PART II

Evaluating the public health significance of micronutrient malnutrition

Introduction

The chapters in Part II of these guidelines provide more detailed background information on the prevalence, causes and health consequences of various micronutrient deficiencies, and review the available evidence regarding the benefits of their control. They are intended to assist planners not only in their evaluation of the micronutrient deficiency situation in their own country, but also to assess the need for, and potential benefits of, food fortification with specific micronutrients.

Chapter 3 looks at iron, vitamin A and iodine deficiencies, which, owing to their widespread occurrence globally, have received the most attention to date. A large amount of information is now available regarding the prevalence, the causes and the control of deficiencies in these three micronutrients. Various studies on the efficacy and effectiveness of interventions to control deficiencies in iron, vitamin A and iodine, are briefly described here (and in the opening chapter of this document; see section 1.3), but are reviewed in greater depth elsewhere (*73*). Chapter 4 focuses on a range of other micronutrients, which, in comparison, have hitherto been somewhat neglected. Deficiencies in at least some of these "neglected" micronutrients (i.e. in zinc, vitamins B_2 and B_{12}, niacin, vitamin D and calcium) are likely to be common throughout much of the developing world and among the poorest populations in the industrialized nations. Fortification provides a means of lowering the prevalence of deficiencies in all of these micronutrients, and their inclusion in mass fortification programmes, in particular, could produce significant public health benefits. Since there is less information about these micronutrient deficiencies in the literature, a concerted effort has been made to summarize what is known about them in these guidelines.

In both chapters, micronutrients are discussed in order of their perceived public health significance, and in each case the recommended or the most commonly used biochemical status indicators are critically reviewed. For some micronutrients, however, biochemical data reflecting nutritional status will be inadequate for assessing the prevalence of deficiencies. Suggestions for dealing with this situation, for example, by using food intake data to estimate the prevalence of inadequate intakes, are provided in Part IV of these guidelines (see section 7.3.2).

Other than a low dietary intake, important causes of MNM include poor bioavailability from foods (especially for minerals), frequent infection with parasites, diarrhoea, and various malabsorption disorders. The presence of any of these risk factors can lead to an underestimation of the prevalence of deficiency in a population if this is calculated on the basis of micronutrient intakes alone.

Risk factors for micronutrient malnutrition

- Monotonous diet resulting in low micronutrient intake, and poor bioavailability, especially of minerals.
- Low intake of animal source foods.
- Low prevalence of breastfeeding.
- Low micronutrient density of complementary foods.
- Increased physiological demands for growth during pregnancy and lactation.
- Increased demand due to acute infection (especially if infection episodes are frequent), chronic infection (e.g. tuberculosis, malaria and HIV/AIDS) and disease (e.g. cancer).
- Poor general nutritional status, in particular, protein–energy malnutrition.
- Malabsorption due to diarrhoea or the presence of intestinal parasites (e.g. *Giardia lamblia*, hookworms).
- Increased excretion (e.g. due to schistosomiasis).
- Seasonal variations in food availability, food shortages.
- Social deprivation, illiteracy, low education.
- Poor economic status and poverty.

CHAPTER 3

Iron, vitamin A and iodine

3.1 Iron deficiency and anaemia

Most of the iron in the human body is present in the erythrocytes as haemoglobin, where its main function is to carry oxygen from the lungs to the tissues. Iron is also an important component of various enzyme systems, such as the cytochromes, which are involved in oxidative metabolism. It is stored in the liver as ferritin and as haemosiderin.

Iron deficiency is the most common and widespread nutritional disorder in the world, and is a public health problem in both industrialized and non-industrialized countries. Iron deficiency is the result of a long-term negative iron balance; in its more severe stages, iron deficiency causes anaemia. Anaemia is defined as a low blood haemoglobin concentration. Haemoglobin cut-off values that indicate anaemia vary with physiological status (e.g. age, sex) and have been defined for various population groups by WHO (*1*).

