency, so really maybe we should now revise these recommendations based on more evidence of low vitamin A intakes or status. There seems to be good evidence at least from Asia that this universal vitamin A supplementation decreases mortality in children less than 6 months of age even when this new India study was included in the meta-analysis. There is less good information from Africa and from Mexico, where there is less vitamin A deficiency, and larger doses of vitamin A to children with no vitamin A deficiency seem to increase infections or respiratory infections and HIV and maybe also antagonize vitamin D metabolism. This was my first point.

The second point, large doses of vitamin A may specifically improve only measles outcome, so the effectiveness in measles-vaccinated children is less clear, and the effectiveness in other disease such as diarrhea may be a little bit clearer. Concerning respiratory infections in the children less than 6 months of age, there seems to be no effect of vitamin A supplementation, so maybe as Lindsay Allen was saying we should target lactation. Inconsistent results with newborns, interactions with vaccinations and perhaps universal supplementation with vitamin A should be rethought, and more evidence should be put in the area of food fortification. This would improve the quality of the diet.

With the iron absorption, ensuring adequate absorption from iron-fortified foods is not easy, it’s a challenge in addition to the iron compound inhibitors, etc. We need now to reflect on inflammation, hepcidin and decreased absorption. It’s still not clear whether efficacy is affected or whether erythropoiesis overrules inflammation. The decrease in absorption has only been demonstrated with malaria and overweight, and we need to look at other more common infections. It’s still not clear what iron requirements are in a population with widespread infections or inflammatory disorders including overweight. We need to think about that as well as fortification levels.
The last points will be on the iron supplementation. It’s accepted that with inadequate health care and not good malaria surveillance, iron supplementation to children in malaria-endemic areas can increase morbidity and mortality. The negative impacts on other infections, as Gary Brittenham said, are expected but not yet demonstrated. The most plausible mechanism is still the non-transferrin-bound iron (NTBI); at the beginning we didn’t really believe it existed, but it does exist, and Noel Solomons has also reported some studies with NTBI; it is possibly coupled with the stimulation of the growth of the pathogenic organisms by the iron which goes through to the gut; the most likely explanation is the sequestration of the infected erythrocytes in the brain or the villi, and the villi could lead to the breach of the intestinal barrier and bacteremia. I think the strategy is by understanding the mechanism of this negative effect with supplementation, we should be able to confirm the safety of food fortification and hopefully also the Sprinkles.

Richard F. Hurrell
Are Weaning Infants at Risk of Iodine Deficiency Even in Countries with Established Iodized Salt Programs?

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Abstract

Because iodine deficiency (ID) during infancy can irreversibly impair neurodevelopment and increase mortality, it is critical that dietary iodine is adequate in this vulnerable group. Lactating mothers consuming iodized salt can transfer adequate iodine to the infant via breast milk, but during the weaning period, infants are at risk for ID for several reasons: (1) requirements per kg bodyweight for iodine and thyroid hormone during infancy are higher than at any other time in the life cycle; (2) experts recommend no extra salt (iodized or not) be given to infants during the first year; (3) cow’s milk (a major source of dietary iodine in many countries) is also not recommended for infants during the first year; and (4) iron deficiency, a common disorder during infancy, can impair iodine metabolism and reduce thyroid hormone production. For many weaning infants in industrialized countries, iodine fortified into commercial infant foods becomes important. This has recently been demonstrated in Switzerland, where a long-standing iodized salt program provides adequate iodine to pregnant women and school-age children, but new national data suggest weaning infants not receiving iodine-containing commercial baby foods have inadequate iodine intakes. Thus, even in countries with effective iodized salt programs, infants may be at risk of ID during weaning and may need additional dietary and/or supplemental sources of iodine.

Iodine deficiency (ID) during early life may cause irreversible damage to the developing brain [1]. In regions of moderate-to-severe ID, many infants and pregnant women have low circulating levels of thyroid hormone [2]. Thyroid hormone is required for normal neuronal migration and myelination of the brain during fetal and early postnatal life. Hypothyroidism during these
critical periods can cause mental retardation and neurological abnormalities [3]. Prevention of ID during infancy not only improves neurodevelopment, it may also reduce infant mortality. In a randomized, placebo-controlled trial of oral iodized oil (100 mg iodine) in Indonesian infants (n = 617) treated at ≈6 weeks of age, there was a 72% decrease in risk of infant death [4]. Similarly, in a large cross-sectional study in Indonesia, use of adequately iodized salt was associated with a lower infant mortality rate [5]. The potential adverse effects of mild-to-moderate ID during infancy remain unclear, as no well-controlled studies have tested the effects of iodine repletion at this age. However, infant requirements per kg bodyweight for iodine and thyroid hormone are much higher than later in life. Even in areas of iodine sufficiency, a newborn’s thyroidal iodine reserve is small, only ≈300 μg [6], and thyroxine turnover is high, with estimated production rates of 5–6 μg/kg bodyweight per day in infancy [7]. Thus, the infant needs a regular supply of dietary iodine to maintain euthyroidism.

National programs to control ID should therefore emphasize this vulnerable period. In most countries, the most effective strategy against ID is salt iodization because salt is one of few foodstuffs regularly consumed by most of the population through the year [8]. Since 1990, a major global effort has increased the number of households using iodized salt from <20% to >70%, dramatically reducing ID [9]. However, the International Council for the Control of Iodine Deficiency Disorders (ICCIDD) estimates 1.88 billion individuals globally still have an insufficient iodine intake, including nearly 1/3 of all school-age children, and ID remains a public health problem in 32 countries [10]. ID is not only a problem in developing regions; it also affects many industrialized countries. About 50% of continental Europe remains mildly iodine deficient, and iodine intakes in other industrialized countries, including the US, the United Kingdom and Australia, have fallen in recent years. ID has reappeared in Australia and the United Kingdom, mainly due to declining iodine residues in milk products because of decreased iodophor use by the dairy industry. In the US, the median UI is 160 μg/l (95% CI: 146–172), still adequate but half the median value of 321 μg/l found in the 1970s [11], and pregnant women who consume few dairy products may be at risk of ID [12]. In most industrialized countries, because 70–90% of salt consumption is from purchased processed foods, if only household salt is iodized, it will not supply adequate iodine. In order to successfully control ID, it is critical that the food industry use iodized salt whenever possible [13]. If both household and industry salt are iodized, salt iodization can deliver adequate iodine to most age groups, including children and adults. Whether it can also deliver adequate iodine during infancy is, however, uncertain [14].

A central problem to monitoring iodine status in infancy is the iodine requirement for this age group has not been well defined. The US Institute of Medicine [15] estimated an adequate intake (AI) for infants because there was not sufficient scientific evidence to calculate an Estimated Average Requirement. The AI, 110 μg and 130 μg/day for ages 0–6 and 6–12 months, respectively, is derived
from the mean iodine intake of healthy infants fed human milk, based on a median breast milk iodine content (BMIC) of 146 μg/l in US women in the early 1980s [15]. But because iodine intakes in the US population were excessive at that time, the BMIC used to set the AI was at the upper end of the range of 78–167 μg/l reported for iodine-sufficient countries [16]. Although high maternal iodine intakes can result in high BMIC, iodine intakes by the infant greater than requirements will simply be excreted in the urine. Thus, iodine requirements during lactation should be based on infant balance studies, and a single small balance study in Belgian infants found iodine retention was 7.3 μg/kg per day [17]. If the reference bodyweight at 6 months of age is 7 kg [15], an infant at this age would be in positive balance at a daily intake of ≈50 μg. The WHO has set a Recommended Nutrient Intake (RNI) of 90 μg/day for iodine during infancy [8]. But clearly more data are needed, preferably from a large, carefully controlled balance study, to better define the iodine requirement during infancy.

Lactating mothers consuming iodized salt transfer iodine to the infant via breast milk. During lactation, the mammary gland actively concentrates iodine via the Na/I symporter [18] and secretes it into breast milk. Because the gland is able to concentrate iodine, iodine supply to the newborn via breast milk may be at least partially maintained even during maternal ID [19]. However, a recent New Zealand study in iodine-deficient mothers reported a significant fall in BMIC during the first 6 months of lactation [20]. In contrast, in iodine-sufficient lactating women in the US with a median urinary iodine concentration (UIC) of 114 μg/l, the median BMIC was 155 μg/l [21]. A review of BMIC in iodine-sufficient countries found a wide range of mean or median concentrations, from 50 μg/l in Finland to 270 μg/l in the US, but sample sizes were small and not representative [16].

Although breast milk can supply adequate iodine to infants, as they are weaned from breast milk, usually in the second half of the first year, their dietary iodine intakes may fall. With the exception of some sea foods, the native iodine content of most foods is low [22, 23]. Residues from iodophors used during dairying and transport of milk can increase the iodine content of dairy products [13]. But iodized salt, either used in the household or added to processed foods, is the primary source of iodine in the diets of many countries. For example, the two main sources of dietary iodine in the US and Switzerland are bread containing iodized salt and dairy products [23, 24]. However, iodized salt may not contribute significantly to infant iodine intakes because pediatricians and nutritionists recommend no extra salt (iodized or not) be given to infants during the first year. After breastfeeding for 6 months, mothers are encouraged to feed their infants home-prepared complementary foods (CF) without added salt [25, 26]. Moreover, cow’s milk (a major adventitious source of dietary iodine in older children) is also not recommended for infants during the first year [26].

Thus, for many weaning infants in industrialized countries, iodine added to commercial infant foods becomes an important iodine source. In the New
Zealand Total Diet study, which simulated typical diets, iodine-containing infant formula/foods provided 60% of iodine intakes for infants older than 6 months [27]. In the US Total Diet Study, 90% of iodine intake in infants older than 6 months was provided by infant formula/foods and dairy products [28].

In national programs to control ID, regular monitoring of iodine status in target populations is important to detect both low and excessive intakes of iodine. For monitoring, WHO recommends using the median UIC from a representative sample of spot urine collections to classify a population’s iodine status [8]. Because >90% of dietary iodine eventually appears in the urine, UIC is an excellent indicator of recent iodine intake. In monitoring programs, it has been traditionally assumed that if the general population is iodine sufficient, infants will be also iodine sufficient. But this assumption has not been rigorously tested as there have been no national studies assessing infant iodine status in countries with established iodized salt programs where the general population has adequate iodine intakes. One reason for the lack of data in infants is the difficulty of obtaining spot urine samples to measure UIC. However, a simple noninvasive method for collection of spot urine samples from infants has recently been developed and validated [29]. An absorbent pad (e.g. a feminine hygiene pad that is free of iodine) is inserted inside the diaper and the infant is breastfed. Several milliliters of urine absorbed by the pad can be aspirated into a syringe for measurement. WHO recommendations state a median (m)UIC ≥100 μg/l in a representative population of infants indicates they have adequate iodine nutrition [8].

Switzerland has a model iodized salt program that was initiated in 1922; in national surveys in 1999 and 2004, >90% of households were using iodized salt, and school children were iodine sufficient [30, 31]. The objectives of a recent Swiss study [32] were to first measure UIC in a national sample of pregnant women and school children to confirm that the Swiss population remains iodine sufficient, and then to collect UIC data from a nationally representative sample of infants. The mUIC (95% CI) in school children (n = 916) and pregnant women (n = 648) was 120 (120–128) and 162 (144–177) μg/l, respectively, indicating iodine sufficiency in both groups. Spot urine samples were collected from infants at 3–4 days after delivery, at 6 months ± 6 weeks or at 12 months ± 6 weeks. Inclusion criteria for the mother-infant pairs were: (1) full-term, healthy pregnancy; (2) parental residence in Switzerland for ≥12 months before delivery and since delivery; (3) no history of thyroid disorders in the mother; (4) no ingestion of iodine-containing drugs or contrast media during gestation; (5) delivery without use of iodine-containing disinfectants, and (6) no health problems in the infant. Breast milk iodine concentrations (BMIC) were measured in the mothers of the infants at 6 and 12 months. The iodine concentration of commercial infant foods was directly analyzed, including infant formulas, follow-on formulas and commonly-consumed baby cereal products. The relative contributions of BMIC, infant formula milk (IFM) and CF to iodine intakes in the infants were estimated.
Twenty-four participating clinics provided samples from exclusively breastfed infants on days 3 or 4 after birth (n = 368: day 3, n = 248, day 4, n = 120). Overall, mUIC was 91 μg/l; at day 3, mUIC was 87 μg/l and at day 4, it was 100 μg/l (table 1). Among the mothers, 65% (n = 241) were taking supplements, but only 0.8% (n = 3) were consuming iodine-containing supplements (during pregnancy or currently), and 12% (n = 42) were using non-iodized salt.

For the older infants, eighteen clinics provided 507 infant/mother pairs. The mUICs (95% CI) in the 6- and 12-month-old infants were 91 (79–103) and 103 (92–116) μg/l, respectively, and were not significantly different (table 1). The overall infant mUIC was 98 μg/l (95% CI: 89–105; table 1). Girls had higher UICs than boys (mUIC, 103 vs. 88 μg/l; p < 0.05). Among the mothers, mUIC was 75 μg/l (95% CI: 69–81) and mBMIC was 49 μg/kg. Fifty-seven percent of the 6-month-old infants and 18% of the 12-month-old infants were being breastfed fully or partly at the time of sampling. Breastfed infants with or without IFM had a lower mUIC than infants not currently breastfed (82 μg/l, n = 196, vs. 105 μg/l, n = 311; p < 0.001). About 60% of all infants were receiving IFM, and their mUIC was higher than in those not receiving IFM (109 μg/l, n = 304, vs. 73 μg/l, n = 203; p < 0.001). Infants (breastfed and/or CF) receiving IFM had higher mUIC than breastfed weaning infants who did not receive IFM (109 μg/l, n = 304 vs. 70 μg/l, n = 131; p < 0.01; fig. 1). Weaned infants not receiving breast milk or IFM did not differ in UIC (89 μg/l, n = 72) from the other two groups. Eighty four percent of mothers were using iodized salt at home, 8% of mothers were not using iodized salt and 8% were unsure; there were no significant differences in UIC of the mothers.

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<th>Table 1. UICs in infancy in Switzerland (2005–2009)</th>
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Data from Andersson et al. [32].
or infants among these 3 groups. Among the 6-month-old infants, nearly 4 out of 5 were already receiving CF, and at 6 and 12 months, 7 and 95% of infants were receiving some foods from the family table. The mUIC of infants receiving some iodized salt in CF (103 μg/l, n = 287) was higher, but not significantly different from the mUIC of infants not receiving iodized salt (89 μg/l, n = 189). In a multivariate regression of predictors of UIC in the 6- and 12-month-old infants, BMIC (β = 0.320, p < 0.0001) and current consumption of IFM (yes/no; β = 0.201, p = 0.010) were significant, while gender, age and maternal UIC were not.

Thirty-two percent of the mothers were taking nutritional supplements (n = 158), but only 3% of women were consuming iodine-containing supplements. The agreement between the labeled and analyzed iodine content of the IFMs and infant cereals was high: the mean (± SD) difference (%) between labeled and measured values was 13.5 ± 9.1% for the formulas and 4.5 ± 2.6% for the cereals. None of the formula milks or cereals exceeded the recommended maxima of 50 and 35 μg/100 kcal [18].

This study is important in that it is the first national representative study assessing infant iodine status in a country with an established long-standing

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**Fig. 1.** Differences in median UIC (error bars show the 95% CI) by mode of feeding in 6- and 12-month-old Swiss infants. Group 1 (n = 131): infants receiving breast milk, partly CF, but no IFM; group 2 (n = 304): infants receiving infant formula, partly combined with breast milk and/or CF; group 3 (n = 72): infants receiving no breast milk or infant formula, but CF. * p < 0.01, significant differences between groups 1 and 2 (Mann-Whitney U test with Bonferroni correction). The dashed horizontal line indicates the WHO cutoff value for the median urinary iodine indicating adequate iodine intakes in infancy [8].
iodized salt program where school-age children and pregnant women have adequate iodine intakes [32]. The mUIC in the infants in this study at 3–4 days and at 6 months was just below the 100 μg/l cutoff that indicates iodine sufficiency in infancy [8]. Infants who were not receiving iodine-fortified IFM during the weaning period were clearly deficient, with an mUIC of ≈70 μg/l. Several reports of mUIC in infants (<2-year-olds) in countries with more than adequate or excess iodine intakes have found higher values than in this study [33–36]. But other studies in European infants have generally reported mUIC similar to the Swiss value [33, 37–40]. In the Swiss infants, only 58% of the 6-month-olds and 18% of the 12-month-olds were being breastfed. Infants who were breastfed and given home-prepared CF (that contain little or no added salt) were at highest risk of low iodine intakes. In Germany, using a dietary model, it was estimated that the iodine intake of an 8-month-old breastfed infant who receives home-prepared CF would be only ca. 45 μg/day compared to ≥125 μg/day in a formula-fed infant who receives commercial CF [41].

An additional factor that may aggravate low iodine intakes in weaning infants is the high prevalence of iron deficiency at this age. Iron deficiency reduces heme-dependent thyroperoxidase activity in the thyroid and impairs production of thyroid hormone. In iodine-deficient children, iron deficiency anemia blunts the efficacy of iodized oil and iodized salt [42]. In pregnant women, poor iron status predicts higher thyroid-stimulating hormone and lower thyroxine concentrations in an area of borderline ID [43]. Thus, iron-deficient infants may be at higher risk of poor thyroid function during low iodine intakes. But there are no published studies of the influence of iron status on thyroid function in infants with ID.

The Swiss study and others raise the concern that specific public health measures, in addition to salt iodization, should be considered to improve iodine intakes at this age. These could include increasing public awareness of the importance of providing at least some iodine-containing infant foods/formula during weaning. Infant food manufacturers should be strongly encouraged to fortify their products with iodine [14]. In Europe, the required level of iodization for IFMs is 10–50 μg/100 kcal (2.5 μg/100 kJ), but for cereal-based and other CFs, there are no requirements for minimum iodization while the allowed upper level is 35 μg/100 kcal [44]. In the US, iodine fortification of infant formula is mandatory at a minimum level of 5 μg/100 kcal (maximum level is 75 μg/100 kcal) [45]. In Germany, it is estimated only ≈50% of CFs are fortified with iodine [41].

Another strategy to increase iodine intakes in weaning infants would be iodine supplementation. Supplementation of lactating women can increase BMIC and could provide additional iodine, particularly during weaning when infants are being partially breastfed. In Danish mothers (n = 147), median BMIC on the 5th day postpartum was significantly higher (57 μg/l) in those receiving supplementation with 150 μg/day of oral iodine, compared to those
not supplemented (34 μg/l) [46]. In Germany, 60 mothers who received 200 μg/day of oral iodine had significant higher mean iodine concentrations in breast milk (76 μg/l) than those who did not (55 μg/l) [47]. Thus, iodine supplementation of breastfeeding women can significantly improve iodine supply to the newborn. In the Swiss study, supplements containing iodine were consumed by <5% of lactating women. Alternatively, iodine supplements could be given to infants directly, as is often done for iron or vitamin D. Currently, American and European pediatric societies do not recommend iodine supplements for infants on well-balanced diets [48]. Similarly, in countries such as Switzerland with an effective iodized salt program, WHO does not recommend iodine supplementation for infants or lactating women [49]. These recommendations may need to be reconsidered if the Swiss findings are confirmed in other industrialized countries.

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49 WHO Secretariat on behalf of the participants to the Consultation: Prevention and control of iodine deficiency in pregnant and lactating women and in children less than 2-years-old: conclusions and recommendations of the technical consultation. Public Health Nutr 2007;10:1606–1611.
**Current Challenges in Meeting Global Iodine Requirements**

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\(^a\)International Council for Control of Iodine Deficiency Disorders, Sydney Medical School, University of Sydney, Sydney, NSW, Australia; \(^b\)International Council for Control of Iodine Deficiency Disorders, Nutritional Intervention Research Unit, Medical Research Council, Cape Town, South Africa

**Abstract**

Iodine deficiency is a global problem of immense magnitude afflicting 2 billion of the world’s population. The adverse effects of iodine deficiency in humans, collectively termed iodine deficiency disorders, result from decreased thyroid hormone production and action, and vary in severity from thyroid enlargement (goiter) to severe, irreversible brain damage, termed endemic cretinism. Thyroid hormone is essential throughout life, but it is critical for normal brain development in the fetus and throughout childhood. During pregnancy, maternal thyroid hormone production must increase by 25–50% to meet maternal-fetal requirements. The principal sources of iodine in the diet include milk and dairy products, seafoods and foods with added iodized salt. Vegetables, fruits and cereals are generally poor sources of iodine because most of our soils and water supplies are deficient in iodine. The accepted solution to the problem is Universal Salt Iodization where all salt for human and animal consumption is iodized at a level of 20–40 μg/g. In principle, mandatory fortification represents the most effective public health strategy where safety and efficacy can be assured and there is a demonstrated need for the nutrient in the population. Voluntary fortification of salt and other foods has many limitations and few benefits. Iodine supplementation is a useful, but expensive, inefficient and unsustainable strategy for preventing iodine deficiency. The current worldwide push to decrease salt intake to prevent cardiovascular disease presents an entirely new challenge in addressing iodine deficiency in both developing and developed countries.

**Introduction**

Iodine is a simple element that is widely distributed in nature occurring in soils and food, but found most abundantly in the ocean in marine creatures and seaweeds. Its importance as a micronutrient rests on its essential role as
Iodine is an integral part of the thyroid hormone molecule, making up approximately two thirds of the mass of T3 and T4. The thyroid secretes predominantly T4, with only a small amount of T3 coming directly from the gland. T4 is a prohormone, converted in peripheral tissues by deiodinase enzymes to the more metabolically active T3. Thyroid hormone exerts multiple physiologic actions in the developing fetus, growing child and the mature adult. Besides being the principal regulator of the metabolic rate in humans, it is the most potent hormonal stimulus for growth and maturation of both the brain and skeleton [1].

The distribution of iodine in soil and water is quite variable, generally being adequate or abundant in coastal regions and becoming deficient the further one travels inland. Severe environmental iodine deficiency is invariably present in the mountainous zones of the world. By contrast, there are areas where iodine is present in excessive quantities in the environment, usually related to subterranean water sources. While iodine deficiency is an important and serious cause of disease, iodine excess may also cause illnesses in predisposed individuals [2]. The major consequences of deficient or disordered iodine metabolism are a result of too little (hypothyroidism) or too much (hyperthyroidism) thyroid hormone [3, 4] (fig. 1).

**Iodine Metabolism and Thyroid Hormone Production**

Iodine is readily absorbed, in the form of iodide, into the blood from the gastrointestinal tract, and is actively transported by an iodine transporter into thyroid
cells where it is concentrated by the thyroid gland. Iodide rapidly inhibits its own transport when the thyroid gland is exposed acutely to an abrupt increase in the plasma iodide concentration. Once incorporated into the thyroid gland, the iodide is oxidized and bound to tyrosine residues in the thyroglobulin molecule to form T3 and T4. These processes are enzymatically regulated within the gland and are under the overall control of thyrotropin secreted by the pituitary gland. The normal adult thyroid needs to trap at least 60 μg of iodide daily to maintain adequate thyroid hormone production. The newborn infant’s thyroidal iodine reserve is very small and turnover very rapid. The body conserves iodine by recycling iodine enzymatically stripped off the T3 and T4 molecules during metabolic breakdown. Approximately 90% of absorbed iodine is excreted each day in the urine with insignificant quantities appearing in the feces. Given this relationship between the amount absorbed and the amount excreted, measurement of urinary iodine excretion (UIE) accurately reflects daily iodine intake, and has become the most widely used index of nutritional iodine status [5].