3.1.1 Prevalence of deficiency

The terms, "iron deficiency" and "iron-deficiency anaemia" are often used synonymously although they are in fact not the same conditions. About 40% of the world's population (i.e. more than 2 billion individuals) is thought to suffer from anaemia, i.e. low blood haemoglobin (**see Table 1.1**). The mean prevalences among specific population groups are estimated to be:

— pregnant women, infants and children aged 1–2 years, 50%;

— preschool-aged children, 25%;

— schoolchildren, 40%;

— adolescents, 30–55%;

— non-pregnant women, 35%.

These average figures obscure the fact that iron deficiency and iron-deficiency anaemia are even more prevalent in some parts of the world, especially in the Indian subcontinent and in sub-Saharan Africa, where, for example, up to 90% of women become anaemic during pregnancy.

The prevalence of anaemia caused by iron deficiency, usually referred to as iron-deficiency anaemia, is less certain because the specific indicators of iron status, such as serum ferritin, transferrin saturation, zinc protoporphyrin and serum transferrin receptors, are measured less often than blood haemoglobin (**Table 3.1**). Most indicators of iron status – with the possible exception of serum transferrin receptors – are also affected by the presence of infection and can therefore be misleading (*74*). Indeed, every indicator listed in Table 3.1 has its own set of limitations, and so iron status is best assessed by a combination of indicators (*74*).

It is generally assumed that, on average, around 50% of the cases of anaemia are due to iron deficiency, as opposed to malaria (which causes anaemia because the malaria parasite destroys erythrocytes), the presence of infection or other nutrient deficiencies. However, the proportion is probably higher in infants and preschool-aged children than in older children or women (*75*), and is likely to vary by location. Although anaemia usually occurs when iron stores are depleted, the prevalence of iron deficiency will often be substantially higher than the prevalence of iron-deficiency anaemia. However, in iron-deficient populations with endemic malaria, the prevalence of anaemia will be greater than, or similar to, the prevalence of iron deficiency (*75*). Furthermore, the use of serum ferritin as an indicator of iron status may well *overestimate* the prevalence of iron deficiency in malaria endemic areas; this is because serum ferritin levels are elevated by the presence of infections such as malaria (Table 3.1), and also the reason why, traditionally, the cut-off level that defined iron deficiency in individuals with malaria was higher (<30 µg/l) than that used for individuals free from infection (<15 µg/l).

Anaemia is considered to be a public health problem when the prevalence of low haemoglobin concentrations exceeds 5% in the population (*1*). The severity of the public health problem of anaemia is classified as mild, moderate or severe according to the prevalence of anaemia (**Table 3.2**).

3.1.2 Risk factors for deficiency

The main risk factors for iron deficiency have been summarized in **Table 1.2**. They include:

- a low intake of haem iron (which is present in meat, poultry and fish);
- an inadequate intake of vitamin C (ascorbic acid) from fruit and vegetables (the presence of vitamin C enhances the absorption of iron from the diet);
- poor absorption of iron from diets high in phytate (including legumes and cereals) or phenolic compounds (present in coffee, tea, sorghum and millet);

TABLE 3.1

Indicators for assessing iron status at the population level[a]

Indicator	Sample	Population group	Cut-off to define deficiency		Comments
			Mild	Severe	
Haemoglobin[b]	Blood	Children 6–59 months	110 g/l	Not defined	Blood haemoglobin is primarily an indicator of anaemia but can provide useful information regarding iron status, as follows: — An increase of at least 10 g/l in blood haemoglobin after 1 or 2 months of iron supplementation is indicative of baseline iron deficiency. — Where poor availability of dietary iron is the main cause of anaemia, children and women have disproportionately low haemoglobin values, while those of adult men are virtually unaffected. Where other factors, such as parasites, contribute significantly, adult men are more likely to also have low haemoglobin values.
		Children 5–11 years	115 g/l		
		Children 12–14 years	120 g/l		
		Men over 15 years	130 g/l		
		Women over 15 years (non-pregnant)	120 g/l		
		Pregnant women	110 g/l	<70 g/l	
Ferritin	Serum or plasma	Under 5 years	<12 µg/l	Not defined	Useful indicator of iron status and also for monitoring interventions for iron deficiency.
		Over 5 years	<15 µg/l	Not defined	Reflects total body iron stores and is decreased in deficient subjects. Elevated in the presence of infection or inflammatory process and should thus be measured, if possible, in combination with another acute phase protein (CRP or AGP), which indicate the presence of infection. Levels of >200 µg/l in adult males (or 150 µg/l in adult females) indicates severe risk of iron overload.