**Iodine Deficiency Disorders**

The adverse effects of iodine deficiency in humans are collectively termed iodine deficiency disorders or IDD (table 1) [6]. Until recently, iodine deficiency in a population was usually equated with endemic goiter, without recognition of the widely prevalent, often subtle, and devastating neurological or psychomotor

---

**Table 1. Spectrum of IDD throughout the human life cycle**

<table>
<thead>
<tr>
<th>Life stage</th>
<th>Clinical expression and adverse outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetus</td>
<td>Miscarriage or stillbirth</td>
</tr>
<tr>
<td></td>
<td>Increased perinatal morbidity and mortality</td>
</tr>
<tr>
<td></td>
<td>Neurological and/or myxoedematous cretinism</td>
</tr>
<tr>
<td></td>
<td>Decreased IQ, deafness and neuropsychomotor deficits</td>
</tr>
<tr>
<td>Neonate</td>
<td>Neonatal goiter</td>
</tr>
<tr>
<td></td>
<td>Neonatal hypothyroidism</td>
</tr>
<tr>
<td></td>
<td>Decreased IQ and neuropsychomotor deficits</td>
</tr>
<tr>
<td>Child</td>
<td>Endemic goiter</td>
</tr>
<tr>
<td></td>
<td>Juvenile hypothyroidism</td>
</tr>
<tr>
<td></td>
<td>Stunted physical growth and impaired mental development</td>
</tr>
<tr>
<td>Adult</td>
<td>Endemic goiter</td>
</tr>
<tr>
<td></td>
<td>Hypothyroidism and its complications</td>
</tr>
<tr>
<td></td>
<td>Risk of iodine-induced hyperthyroidism</td>
</tr>
</tbody>
</table>

Data from Hetzel [6].
damage in these populations (fig. 2 and 3). It has long been known that very severe iodine deficiency affecting people in remote underdeveloped regions of the world is associated with severe mental and physical impairment (termed endemic cretinism), but it was not appreciated until recently that less severe forms of brain damage were so highly prevalent [6, 7]. The WHO says: ‘Iodine deficiency is the world’s most prevalent – yet easily preventable – cause of brain damage’ [8]. Iodine deficiency affects approximately 2 billion people worldwide, but the prevalence has decreased by half over the past 2 decades [9]. In populations where iodine deficiency is severe, IQ levels are, on average, 13–15 points below comparable iodine-replete populations, and irreversible neurodevelopmental abnormalities are highly prevalent [5, 7]. However, there remains considerable debate about the consequences in the offspring born to mothers with mild to moderate iodine deficiency.

Thyroid hormone is essential throughout life, but it is critical at certain life stages. Maternal T4 is the only source of hormone for the fetus early in gestation before the development of the fetal thyroid (fig. 4) [10]. The adverse effects of IDD are dependent on the timing of the iodine deficiency and the severity of the insult from lack of thyroid hormone. During pregnancy, thyroid hormone synthesis must increase by between 25 and 50% to meet maternal fetal requirements [11]. The damage to the developing brain of the fetus, neonate and infant from lack of thyroid hormone due to iodine deficiency is largely irreversible [12]. Coexisting deficiencies of selenium, iron and vitamin A may contribute to the severity of the expression of iodine deficiency [5]. Therefore, our efforts to prevent brain damage mean we must target our prevention programs at the pregnant woman and developing child.

**Fig. 2.** Large multinodular goiter in a mature-age woman from an area of moderate to severe iodine deficiency in northern Thailand.
Fig. 3. A man afflicted by neurological cretinism living in a rural area of severe iodine deficiency in China.

Fig. 4. Diagrammatic representation of the relationship between gestational age, neurological development in the fetus and transfer of thyroxine from mother to fetus. Adapted from Morreale de Escobar et al. [10].
The principal sources of iodine in the diet vary from one country and region to another. In less developed regions of the world lacking iodine in the soil or water, the most valuable source is likely to be iodized salt. By contrast, in developed countries where most varieties of food are readily available, milk and dairy products, seafood, and the discretionary use of iodized cooking and table salt are the major sources of iodine in the diet (Table 2). Vegetables, fruit and cereals are generally poor sources of iodine because most of our soils and water supplies are deficient in iodine. The quantity of iodine in salt-water fish and shellfish is very variable and small compared with its abundance in certain seaweeds. This is the reason for the very high iodine intake in the traditional diet of the Japanese and Korean peoples. Other common adventitious sources of iodine include medications such as amiodarone, cough mixtures, throat lozenges, iodine-containing antiseptics, multi-vitamin preparations, kelp tablets, and foods and beverages containing the iodine-containing food coloring agent erythrosine. Many of the diagnostic contrast agents employed in medical imaging, for example CT scans, contain huge amounts of iodine.

While a proportion of the iodine in dairy milk comes naturally from pastures, most is the result of contamination by iodine residues in the milk from iodine-containing sanitizers called iodophors. The dairy industries in Australia and New Zealand – as many other countries in the world – have employed iodophors as sanitizing agents for the past 4–5 decades [13]. These chemicals are disinfectants consisting of iodine coupled to an anionic detergent. Iodine is released into the solution to exert its bactericidal activity. When used in the dairy industry, the amount of residual iodine in milk is highly variable and, in our experience, may result in anything from negligible quantities to 500 μg/l. Iodine-contaminated milk has been the major source of this micronutrient in

<table>
<thead>
<tr>
<th>Food</th>
<th>Level of iodine, μg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit 1 serve</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Vegetables 1 serve</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Cheese 40 g</td>
<td>13.6</td>
</tr>
<tr>
<td>Seaweed (nori) 1 sheet</td>
<td>16</td>
</tr>
<tr>
<td>Eggs 55 g</td>
<td>20</td>
</tr>
<tr>
<td>Milk 200 ml</td>
<td>30–60</td>
</tr>
<tr>
<td>Iodized salt 1 g</td>
<td>20–40</td>
</tr>
<tr>
<td>Sea fish 100 g</td>
<td>35</td>
</tr>
</tbody>
</table>

Adapted from FSANZ [21].

Sources of Iodine in the Diet

The principal sources of iodine in the diet vary from one country and region to another. In less developed regions of the world lacking iodine in the soil or water, the most valuable source is likely to be iodized salt. By contrast, in developed countries where most varieties of food are readily available, milk and dairy products, seafood, and the discretionary use of iodized cooking and table salt are the major sources of iodine in the diet (Table 2). Vegetables, fruit and cereals are generally poor sources of iodine because most of our soils and water supplies are deficient in iodine. The quantity of iodine in salt-water fish and shellfish is very variable and small compared with its abundance in certain seaweeds. This is the reason for the very high iodine intake in the traditional diet of the Japanese and Korean peoples. Other common adventitious sources of iodine include medications such as amiodarone, cough mixtures, throat lozenges, iodine-containing antiseptics, multi-vitamin preparations, kelp tablets, and foods and beverages containing the iodine-containing food coloring agent erythrosine. Many of the diagnostic contrast agents employed in medical imaging, for example CT scans, contain huge amounts of iodine.

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the Australian diet for the past 40 years [13, 14]. This has been termed silent prophylaxis resulting in ‘an accidental public health triumph’. Unfortunately, over the past decade there has been a substantial decline in the sales of iodophors in Australia with chlorine-based disinfectants replacing the iodine-based ones. We have been monitoring iodine concentrations in retail milk samples in Sydney and found that these levels have been declining and currently are between 90 and 200 μg/l with an average of approximately 130 μg/l [14]. These levels are less than half of what we measured a decade or so ago, and have resulted in the reemergence of iodine deficiency in Australia and New Zealand [15]. It is quite an extraordinary situation that the Australian population has, for decades, relied upon this adventitious source of iodine to maintain adequate iodine nutrition, and we are now facing a significant public health problem because of declining iodine contamination of dairy milk.

Recommended Intakes of Iodine at Different Stages of Life

Several countries and organizations throughout the world have adopted recommended dietary intakes, allowances or reference values for iodine nutrition (table 3) [3]. Most of these recommendations are very similar; however, recently there has been a tendency to increase recommended intakes. The recommendations are very arbitrary, and most are not based on solid scientific evidence. Unfortunately, we do not have rigorous experimental-based evidence to make these recommendations and must rely on indirect evidence. Indeed, most of the early recommendations on iodine intake were aimed at providing sufficient iodine in the diet simply to prevent development of palpable or visible goiter, and were made in the absence of

<table>
<thead>
<tr>
<th>Population group</th>
<th>Recommended daily intake of iodine, μg/day</th>
<th>Excessive iodine intake, μg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WHO</td>
<td>IOM (USA)</td>
</tr>
<tr>
<td>Infants</td>
<td>90</td>
<td>110–130</td>
</tr>
<tr>
<td>Children</td>
<td>120</td>
<td>90–120</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult men and women</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Pregnant women</td>
<td>250</td>
<td>220</td>
</tr>
<tr>
<td>Breastfeeding women</td>
<td>250</td>
<td>290</td>
</tr>
</tbody>
</table>

Data sourced from Eastman and Zimmermann [3]. IOM = Institute of Medicine; NHMRC = National Health and Medical Research Council of Australia.
our understanding of subtle but significant deficits in neurological and intellectual function occurring in response to minor decreases in maternal thyroid function. Recommended daily intake for iodine is the average daily intake to meet the iodine requirement of 97.5% of healthy individuals at any specific life stage.

**Excessive Iodine Intake and Recommended Upper Limit of Normal Intake**

The normal adult thyroid gland adjusts to both abrupt and sustained increases in iodine intake by in-built mechanisms that regulate absorption of iodine at the level of the thyroid cell. However, fetal and neonatal thyroid glands cannot do this very well, and exposure to large quantities of iodine may cause goiter and hypothyroidism in the developing baby. Similarly, individuals suffering from autoimmune thyroid disease are unable to compensate and may develop hypothyroidism or hyperthyroidism when exposed to iodine excess [4]. An arbitrary iodine intake of around 1,000–1,100 μg/day has been set as the upper limit of normal in Australia and most other countries in the world. While most of the public health emphasis has been on efficacy to ensure an adequate daily intake, insufficient emphasis has been placed on the concept of safety and how these two objectives can be satisfactorily achieved.

**Current Scientific Issues in Iodine Deficiency Disorders Research**

Zimmermann has recently emphasized the need for more research to resolve issues relating to (a) iodine nutrition in pregnancy and infancy, (b) potential adverse effects of iodine deficiency on cognitive development, especially the relative contributions of maternal versus childhood iodine deficiency to neurodevelopment, (c) safe upper limit of iodine intake, (d) laboratory methods for monitoring salt iodine content, (e) metabolic interactions of iodine and other micronutrient deficiencies.

**Fortification of Food with Iodine**

In principle, mandatory fortification represents the most effective public health strategy where safety and efficacy can be assured, and there is a demonstrated need for the nutrient in the population. Voluntary fortification has many limitations and few benefits. [16]. It is generally inequitable, poses major difficulties in monitoring, and it usually fails because it is not sustainable. By contrast, mandatory fortification is a more equitable strategy, reaching most of the population, and is readily monitored allowing adjustment in the fortification level, and hence is more likely to be sustainable (table 4) [16].
Universal Salt Iodization: Salt Fortification

The recommended strategy for control and prevention of iodine deficiency is mandatory universal salt iodization (USI) that requires the iodization of all salt for human and animal consumption at a level to provide sufficient iodine in the diet for most of the population without exposing them to an excessive iodine intake. The recommended level of iodization is 20–40 mg iodine/kg salt [17]. Salt is usually considered the best food to fortify with iodine because it is widely consumed by most people in the world regardless of income; it is consumed consistently in small amounts by individuals; it is inexpensive to iodize, and there is no chemical reaction altering taste or color of the salt.

The WHO estimates that approximately two thirds of 5 billion people living in areas of iodine deficiency now have access to iodized salt. Over the past decade, the number of countries employing USI has doubled from around 50 to over 100, but there are over a dozen developing countries where there has been little or no progress [9, 17]. In the developed world, USI has not generally been adopted. Australia is a good example. Iodized salt is available in Australia, but the market size for iodized salt is only 25% of the total edible salt market. Food standard codes regulate iodine concentrations at between 25 and 45 μg/g. Why are the sales of iodized table salt in Australia so poor compared with non-iodized salt? We do not have a definitive answer to this question, but it appears to be due to several factors; the consumer is unaware of the benefits of iodized salt, health authorities do not promote its use and the food industry does not market it. To our knowledge, iodized salt is not used in the food manufacturing industry or at the retail or fast food outlets. With increasing consumer awareness of the adverse effects of high salt intake on blood pressure and cardiovascular disease, we might reasonably expect that iodine intake from iodized salt will decrease even further with time. It is likely that the informed consumer will first reduce the discretionary salt intake and hence exacerbate iodine deficiency.

<table>
<thead>
<tr>
<th>Effectiveness</th>
<th>Mandatory USI</th>
<th>Voluntary fortification</th>
<th>Fortification of other foods, e.g. water, bread</th>
<th>Supplements</th>
<th>Dietary education</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equity</td>
<td>yes</td>
<td>possible</td>
<td>incomplete</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Efficiency</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Certainty</td>
<td>yes</td>
<td>no</td>
<td>yes/no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Feasibility</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Sustainability</td>
<td>yes</td>
<td>probably not</td>
<td>yes</td>
<td>probably not</td>
<td>probably not</td>
</tr>
</tbody>
</table>

Table 4. Assessment of potential public health strategies to correct iodine deficiency in a population [16]
Barriers to Adoption of Universal Salt Iodization

Major efforts have been made throughout the world to achieve the goal of USI, particularly in developing countries with high prevalence of iodine deficiency. There are many outstanding examples of countries who have achieved a penetration of over 90% of households using iodized salt. Two of the best examples in the Asia-Pacific region are the People's Republic of China and Vietnam. Essential elements in successful programs require legislation underpinning USI and national monitoring programs to assess the effectiveness of these programs. Many countries claim to have implemented USI, but in effect the iodization of salt is not universal. Despite the continuing belief by international agencies that USI can be implemented universally and can be sustained, this is simply not possible in many countries for several reasons.

In developed countries, barriers to USI include:

- Most of the salt intake in the diet (>75%) comes from processed foods not from the discretionary use of household salt. While most developed countries have household iodized salt available for purchase in food stores, non-iodized salt is also readily available.
- Large multinational food-producing companies resist the pressure to use iodized salt in food processing and manufacturing. The reasons for this resistance include costs for production and labeling, costs for meeting regulatory requirements and trade barriers erected by countries who have legislated against the importation of foods containing iodized salt.
- Increasingly successful campaigns to reduce population salt intake [18].

In developing countries, barriers to USI include:

- A large proportion of the salt intake in the diet comes through salty condiments not through the use of household salt in many countries, particularly Asian countries; iodized salt is not employed in the manufacture of these products.
- The inability of countries to implement and/or police iodized salt legislation; the lack of political will is the usual cause for lack of implementation.
- The ready availability of less expensive non-iodized salt in the marketplace.
- Lack of political will and commitment to sustaining USI.
- Concerns about the safety of USI and the fear of potential adverse effects.

The well-orchestrated salt-reduction campaign originating in the UK in 2005 is now active in 80 countries and expanding rapidly [18]. This campaign is founded on the premise that the current estimated high salt intake, in excess of 10 g/day in developed countries, is a major cause of hypertension and cardiovascular disorders [19]. The primary goal is to decrease salt intakes to the WHO target of <5 g/day. As this movement becomes more widespread, International Agencies (UNICEF, WHO, ICCIDD) will have to revise the recommended levels of iodine in edible salt based upon population monitoring. In countries like the People's Republic of China there has been a public backlash against USI with claims by several medical professional groups that the Chinese population is
ingesting excessive quantities of iodine, as a consequence of USI, and this is causing a rising incidence of autoimmune thyroid disease and thyroid cancer [20].

**Fortification of Other Foods**

The addition of iodine to bread, drinking water, rice, fruits, eggs and fish sauce, and complementary foods has been tried in several countries with varying degrees of success. The Netherlands has continued the practice of using iodized salt in bread for several decades without any problems. Australia and New Zealand mandated the use of iodized salt in bread in 2009 in an attempt to address the reemergence of iodine deficiency in both of these countries, but recognized this initiative would not satisfy iodine requirements in pregnant and breastfeeding women, the newborn and young infants [21]. Therefore, these strategies may be useful and effective in limited geographical situations, but will need to be complemented by iodine supplementation directed to the most vulnerable groups [22]. It is a matter of ‘think globally but act locally’.

**Iodine Supplementation**

Iodine supplementation to specific individuals or subgroups of an iodine-deficient population has been widely practiced, but remains a controversial strategy. Until recently, this strategy did not receive much support from the international agencies, such as WHO/UNICEF/ICCIDD who argued that supporting iodine supplementation programs would detract from USI. Dietary education and supplementation are generally inefficient public health strategies because of the level of investment necessary to implement such interventions, and they are confounded by equity and access issues [16]. Those with higher socioeconomic and educational levels are more likely to benefit from education and promotion of supplements frequently making the strategies inequitable. Education of the population and health care providers is a prerequisite for any supplementation program. However, there is a place for iodine supplementation during pregnancy and breastfeeding as a complementary strategy, particularly where voluntary or mandatory salt iodization has not been implemented or is ineffective [22].

Supplements can be given on a daily basis by oral administration of potassium iodide pills (150–250 μg/day) or annually by way of iodized oil capsules (400 mg dose) which are slow-release depot preparations that provide enough iodine for up to 12 months’ duration [17]. While there are numerous studies demonstrating the effectiveness of iodized oil in preventing IDD, there are no data from randomized control trials and very little in the way of information confirming the safety of this material. The current recommendations agreed by WHO, ICCIDD and UNICEF are for the administration of iodine supplements
to pregnant and breastfeeding women and infants up to 2 years of age living in areas where USI has not been implemented or is ineffective [17]. Several influential scientific and medical bodies – including the American Thyroid Association, the American Endocrine Society and the National and Medical Research Council in Australia – now recommend daily iodine supplementation for pregnant and breastfeeding women. Recommendations for infants and children and the need to ensure optimal iodine intake in complementary foods are covered elsewhere in this symposium.

**Monitoring and Sustainability**

The success of all iodine fortification or supplementation programs is dependent on population monitoring. In the past, monitoring has targeted school-age children, measuring goiter prevalence and UIE. It is clear that monitoring should be directed to the most vulnerable groups, namely pregnant and lactating women, neonates and infants.

**References**

Folate and Vitamin B\textsubscript{12}: Function and Importance in Cognitive Development

Aron M. Troen

Nutrition and Brain Health Laboratory, Institute of Biochemistry, Food Science and Nutrition, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel

Abstract

The importance of the B vitamins folate and vitamin B\textsubscript{12} for healthy neurological development and function is unquestioned. Folate and vitamin B\textsubscript{12} are required for biological methylation and DNA synthesis. Vitamin B\textsubscript{12} also participates in the mitochondrial catabolism of odd-chain fatty acids and some amino acids. Inborn errors of their metabolism and severe nutritional deficiencies cause serious neurological and hematological pathology. Poor folate and vitamin B\textsubscript{12} status short of clinical deficiency is associated with increased risk of cognitive impairment, depression, Alzheimer’s disease and stroke among older adults and increased risk of neural tube defects among children born to mothers with low folate status. Folate supplementation and food fortification are known to reduce incident neural tube defects, and B vitamin supplementation may have cognitive benefit in older adults. Less is known about folate and vitamin B\textsubscript{12} requirements for optimal brain development and long-term cognitive health in newborns, children and adolescents. While increasing suboptimal nutritional status has observed benefits, the long-term effects of high folate intake are uncertain. Several observations of unfavorable health indicators in children and adults exposed to high folic acid intake make it imperative to achieve a more precise definition of folate and B\textsubscript{12} requirements for brain development and function.

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Introduction – Biochemical Function of Folate and Vitamin B\textsubscript{12}

The importance of the B vitamins folate and vitamin B\textsubscript{12} for healthy neurological development and function is unquestioned. As essential cofactors for one-carbon metabolism, folate and vitamin B\textsubscript{12} are required for biological methylation and DNA synthesis. Methylation activity is essential for many processes that
are critical for central nervous function, such as the epigenetic regulation of gene expression by DNA and histone methylation, the methylation of myelin basic protein and membrane phosphatidylcholine, the synthesis of hormones and neurotransmitters such as melatonin and epinephrine, and the inactivation of dopamine and catecholamines. Folate (in the form of 5-methyltetrahydrofolate) and vitamin B\textsubscript{12} are required in all tissues to maintain adequate cellular methionine, which in turn is converted to S-adenosylmethionine the universal methyl donor for these reactions. Methylation produces the sulfur amino acid homocysteine, which serves as precursor for the cyclical regeneration of methionine. Choline is another important dietary methyl donor, providing an alternative source of labile methyl groups for regeneration of methionine by an alternative pathway which is only expressed in the liver and kidney.

Disrupting these pathways can have wide-ranging direct and indirect consequences for neural tissue. Folate and B\textsubscript{12} deficiency inhibit methylation and cause homocysteine to accumulate in circulation. Homocysteine may be cytotoxic, and mildly elevated plasma homocysteine is associated with increased risk of neurodegenerative and cardiovascular disease. By increasing choline utilization or by depleting membrane phosphatidylcholine, folate deficiency might also indirectly limit the synthesis of the key neurotransmitter acetylcholine. Folate and B\textsubscript{12} are also required for activity that is independent of each other. Folate is needed to synthesize thymidylate and purine nucleotides. Thus, folate deficiency can compromise DNA replication and integrity. Vitamin B\textsubscript{12} serves as a coenzyme for methylmalonyl-CoA mutase which catalyzes the final step in the mitochondrial degradation of odd chain fatty acids and branched amino acids. Thus, in addition to impinging upon folate and methylation, B\textsubscript{12} deficiency leads to abnormal membrane fatty acid composition and elevation of circulating methylmalonic acid. These biochemical effects of deficiency can be theoretically linked to the observed neuropathological, cognitive and developmental abnormalities that are associated with deficiency of these vitamins [1].