TABLE 3.1

Indicators for assessing iron status at the population level[a] (*Continued*)

Indicator	Sample	Population group	Cut-off to define deficiency		Comments
			Mild	Severe	
Transferrin receptors	Serum	Can be applied to all population groups	Cut-off values vary with method used		Useful indicator of iron status; not affected by infection and thus can be used in combination with measurement of serum ferritin to confirm deficiency in cases of infection. No universally agreed cut-offs; reference materials still need to be standardized.
Transferrin saturation	Serum	Can be applied to all population groups	<16%	Not defined	Pronounced diurnal variation and not very specific. Elevated in the presence of infection. No universally agreed cut-offs.
Erythrocyte protoporphyrin	Erythrocytes (RBC)	Under 5 years Over 5 years	Normal Normal	>70 µg/dl >80 µg/dl	Elevated when iron supply is inadequate for haem production. Elevated in the presence of infection, lead poisoning and haemolytic anaemia.

AGP, Alpha 1 acid glycoprotein; CRP, C-reactive protein; RBC, red blood cell.

[a] Every indicator of iron status has limitations so the best way to assess iron status is to use a combination of indicators.

[b] Haemoglobin values for populations living at sea level require adjustment for selected variables, including altitude and tobacco consumption.

Sources: reference (*1,74*).

TABLE 3.2

Criteria for assessing the public health severity of anaemia

Severity of the public health problem	Prevalence of anaemia[a] (% of the population)
None	≤4.9
Mild	5.0–19.9
Moderate	20.0–39.9
Severe	≥40

[a] Anaemia is defined on the basis of blood haemoglobin concentrations (see **Table 3.1**)

Source: reference (*1*).

TABLE 3.3

Classification of usual diets according to their iron bioavailability

Category	Iron bioavailability (%)	Dietary characteristics
Low	1–9	Simple, monotonous diet based on cereals, roots or tubers, with negligible amounts of meat, fish, poultry or ascorbic acid-rich foods. Diet high in foods that inhibit iron absorption such as maize, beans, whole wheat flour and sorghum.
Intermediate	10–15	Diet of cereals, roots or tubers, with some foods of animal origin (meat, fish or poultry) and/or containing some ascorbic acid (from fruits and vegetables).
High	>15	Diversified diet containing greater amounts of meat, fish, poultry and/or foods high in ascorbic acid.

Sources: adapted from references (*78,79*).

- periods of life when iron requirements are especially high (i.e. growth and pregnancy);
- heavy blood losses as a result of menstruation, or parasite infections such as hookworm, ascaris and schistosomiasis.

As mentioned above, acute or chronic infections, including malaria, can also lower haemoglobin concentrations (*76*). The presence of other micronutrient deficiencies, especially of vitamins A and B_{12}, folate and riboflavin, also increases the risk of anaemia (*77*).

The dietary habits of a population group strongly affect the bioavailability of both dietary iron and added fortificant iron. Estimates of the average bioavailability of iron from different types of diets are provided in **Table 3.3**. Although the efficiency of iron absorption increases substantially as iron stores become

depleted, the amount absorbed from foods, especially where diets are low in meat, fish, fruit and vegetables, is not enough to prevent iron deficiency in many women and children, especially in the developing world.