**Neurological Consequences of Acquired and Congenital Defects in One-Carbon Metabolism**

The importance of the B vitamins folate and vitamin B\textsubscript{12} for healthy brain development and function is evident in the serious neurological consequences of acquired nutritional deficiencies and inborn errors of their metabolism. Severe acquired deficiencies of vitamin B\textsubscript{12} and folate manifest with either hematological or neurological abnormalities or a combination of both [2–6]. A large proportion of cases develop neurological symptoms without exhibiting any major hematological abnormality, and this tendency is thought to be more prevalent among the elderly [7]. The primary neurological feature is a progressive
neuropathy in which sensation and motor control are gradually lost, typically beginning with the lower limbs [6]. In addition, these conditions are often accompanied by behavioral changes ranging from mild irritability to severe depression, hallucinations, confusion and memory loss [8]. If the condition is left untreated, it may eventually lead to sub-acute combined degeneration of the spinal cord. The dominant histopathological finding in this condition is a patchy loss of myelin in the white matter of the spinal cord where myelin-laden macrophages surround the empty spaces, although brain and peripheral myelin are also affected [7]. Prognosis depends on intervention. Although vitamin supplementation is an effective treatment in many cases, cumulative neurodegenerative damage may become irreversible if it is allowed to progress too far. It is not clear whether the behavioral changes are due to vitamin-related neurochemical imbalances or to structural changes in the brain caused by demyelination [8].

Genetic impairment of one-carbon metabolism can produce similar pathology. For example, mutations that severely limit the synthesis of 5-methyltetrahydrofolate by methylene tetrahydrofolate reductase (MTHFR) typically result in abnormal neurological development, progressive demyelinating neuropathy and cognitive impairment [9–11]. Although congenital defects in MTHFR activity usually become evident soon after birth, they have been known occasionally to remain silent until the onset of neurological symptoms and psychotic or schizophrenic behavior in adolescence or adulthood [12–15]. Gene-gene and gene-nutrient interactions appear to influence the severity and expression of phenotype in these cases. This is implied by cases where some family members carrying the same mutation are symptomatic while others are not [13, 14]. In many cases, MTHFR deficiency is partially responsive to supplementation with folate or betaine [12, 13]. Similar findings are observed in mice with a targeted mutation in the MTHFR gene. In homozygotes for the mutation, CNS development is compromised, but this can be partially mitigated by dietary supplementation with betaine [16]. Cerebral folate deficiency is another example of folate-dependent impairment of CNS development and function. In this rare pediatric autoimmune condition, auto-antibodies to the choroid plexus high-affinity folate transporter are associated with low cerebrospinal folate despite normal serum folate, and with progressive impairment of neurological development, myelination and cognition [17]. In some cases, the symptoms can be partially mitigated, at least temporarily, by folinic acid (5-formyl tetrahydrofolate), which can enter the CNS through an alternative reduced folate transporter. Vitamin B12 deficiency in newborns and infants who have smaller liver and tissue stores [18] is often due to maternal deficiency. Symptoms of clinical deficiency can be treated with intramuscular injections and high-dose oral supplementation, leading to hematologic and neurologic recovery; however in the long-term, resolving the deficiency and acute pathology does not guarantee full restoration of cognitive development [19].
Subclinical Deficiencies in CNS Development and Aging

The neuropathology common to both congenital and severe acquired vitamin B₁₂ and folate deficiencies demonstrates the importance of methylation for brain development and function, yet these deficiencies are relatively rare. More often, individuals who fall in the lower range of population folate and vitamin B₁₂ intake and plasma concentrations, but who are not clinically deficient, have significantly higher risk of poor neurological and cognitive outcomes. These include increased risk of neural tube defects (NTDs) among children born to mothers with low folate status, and increased risk of cognitive impairment, depression, Alzheimer’s disease and stroke among older adults.

With respect to aging, the association between low B vitamin status and increased risk of neurodegenerative disease has been extensively reviewed elsewhere [20, 21]. Data from over 100 observational cross-sectional and prospective studies, encompassing over 50,000 subjects in total, provide compelling evidence for the association, with approximately 90% of the studies reporting significant associations and the remainder not. Taken together, they provide the basis for the tenable hypothesis that 'low-normal intake or blood concentrations of B vitamins (folate, B₁₂ and B₆) and/or moderately elevated plasma total homocysteine increase the risk of brain atrophy and developing cognitive impairment in the elderly' [1, 21, 22]. As demyelination is not a dominant feature of these diseases, a variety of alternative mechanisms has been proposed to account for these associations, relating both vitamin deficiencies and elevated homocysteine to different aspects of cognitive dysfunction and neuropathology [23]. The efficacy of B vitamin supplementation for neurocognitive protection has yet to be determined, with several trials reporting null findings [24–26] and others suggesting benefit [27, 28].

With respect to early life, the prevention of NTDs by folic acid provides strong evidence of folate’s critical importance to CNS development, although the precise mechanism for the phenomenon is still unknown. Early observations that poor folate status is associated with increased risk of spina bifida and anencephaly led to highly successful clinical trials in which use of periconceptual folic acid supplements reduced the risk of incident NTDs by as much as 50% or more [29]. Further evidence for folate’s importance for neural development comes from genetic association studies. A meta-analysis of more than 17 different studies including several thousand subjects calculated a pooled odds ratio giving an approximately twofold increase in risk of an NTD for mothers and children who are TT homozygotes compared with CC homozygotes for the C677->T single nucleotide polymorphism in the MTHFR gene, where the TT genotype encodes a slightly less active enzyme. Risk for NTDs among the TT homozygotes decreased with increased folate status [30]. Folate supplementation before conception is critical for preventing NTDs given that neural tube closure occurs early in embryonic development when the mother may not be
aware of the pregnancy. Despite this, most women of childbearing age do not take supplements, particularly those at risk for nutritional deficiency. For this reason, mandatory food fortification of all cereal grains with folic acid has been enacted in over 50 countries. These public health programs have been highly successful with respect to reduction of incident NTDs. They have also significantly increased folic acid intake across populations and lowered the prevalence of hyperhomocysteinemia. In the Framingham study in the USA, the prevalence of folate deficiency decreased from 4.9 to 1.9% after fortification [31], but at the same time, the proportion of individuals exceeding the recommended upper intake limit of 1 mg folic acid/day grew from 1.3 to 11.3% [32]. Interestingly, following fortification, vitamin B$_{12}$ and choline status have been found to determine NTD risk [33, 34].

**Folate and B$_{12}$ Supplementation and Cognitive Development**

All of these examples relate to pathological consequences of vitamin deficiency. But what effect does supplementation beyond replacement have on brain development and attainment of full intellectual potential? Here, the data are surprisingly scarce. Answering this question is important in light of the possibility that exposure to high levels of folic acid may have unintended effects, for better or for worse. The upper limit of folate intake for adults was conservatively set at 1 mg per day out of concern for the possibility that in individuals with frank B$_{12}$ deficiency, high folate might either exacerbate the symptoms or ‘mask’ the associated anemia, delaying early detection and treatment and thus allowing it to progress [35]. This concern relates to a relatively small though not insignificant proportion of the population. There is deeper controversy over whether fortification has increased cancer risk and decreased risk of cardiovascular disease and stroke [36–38]. Finally, despite scant data, concerns have also been raised over theoretical mechanisms through which high folate intake might exert subtle but substantial life-long effects on metabolism and neurological development through altered epigenetic programming [39, 40].

A small number of recent observations show unexpected evidence for metabolic interaction of high folate status with low-marginal B$_{12}$ status. Cross-sectional data from the NHANES and SALSA cohorts show that the metabolic effects of vitamin B$_{12}$ deficiency are more pronounced in adults with high folic acid intake. MMA and Hcy levels are elevated in individuals with low B$_{12}$ and high folate status compared to those with normal folate and normal or low B$_{12}$ [41, 42]. These metabolic findings are mirrored by poorer neuropsychological test scores on the symbol digit test and by lower hemoglobin values [43]. Similar associations between worse metabolic and functional impairments with B$_{12}$ deficiency were found when unmetabolized folic acid in plasma was used as a putative proxy for high folic acid intake [44]. Irrespective of B$_{12}$ status, high folic acid intake has also
been observed to associate with more rapid cognitive decline in older adults [45] and with decreased immune function in older postmenopausal women [46].

The same pattern of a potentially undesirable interaction between high folate and low B12 has been seen in children. A large study of 2,812 Columbian school children aged 5–12 years found that hemoglobin concentrations decreased with increasing erythrocyte folate [47]. There was a significant interaction between folate and B12 status such that differences in hemoglobin concentrations between the highest and lowest quartile of folate were greatest in children with low B12 status (<148 pmol/l) followed by marginal B12 status (between 188–221 pmol/l) and least in B12-sufficient children. Notably, the prevalence of anemia in this cohort was only 3.7%, and data were adjusted for relevant biological and socioeconomic factors. The same interaction pattern has been observed in an entirely different setting, the Maternal Nutrition Study in Pune, India, where higher maternal folate during pregnancy predicted greater insulin resistance in offspring 6 years later, particularly in mothers with low B12 [48].

Cognitive development was not reported in these studies; however, a handful of other studies yield inconsistent associations between maternal folate and B12 status with cognitive development in children. In a cohort of 536 children from Mysore, India, higher maternal folate was associated with better performance on a battery of neuropsychological tests after appropriate adjustment for confounding, irrespective of vitamin B12 status, and despite the fact that 42.5% of mothers had low vitamin B12 during pregnancy. In this cohort, maternal B12 and homocysteine concentrations did not predict children's cognitive performance [49]. In contrast to these findings, maternal B12 status predicted cognitive performance in 9-year-old offspring in a small subset of the Pune cohort. Children born to mothers in the lowest B12 decile (<77 pmol/l) performed significantly worse than those whose mothers were in the highest decile of B12 (>224 pmol/l) on two out of 7 neuropsychological tests used for the examination (digit span backward and color trail test A). Plasma folate, homocysteine and methylmalonic acid were not associated with cognition [50]. A cross-sectional study of 598 Indian children aged 6–10 years revealed an unexpected inverse association between plasma B12 status and factor scores for cognitive domains of short-term memory, retrieval ability and an overall composite score for mental processing. In this cohort, increasing hemoglobin concentration was positively associated with better cognitive performance, but iron, iodine, fatty acids and folate showed no association [51]. A comparison of cognitive performance among adolescents (age 10–16) who were either vegan-macrobiotic or omniverous until the age of 6, found poorer performance on cognitive tests among the previously macrobiotic group, which also had a higher prevalence of low but not deficient vitamin B12 status. Finally, an early study found severe mental retardation among children born to mothers with folate deficiency [3], but a more recent study found no relation of neuropsychological development in 5-year-old children to maternal folate status during the second half of pregnancy [52].
A Role of Folate and Vitamin B₁₂ in Epigenetic Programming of Neurocognitive Development?

The prevention of NTDs by relatively modest doses of folic acid demonstrates the profound effect that folate-dependent metabolism has during CNS development. However, there is no reason to assume that the mechanism for this effect is specific only to neural tube closure. A leading hypothesis for the mechanism involves epigenetic mechanisms – the stable and heritable regulation of gene without altering gene sequence that is regulated by DNA and histone methylation. Epigenetic patterns that are established early in life program life-long effects on development, function and aging. Aberrant DNA methylation is critically involved in the neurodevelopmental disorders Praeder Willi and Angelman syndromes, and is also hypothesized to be involved in Rett syndrome, schizophrenia and autism [53, 54]. Moreover, recent animal studies have shown that experience-dependent, reversible changes in DNA and histone methylation are critically involved in the regulation of synaptic plasticity, learning and memory in nondividing neurons of the adult brain. Thus, it is reasonable to hypothesize that even mild alterations of methylation during brain development as a function of intake of folic acid, vitamin B₁₂, choline or other nutrients would have long-lasting impact on brain development and function [55, 56].

Evidence that methyl donor availability can determine stable epigenetic changes in brain development and cognitive function comes largely from animal studies. In rodents, providing dams with choline at critical windows during embryonic development changes DNA methylation and gene expression in the brain of the offspring, induces structural changes involving neuronal stem cell proliferation and angiogenesis in hippocampus, and enhances memory. Moreover maternal choline supplementation prevents normal age-related cognitive decline in elderly rats [57]. However, the beneficial effects of supplementation may have upper limits. Folic acid has been shown to significantly improve nerve regeneration following spinal cord injury in adult rats. The effect of folate followed a nonlinear dose-dependent relationship with regeneration, where the enhancement of regeneration peaked at 80 μg folic acid per kg body weight and declined significantly thereafter. This inverse U-shaped relationship was paralleled by increasing expression of the DNA methyltransferase enzyme 3 (DNMT3) up to 80 μg/kg and decreased expression at higher doses [58].

Although data from human studies are limited, the available data show that even modest supplementation within the normal range of dietary intake can have significant epigenetic effects. A recent study documented the effect of using periconceptual folic acid (400 μg/day) on epigenetic regulation of the insulin-like growth factor 2 gene (IGF2) in offspring. Lymphocyte DNA methylation in the promoter region of the IGF2 gene was significantly (4.5%) higher in children born to mothers who took folic acid supplements (n = 86), compared to those not exposed to folic acid (n = 34). Although birthweight was similar in the
two groups, a 1.7% higher methylation was associated with one standard deviation decrease in birthweight (584 g) independent of periconceptual exposure to folic acid or gestational age at delivery [59]. Another human study found a nonlinear U-shaped association of plasma folate with DNA telomere length in a non-fortified population of older Italian adults. Telomere length (a marker for biological aging) declined with increasing folate until the median folate level, and then increased again as folate increased above the median [60]. The authors speculated that epigenetic mechanisms were involved. If such changes to peripheral DNA are also reflected in the brain, this would suggest that brain development, cognitive function and aging can be modulated by folate and B<sub>12</sub> availability across the current range of vitamin intake from diet, fortification and supplement use. Given the clear importance of folate and vitamin B<sub>12</sub>-dependent metabolism in brain development and function, it would not be surprising if these processes also influenced the development and realization of full cognitive and intellectual potential.

Conclusions

Taken together, the demonstrable importance of folate supplementation for NTD prevention, the paucity of data on the impact of folic acid and B<sub>12</sub> supplementation on neurocognitive development in human populations, the observation of both favorable and potentially unfavorable outcomes in association with folate intake alone or in association with low B<sub>12</sub> status, and the uncertainty regarding the mechanisms that underlie such observations all suggest it is important to improve folate and vitamin B<sub>12</sub> status in deficient populations, possibly in conjunction with choline, and to avoid excess. In order to improve on current guidelines and recommendations by revisiting the upper limits, and to delineate their risks and benefits, we require a much more precise understanding than is currently available of the impact of these nutrients on brain development and cognitive function, and the mechanisms through which they act on the brain throughout the lifespan.

References


Discussion on Folate and Vitamin B\textsubscript{12} Importance in Cognitive Development

Dr. Troen has provided a contemporary insight into the ways in which folic acid and vitamin B\textsubscript{12}, their interactions and deficiencies might impact upon cognitive and brain development in the child and even at the other extremity of the life cycle, older age. We need to remember that when we speak about folic acid, we need to add our specificity, especially when we refer to the possibility of adverse effects. Folic acid is a synthetic form of folate. The synthesis was accomplished in the late 1940s. The synthetic form of the folic monoglutamate is not the same as folate in foods which tend to be reduced and tend also to be in the conjugated form of polyglutamates. As we think about the effects of folic acid administered as a supplement before or during pregnancy, we need to speak in terms of whether there are special effects of synthetic folic acid, which has to traverse a somewhat different metabolic pathway than so-called ‘natural’ folate. That distinction is probably going to turn out to be important as we explore further the matter of epigenetics and the way in which folic acid or other methyl donors may be influencing gene and gene expression. Also the pathways leading to methylation of the DNA genome are going to be a very interesting approach to epigenetic regulation.

Regarding brain development, as with physical growth and development, deficiency of almost any essential vitamin and mineral (as we have heard with iodine and iron and other B vitamins, thiamine, niacin and vitamin B\textsubscript{6}) can affect brain development and cause disease. The complex relationship between nutritional status of micronutrients and brain development is before us. The special consideration that was emphasized by Dr. Troen is the interaction of the last two of the discovered vitamins. I remind you that the last two vitamins that were discovered in the 1940s and 1950s were folic acid (which became vitamin B\textsubscript{9}) and vitamin B\textsubscript{12}. (It turned out that vitamin B\textsubscript{10} and B\textsubscript{11} were both forms of folic acid.) But the history is entrained in a very important way so that before we had the identification of vitamin B\textsubscript{12}, while we already had synthetic folic acid, folic acid was used in the treatment of pernicious anemia even before it was known that pernicious anemia was vitamin B\textsubscript{12} deficiency conditioned
by an absorption defect of intrinsic factor. The result was that many patients were reported to have a worsening of their neurologic problems when they were treated with high doses of folic acid before we learned that vitamin $B_{12}$ was the therapeutic need. These observations had a large impact on the later definition of the safe upper level of folic acid, which was defined in the DRI (Dietary Reference Intake) report for the US Institute of Medicine in terms of what would happen if you gave large doses of synthetic folic acid in the presence of limiting vitamin $B_{12}$ status. That continues to be a challenge for us now, and it reemphasizes the importance of updating our information about what should be the safe levels of folic acid supplementation using synthetic folic acid. I am not talking about any adverse effect of folate as food folate. Perhaps in the next version of the DRIs we are going to have to look at a much more complex interaction using some of the kinds of methodologic evaluations that Dr. Troen presented (including epigenetic gene expression) to arrive at an understanding of not only how folate and $B_{12}$ influence brain development and cognitive development but perhaps how their interaction might be important in the generation and maintenance of health.

When Dr. Troen was asked whether he thinks that the closure of the developing neural tube in the presence of perhaps adequate amounts of folic acid is a good model for us to be thinking about the effect of folic acid on brain development, he replied that we still don’t understand the mechanism despite the powerful effect of folic acid supplementation. There are clear trophic effects of this metabolic pathway on different aspects of brain development at different critical windows. Perhaps the problem we have with fortification is that we undertook the public health initiative to prevent neural tube defects without full knowledge of observations in populations that are exposed with respect to a whole range of other important health outcomes and risks.

Dr. Haschke added two questions related to epigenetic phenomena which Dr. Troen described, the first one is the Indian study on maternal $B_{12}$ and cognitive function at 9 years of age. We must be very careful because this is a highly selective population in terms of nutrition. The low vitamin $B_{12}$ cohort is probably a vegetarian cohort, and it might be that the children have also very low $B_{12}$ intake as children from parents who are vegetarians. And the second phenomenon described is the periconceptional folic acid supplementation. The study by Steeger seems to show lower birthweight if the mothers are supplemented. This was not the primary outcome of the study, and the sample size is much too small to really show differences in birthweight. You need at least 80–100 per group to rule out the type 2 error. If folic acid when given perinatally results in lower birthweight, this potentially important finding has to be confirmed in adequate studies.

Dr. Troen added that we don’t know that the Steeger’s study demonstrates that there is an effect of periconceptional folic acid on birthweight. We are looking at one gene. His point was that by virtue of our standard practice we can
show a measurable effect on a measurable biological change that has a known consequence in terms of expression of the gene product and downstream effect. But that’s one gene out of our entire genome, the entire genome is exposed, the whole panel of imprinted genes is exposed, we don’t know what this does overall. So, he was not unduly concerned, and he doesn’t think that we have reduced birthweight across the folic acid-fortified population.

Irwin H. Rosenberg
Pros and Cons of Increasing Folic Acid and Vitamin B₁₂ Intake by Fortification

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Abstract

There is no doubt that folic acid fortification can be effective for reducing the incidence of neural tube defects. The degree of efficacy depends on both the level of folate depletion and other, yet to be fully characterized, genetic and/or environmental factors. This article summarizes briefly data on neural tube defect reduction and other benefits of folic acid fortification as these have been reviewed in more detail elsewhere. More attention is drawn to questions that have been raised about the possible adverse effects of folic acid fortification including the incidence of colorectal cancer and immune function. The main question addressed here is whether folic acid fortification can exacerbate the adverse effects of vitamin B₁₂ deficiency. Most analyses of this question have been conducted in wealthier countries based on data from elderly populations – which have the highest prevalence of vitamin B₁₂ deficiency. However, of potentially greater concern is the increasingly common practice of folic acid fortification in developing countries, where folate status is probably often adequate even prior to fortification, and vitamin B₁₂ depletion or deficiency is common. To add to this information, data from a group of Chilean elderly with a range of vitamin B₁₂ status and exposed to high levels of folic acid fortification will be presented.

Introduction

Flour fortification with folic acid has the primary goal of preventing neural tube defects (NTDs) by improving the periconceptional folate status of women at risk of delivering an infant with these abnormalities. At least 52 countries are currently mandating flour fortification with folic acid [1], although none of them are in Europe where there are stronger concerns about the safety of folic acid fortification and/or its likely effectiveness in regions where industry is already
voluntarily fortifying foods, such as cereals, with the vitamin. After mandatory or voluntary fortification was instigated in different locations, reports began to appear of associations between the timing of fortification, and/or folate status, and a range of positive and negative health outcomes. Mandatory fortification with vitamin B\textsubscript{12} is much less common, although the prevalence of deficiency is probably much higher. There are numerous documented and potential adverse effects of this nutrient deficiency that could justify fortification policies. Dual fortification with both vitamins may make sense based on their metabolic interactions and concern about the potential (as yet unproven) for folic acid fortification to exacerbate signs and symptoms of vitamin B\textsubscript{12} deficiency.

**Folic Acid Fortification**

**Advantages**

Benefits of folic acid fortification have been documented in a number of countries. For example, in the United States where fortification of flour became mandatory in 1998, the incidence of NTDs has been reduced by 19–40\% [2–4]. In addition, the prevalence of folate deficiency has fallen from ≈20–25\% to ≈1\% [5–7]. Hyperhomocysteinemia has been reduced from 17 to 9\% [5], which has been associated with reduction in mortality from stroke in the US and Canada [8], although it is not clear that this has had any effect on risk of CVD [9]. High plasma homocysteine in pregnant women is associated with an increased risk of other adverse pregnancy outcomes [10], lower birthweight of the infant [11] and in rats, impaired glucose regulation in the offspring [12], but these associations have not been tested in randomized controlled trials. In Canada, the prevalence of open NTDs fell by 48\%, from 1.13 to 0.58 per 1,000 pregnancies within a few years of the start of fortification [13]. There was also a significant, 6%/year fall in the birth prevalence of severe congenital heart defects [14].

**Potential for Adverse Effects**

Concern has been raised about potential adverse effects of folic acid fortification, as reviewed elsewhere [15, 16]. Folic acid is a substrate for the synthesis of thymidine which is incorporated into DNA, and in folate deficiency there is less thymidine available and more uracil is incorporated into DNA, which can cause breaks in the DNA base sequence. In animal models, this increases the risk of cancer initiation. However, after cancer has been initiated, there may actually be a reduction in proliferation when folate is restricted, which is the basis of treatment with anti-folate drugs such as methotrexate. Thus, fortification with folic acid can theoretically prevent cancer initiation in depleted individuals but stimulate proliferation of preexisting tumors. This hypothesis is supported by an increase in colorectal cancer rates in the United States since folic acid addition to foods commenced [17] and a similar situation in Chile [18]. Although
improvements in ability to detect and diagnose colon cancer were ruled out by the investigators as the explanation for the increase in colon cancer, it is clear that more research is needed before causality can be proven. Moreover, increased mortality from colon cancer has not been demonstrated.

Questions have also been raised about whether high folic acid intakes reduce natural killer (NK) cell cytotoxicity, which is an important component of immune function. Postmenopausal women in the US whose diet was low in folate (<233 μg/day) had better NK cell cytotoxicity if they took folic acid supplements, but those who had a folate-rich diet and in addition took >400 μg/day as a supplement had poorer NK cell cytotoxicity [19]. This association was supported by the cytotoxicity being inversely related to levels of unmetabolized folic acid in plasma; the latter increase with folic acid intake.

Potential for High Folate Status to Exacerbate Vitamin B₁₂ Deficiency
Evidence has been accumulating to suggest that high folic acid intakes might exacerbate the adverse effects of vitamin B₁₂ deficiency. Concerns were first raised in the 1940s and 1950s about the possibility that folic acid supplementation might exacerbate both the hematological and the neurological symptoms of vitamin B₁₂ deficiency. These concerns arose during clinical examination of patients with megaloblastic anemia who were treated with folic acid because they were mistakenly diagnosed with folate deficiency, when in fact they were B₁₂ deficient. The folic acid ameliorated the anemia for a few years but the neurological symptoms of vitamin B₁₂ deficiency were reportedly exacerbated or appeared suddenly when folic acid supplementation was started [20, 21]. Higher doses of folic acid, given for a longer time, were most likely to produce these effects, and of 38 cases of vitamin B₁₂ deficiency treated with <1 mg folic acid, only 6 had neurological deterioration, and those cases had been treated longer [21]. Thus, 1 mg/day is generally accepted as being the upper limit for folic acid intake.

Morris et al. [22] analyzed data from the 1999–2002 National Health and Nutrition Examination Survey (NHANES) to investigate whether individuals with higher serum folate concentrations had poorer vitamin B₁₂ status based on biochemical markers. Values were available for 1,459 persons after excluding those with evidence of renal disease, alcoholism, history of stroke, or liver, thyroid or coronary artery disease. Their analyses revealed that having both vitamin B₁₂ deficiency (serum B₁₂ <148 pmol/l or serum methylmalonic acid, MMA >210 μmol/l) and high serum folate (>59 nmol/l, the 80th percentile of the population) increased the risk of both anemia and cognitive impairment (assessed with the Digit Symbol Coding subtest of the Wechsler Adult Intelligence Scale III, which assesses processing speed and memory). The risk of anemia and cognitive impairment was around three times higher in the low-B₁₂, high-folate group compared to those with serum folate ≤59 nmol/l. On the other hand, high serum folate protected against cognitive impairment in those with adequate B₁₂ status.
The same team later performed similar analyses using data from NHANES conducted in 1991–1994 and from 1999 to 2002, this time adding values for total homocysteine (tHcy) and MMA [23]. In that analysis, they found that persons with vitamin B\textsubscript{12} deficiency – defined as low serum vitamin B\textsubscript{12} (<148 pmol/l) – in combination with high serum folate (>32.6 nmol/l) had significantly higher plasma tHcy and serum MMA than those with B\textsubscript{12} deficiency and lower folate status. In the low serum B\textsubscript{12} group, tHcy and MMA increased when serum folate started to rise above 20 nmol/l. Subsequent analyses explored additional relationships among data from the 1999–2002 NHANES [24]. Low B\textsubscript{12} status was defined as serum B\textsubscript{12} <148 pmol/l or serum MMA >210 nmol/l. Compared to subjects with plasma folate ≤59 nmol/l and normal B\textsubscript{12} status, the odds ratios of having anemia and impaired cognitive function respectively were 2.1 and 1.7 with low B\textsubscript{12} and normal folate, and 4.9 and 5.0 for low B\textsubscript{12} and high folate (>59 nmol/l). With increasing serum folate, in participants with serum B\textsubscript{12} <148 pmol/l there was again a surprising increase in homocysteine and MMA, whereas there was the opposite trend for those with serum B\textsubscript{12} ≥148 pmol/l. These results suggest that the coenzyme functions of vitamin B\textsubscript{12} are more impaired at high serum folate concentrations in people with poor vitamin B\textsubscript{12} status.

In California, we found a similar pattern in elderly Latinos (participants in the Sacramento Area Latino Study on Aging) [25]. In this analysis, vitamin B\textsubscript{12} deficiency was again defined as <148 pmol/l, while high serum folate was defined as >45.3 μmol/l, a cutoff selected because it was the upper limit of accuracy for the assay. The group with B\textsubscript{12} deficiency and high serum folate had a significantly higher plasma tHcy and MMA, and lower holoTC and holoTC:B\textsubscript{12} ratio, than groups with B\textsubscript{12} deficiency and high folate, or adequate B\textsubscript{12} status and normal or high folate. However, neither cognitive function (3MSE and delayed recall) nor depressive symptoms were different across the four groups. In contrast, low serum folate was associated with dementia and impaired cognitive function in the same population [26].

In contrast to these reports, a study from Oxford, UK, found that older individuals with a holoTC concentration in the lowest tertile had a 1.8-fold higher risk of cognitive impairment, while for the lower tertile of plasma folate, it was 1.55-fold higher [27]. However, there was no evidence that high folate status (serum folate >30 or >60 nmol/l) increased the risk of anemia or cognitive impairment.

Possible Explanations for the Observed Adverse Associations with Folate Status
For adverse effects of high folic acid intakes to be plausible, there needs to be a plausible explanation for the observed associations. Several such explanations have been offered. Certainly folate and vitamin B\textsubscript{12} metabolism are closely linked, and both 5-methyl-tetrahydrofolate and B\textsubscript{12} are cofactors for the synthesis of methionine. Thus, an abnormal folate cycle or vitamin B\textsubscript{12} deficiency can
have similar effects on both hematopoiesis and the nervous system. In vitamin B$_{12}$ deficiency plasma folate increases because there is a block in the utilization of methyl folate; this can lead to megaloblastic anemia and neurological problems. In addition, the apparent associations between vitamin B$_{12}$ deficiency and high serum folate may to some extent be explained by this phenomenon rather than high folate causing B$_{12}$ depletion. Some investigators have hypothesized that the increase in circulating folic acid, which rises progressively with intakes of folic acid in supplements or fortification [28], may have adverse effects on immune function or the rate of oxidation of forms of methionine synthase [23]. An alternative interpretation is that individuals with high serum folate are those taking multivitamin supplements containing folic acid, in addition to fortified products [29]. Such supplements usually contain vitamin B$_{12}$, so individuals with low serum B$_{12}$ but high folate may have problems absorbing vitamin B$_{12}$, i.e. they may have pernicious anemia, and ensuing long-term negative effects on hematology or neurological function. Clearly, all of these mechanisms remain to be confirmed, logically with the inclusion of both animal models and clinical trials.

**Estimating the Appropriate Level of Folic Acid Addition to Flour**

Because of the low occurrence of NTDs (0.8/1,000 in the US to 14/1,000 in China, for example), it has been pointed out that relatively few NTDs are prevented relative to the number of people exposed to folic acid fortification [15]. For example, it has been estimated that increasing folic acid intake in the UK may prevent up to 162 NTDs/year in a population of 60 million people. Increasing intake by 0.2 mg/day would prevent 49 NTDs per year in Australia and 11 in New Zealand [30] out of a population of $\approx$27 million people. In the UK, were flour to be fortified with 300 μg/100 g, 77–162 NTDs/year would be prevented, but 370,000–780,000 people exposed to excess folic acid per NTD prevented [15]. Thus, it is important to ensure that the level of exposure of the general public to folic acid is not excessive as a result of fortification.

The underlying metabolic and genetic defects that cause NTDs are not completely understood, but it is apparent that low maternal folate status interacts with genetic and environmental risk factors to increase risk [31, 32]. Thus, folic acid fortification is more effective at reducing NTDs in population groups with poor folate status. The folate status of populations varies widely, and tends to be lower in industrialized countries than in poorer regions of the world where there is a relatively high intake of green leafy vegetables and legumes, which are good sources of the vitamin [33]. Countries such as Chile, the United States, Canada, and China had a low intake and generally poor folate status before fortification, and thus would be expected to benefit more from fortification. Moreover, it is clear that the reduction in NTDs after fortification was greatest in areas with the highest rates of NTD and poorer folate status (e.g. Newfoundland compared to Quebec and Manitoba in Canada, Northern China compared to the South, New England compared to the rest of the US) [34]. The question then arises about
whether it is reasonable to expect similar rates of NTD reduction in developing countries, where folate status might be adequate before fortification. For example, it has been estimated that serum folate needs to be 16 nmol/l for maximum prevention of NTDs, and that this can be achieved with a folic acid intake of 279 μg/day, assuming no other dietary intake [35]. It is rational to obtain data on folate status and intake in populations with a low intake of animal source foods (ASF; and/or vitamin B₁₂ deficiency) prior to assuming that folic acid fortification will have sufficient benefit to outweigh any potential harm.

**Vitamin B₁₂ Fortification**

There are reasons why vitamin B₁₂ fortification should be considered, with or without folic acid fortification, especially for the elderly and in populations with a low intake of ASF.

**Prevalence of Deficiency**

It is now apparent that the prevalence of vitamin B₁₂ deficiency (serum B₁₂ <148 pmol/l or <200 pg/ml) and depletion (serum B₁₂ ≥148 to <221 pmol/l) is high in people of all ages in populations with a low intake of ASF, since these are the only natural source of the vitamin. Based on 2005 data from the World Health Organization, the prevalence of B₁₂ deficiency in adults was highest in India (50%) and was about 5–30% in most other countries [36]. The prevalence of vitamin B₁₂ depletion is likely to be at least as high as that of deficiency; in studies in Latin America, for example, prevalence of serum B₁₂ <148 pmol/l is about 15–20%, while over 20% are depleted. This prevalence also applies to a nationally representative sample in Mexico [37]. In all developing country studies where prevalence was reported, serum vitamin B₁₂ concentrations were correlated with intake of the vitamin, or intake of ASF.

Vitamin B₁₂ deficiency has several well-documented adverse effects and is associated with many others. The severe deficiency that occurs in pernicious anemia, strict vegetarianism, and occasionally in elderly with malabsorption of the vitamin from food due to gastric atrophy can result in megaloblastic anemia. However, populations chronically deficient or depleted in the vitamin due to low ASF intake are much less likely to suffer from anemia as a consequence [38], nor does supplementation with B₁₂ affect any hematological symptoms. Vitamin B₁₂ status was a predictor of NTDs in Ireland where flour is not fortified with folic acid [39], and Canada after folic acid fortification of flour was introduced [40]. Neurological disorders including loss of memory, cognitive impairment [41] and rate of brain volume loss with aging [42, 43] and the severity of white matter lesions [44], and depression [Jones et al., unpubl. data] are all associated with poor vitamin B₁₂ status. The results of supplementation trials are not yet definitive for vitamin B₁₂ alone, but homocysteine lowering as a result of
supplementation with vitamin B₁₂ (0.5 mg/day), vitamin B₆ and folic acid significantly reduced the rate of brain volume loss with aging [44]. A potentially major but poorly documented problem is the low concentration of the vitamin in breast milk produced by mothers who are deficient or depleted in the vitamin [Deegan et al., unpubl. data], which is a concern since infants breastfed by deficient mothers have an increased risk of developmental delays, which may be permanent [45], including delayed motor development [46]. Poor vitamin B₁₂ status has also been implicated in bone loss [47] possibly as a result of hyperhomocysteinemia, although randomized controlled supplementation trials are needed for confirmation.

**Vitamin B₁₂ Fortification**

Based on the high prevalence of vitamin B₁₂ deficiency and depletion, and the confirmed and potential adverse effects of deficiency, it has been recommended that flour be fortified with 2 μg vitamin B₁₂/100 g flour in the form of cyanocobalamin in developing countries [48]. This level of addition is restricted by the relatively high cost of the vitamin; in fortification, it is recommended that the total cost of fortificants should not exceed 2% of the total cost of the product. Unfortunately, there are no large-scale fortification trials of the efficacy of this dose. We have conducted a small-scale pilot study of the bioavailability of the labeled vitamin from fortified bread rolls given to healthy adult volunteers, and found absorption from a 0.8 μg dose to be approximately 50%. The efficiency of absorption of the vitamin decreases substantially as intake increases, to about 30% from 5 μg and 5% with 20 μg; above about 25 μg only 1% will be absorbed, by passive transport [49]. There are no known adverse effects of high intakes of vitamin B₁₂ in supplements, or when taken intramuscularly.

Thus, it may be prudent policy to include both folic acid, at a reasonable level, and vitamin B₁₂ in flour fortification programs for developing countries. Many elderly are also likely to benefit in countries where ASF intake is higher, since vitamin B₁₂ deficiency is more common in this group presumably due to gastric atrophy and poor absorption of the vitamin from food. Clearly, trials of dual fortification should be conducted.

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23 Selhub J, Morris MS, Jacques PF: In vitamin B12 deficiency, higher serum folate is associated with increased total homocysteine and methylmalonic acid concentrations. Proc Natl Acad Sci USA 2007;104:19995–20000.


Discussion on Vitamin \( \text{B}_{12} \) and Folic Acid Fortification

Forty years after the discovery of vitamin \( \text{B}_{12} \), there is still much work left to be done. This connects also with Dr. Troen’s remarks on folic acid. The powerful effects of the randomized controlled trial that led to the decision in some countries to fortify flour with folic acid in order to prevent neural tube defects present additional challenges for interpretation. The message of those trials was not just that there needs to be better folic acid status in the preconceptional period in order to limit, even if not to altogether prevent, the incidence of neural tube defects. Another message of those trials is that we need to be much more concerned about the entire nutritional status of women in the periconceptional period. This isn’t just about folic acid, nor is it even just about those other micronutrients which may be involved in prevention of neural tube defects. We have heard throughout these few days that the maternal nutritional status before as well as during pregnancy and even after pregnancy is a very important target with really substantial public health implications much beyond the prevention of neural tube defects. Perhaps this returns us to the observation that was made by Dr. Jooste. I will paraphrase his story about ‘think globally and act locally’ to say we need to think critically globally and act locally upon a much better nutritional database than we currently have. The notion to me of a flour fortification initiative that says that we need to use data from a limited number of countries to make public health decisions about fortification or even levels of fortification throughout the world, without knowing nearly enough about the status of either folate or \( \text{B}_{12} \) or other factors, strikes me as not thinking critically globally. As Dr. Allen states, we have a need for a lot more information about \( \text{B}_{12} \) status around the world, and about folate status around the world, not just to consider the incidence of neural tube defects.

When asked to comment further on what we know about the efficiency of absorption of either folate or \( \text{B}_{12} \) from breast milk (since one mistake of the DRIs was to suggest that the absorption of breast milk folate was less efficient than the absorption of synthetic folic acid), Dr. Allen stated that it’s clear that
early in infancy the mechanism of absorption is different from later in infancy. Vitamin \(B_{12}\) is probably absorbed bound to haptocorrin early in infancy and then later there is enough intrinsic factor and peptic digestion for \(B_{12}\) to be absorbed as free \(B_{12}\). \(B_{12}\) is bound incredibly tightly to haptocorrin in milk, which is why the assay has been difficult. Interestingly, Dr. Lönnerdal showed that the haptocorrin reduces the growth of \textit{Escherichia coli} in the intestine, so that's another reason why it's also probably really important. She didn't know what percent is broken down and absorbed. Because the assays were wrong formerly, we underestimated requirements of those infants and breastfeeding women. The values are going to be double. There is a big study going on in Denmark where they don't have supplementation of women in pregnancy and lactation to get normal values for breast milk vitamin \(B_{12}\).

In response to Dr. Kalantari’s comment that the \(B_{12}\) content of breast milk is dependent on the daily diet of the mothers, Dr. Allen noted that one interesting thing about \(B_{12}\) is that the efficiency of absorption falls off quickly with increasing doses, even in the usual range of intakes. For example, she has just done a study with eggs labeled with isotopic \(B_{12}\), and you get half the absorption from two eggs as you do from one egg. So, it appears not to make any difference whether you eat one or two eggs. Even in the low range of intake, if you give a dose in one bolus, then you have poor efficiency of absorption, and so that's what we are doing when we give these high-dose supplements to lactating women. That's why when treating \(B_{12}\) deficiency, physicians give high doses, and that's because only 1% of those high doses are absorbed compared to 50% of really low doses. So, you really need a system with \(B_{12}\), where you are delivering it in small amounts during the day to have an effect. And then the other unknown is what comes from the mother's liver. Clearly, mother's status is related to infant's status and infant's liver content even 2 years after they were born. Perhaps some of the \(B_{12}\) in breast milk comes from stores and not from current intake. We are not certain about that with practically any nutrient, how much comes currently from the mother’s diet versus from stores that she already has. But she thinks the efficiency of absorption is the issue here.

Dr. Allen added one really important comment about folate and \(B_{12}\) that the folate concentration in breast milk is not much affected by maternal status. What happens is that the mother gets more and more depleted as lactation goes on if she is not replacing it from the diet, and the breast milk levels change hardly at all, so you don't get infant depletion as a result of low breast milk levels of folate, nor does high folic acid intake affect breast milk levels very much.

Dr. Penny asked whether Dr. Allen could give some idea about how much animal source food is needed and for how long in terms of amount for women and for children.

Dr. Allen replied that in Kenya we fed sort of the RDA as animal source foods as milk or meat to schoolers of whom about 80% were deficient or depleted. After a year, we had a substantial improvement, after 2 years the severe
deficiency went away, but we still had a lot of depletion and even deficient levels. In Guatemala, what I didn’t show were the results of this intervention study where we gave meat to these kids or the RDA as supplements, and that had absolutely no effect on their serum levels of $B_{12}$ over one meal a day feeding 70 g of beef or the equivalent of about a microgram of $B_{12}$. I don’t think it’s malabsorption because those that were drinking cow’s milk were doing just fine, and the cow’s milk had much higher levels of $B_{12}$ in it than we were giving. So, it’s hard to replete a $B_{12}$-deficient person once he/she is depleted, and we need to understand much more about that.

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Perhaps folic acid and vitamin B\textsubscript{12} have been orphan micronutrients for too long. Even though they are the last of the vitamins to have been identified, I think we have had over 60 years to gather more information about the status of these vitamins and their importance, not only in nutritional health but in child development. This challenge was put forward both by Lindsay Allen and Aron Troen. Aron Troen has shown us a number of examples of the significant extent to which our understanding of the biologic importance of these nutrients have advanced. The advances that we heard from Aron Troen on our understanding of the biology of folic acid and folic acid and vitamin B\textsubscript{12} interactions give us a very strong platform for application of knowledge about vitamin status to address some of the important current public health considerations. Lindsay Allen indicated there are more than 52 countries that are fortifying flour with folic acid. This is a remarkable demonstration of thinking globally but not thinking critically. As there is increasing information about those processes in human biology which are influenced by folic acid through methylation and epigenetics, we must be even more cognizant of local information that helps us make decisions about fortification and about interactions. Arguments put forward by Lindsay Allen suggesting that we have inadequate database about vitamin B\textsubscript{12} in a number of countries should raise the question about whether this is a micronutrient that deserves to be increased in the public health priority as a way of promoting health and approaching prevention of deficiency, and whether in fact fortification with vitamin B\textsubscript{12} does have as strong a scientific basis as the fortification with folic acid.

The matter of the interaction of the two vitamins has become more fascinating and current. These two nutrients were intertwined in their early history, and now we realize that we have these national experiments of fortification with folic acid (driven by experiences in North America with control of neural tube defects) in countries which did not even have information about the folic acid status, vitamin B\textsubscript{12} status, or even neural tube birth incidence. We have now the challenge of deciding to obtain information that will allow us to judge the
relative importance and relative risk of fortifying with folic acid in order to prevent neural tube defects in countries with minimal reporting data. While neural tube defect control can be demonstrated to be an important goal of folic acid fortification, the question remains, what is the exposure with regard to safety and interaction of the vitamins? We are talking about synthetic folic acid, not about food folate. We are now able to measure the circulating levels of unmetabolized folic acid and analyze some of these observations in terms of whether we are looking at the effects of folic acid per se or total folate intake in relation to vitamin B₁₂. The approaches that were presented in this session allow us to begin to look at these interactions in a way which is much more analytical in use of growing information about the fact that we actually can measure the status of these nutrients. This should help us put at a higher priority level the consideration of both decisions about folic acid and vitamin B₁₂, and that can be done with an increasing foundation of a solid and growing biological database.

Let me return again to the notion put forward by Pieter Jooste of ‘thinking globally but acting locally.’ This is very much a challenge with many if not all the micronutrients that we have considered throughout these few days. Certainly it’s true of the micronutrients that we considered today with regard to iodine, folate and vitamin B₁₂, and if we are to make our decisions about how to target our public health interventions, we are going to have to upgrade the level of information that we have in individual countries. We should not take as a global imperative or an initiative which is global, arguments for doing universal fortification. Sometimes that would be expedient when there may not be enough information from other sources. Lindsay Allen will remember and as will Richard Hurrell, the WHO consultation on Fortification [1] whose recommendations were clear that the decisions about fortification for any of these nutrients should be based to the extent possible on local information and public health priorities. Sometimes that local information is actually going to be regional. The points that Michael Zimmerman made in his discussion about iodine nutrition focus on ways in which we might improve the information on the infant or the neonatal child by using some new techniques of measurement such as steroid-stimulating hormones. There are ways forward with regard to being able to not only think locally on the basis of local information but to be able to actually define the target populations and whether the intervention that we have in mind is well suited to those target populations. Where a universal approach is not adequate to reach all the target populations, then we are clearly challenged to find ways to improve on the impact of whatever interventions we can do.

Both of the sessions this morning should challenge us to be much more cognizant of the nutritional status, nutritional intake, not just of women in pregnancy, not just women who are breastfeeding and not even perhaps just in the preconceptional period and women of childbearing age, but also that we heard a great deal of support for the importance of targeting even the adolescent women who in many countries will soon become mothers. Their nutritional status will
have a great deal of influence not only on birth outcomes but even on outcomes that can be projected to even later times in the life cycle. Thus, by focusing on a number of these nutrients such as we did in this session and throughout this meeting, we can mount some very strong arguments for targeting our intervention to the populations which are the important targets. I would hope that we can use some of this kind of information and this discussion, to which all have contributed so well, to have this publication lead to a list of important targets and recommendations that could have an influence on how we go forward with health and development programs.

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Meeting macro- and micronutrient requirements during pregnancy and early childhood is crucial for short- and long-term health and cognitive function. Meta-analyses confirm that supplementation or fortification of food with the ‘big four’ (vitamin A, iron, zinc, and iodine) is efficacious to reduce the risk of infectious disease and improves growth and cognitive outcome. More recently, folate and vitamin B₁₂ deficiencies during pregnancy have been shown to be associated with poor neurodevelopmental outcome and childhood obesity.

The papers collected in the book at hand address the fact that maternal and fetal deficiencies can induce inadequate metabolic programming in the offspring, with increased risk for non-communicable diseases later in life. World-renowned experts in the fields of epidemiology and nutritional intervention met with those in genetics, epigenetics, and metabolic outcome to clarify the pathogenesis of micronutrient deficiencies in pregnancy and childhood, preventive methods and strategies, and opportunities for treatment.