3.1.3 Health consequences of deficiency and benefits of intervention

The main consequences of iron deficiency are anaemia, impaired cognitive and physical performance, and increased maternal and child mortality (see **Table 1.2**). Iron deficiency has been shown to reduce physical endurance, even in the absence of anaemia (*80*), and severe anaemia has been associated with an increased risk of both maternal and child mortality (*81,82*). As indicated previously (see section 1.1), there is now substantial evidence to suggest that iron supplementation can reverse the adverse effects of iron deficiency on work capacity and productivity, and on pregnancy outcome and child development (*14–16*). In a study in the United States, for example, iron supplementation during pregnancy reduced the number of preterm deliveries and low-birth-weight infants (*83*).

Improving iron status may have other, but as yet poorly appreciated, benefits for health, most noticeably with respect to the utilization of vitamin A and iodine. That vitamin A (retinol) is mobilized from the liver by an iron-dependent enzyme is well-established fact, but more recently, experimental studies have suggested that in cases of iron deficiency the vitamin is trapped in the liver and thus may be less accessible to other tissues and organs (*84*). Furthermore, iron supplementation of iron-deficient individuals increased plasma retinol in some studies through mechanisms that are as yet incompletely understood (*85*). Similarly, iron is required by the enzymes that synthesize thyroxine, and thus a low iron status may have implications for iodine metabolism. Studies in Côte d'Ivoire have demonstrated that recovery from goitre after iodine treatment is slower in iron-deficient individuals (*86*). In a population of children with a high prevalence of anaemia and goitre, iron supplementation improved the response to iodized oil or iodized salt (*87*) (see also section 1.3.2.3). On the basis of the above findings, it is reasonable to assume that improvements in the iron status of a population may well have benefits for vitamin A and iodine metabolism.

3.2 Vitamin A

Vitamin A is an essential nutrient that is required in small amounts by humans for the normal functioning of the visual system, the maintenance of cell function for growth, epithelial cellular integrity, immune function and reproduction. Dietary requirements for vitamin A are normally provided as a mixture of preformed vitamin A (retinol), which is present in animal source foods, and provitamin A carotenoids, which are derived from foods of vegetable origin and which

have to be converted into retinol by tissues such as the intestinal mucosa and the liver in order to be utilized by cells.

Aside from the clinical ocular signs, i.e. night blindness and xerophthalmia, symptoms of vitamin A deficiency (VAD) are largely non-specific. Nevertheless, accumulated evidence suggests that VAD is an important determinant of child survival and safe motherhood (see section 3.2.3). The non-specificity of symptoms, however, means that, in the absence of biochemical measures of vitamin A status, it is difficult to attribute non-ocular symptoms to VAD and it also complicates the definition of VAD. With these considerations in mind, WHO has defined VAD as tissue concentrations of vitamin A low enough to have adverse health consequences, even if there is no evidence of clinical xerophthalmia (*5*). In more recent years, the term "vitamin A deficiency disorders" has been coined to reflect the diversity of adverse outcomes caused by vitamin A deficiency (*88*).

3.2.1 Prevalence of deficiency

As vitamin A deficiency affects visual function, indicators of vitamin A status have traditionally relied on changes in the eye, specifically night blindness and xerophthalmia (*5*) **(Table 3.4)**. Worldwide, about 3 million preschool-aged children present ocular signs of VAD (*3*). Vitamin A deficiency is, however, more commonly assessed using serum or plasma retinol levels. WHO estimates that 254 million preschool-aged children throughout the world have low serum retinol levels and can therefore be considered to be clinically or subclinically vitamin A deficient (*3*). In the developing world, prevalence rates in this age group range from 15% up to as high as 60%, with Latin America, the Eastern Mediterranean and the Western Pacific being at the low end of this range, and Africa and South-East Asia occupying the high end (*3,89*) (see also Table 1.1). The prevalence of night blindness is also high among pregnant women in many poor regions of the world, with rates varying between 8% and 24% (*89*). Night blindness tends to be accompanied by a high prevalence of low concentrations of retinol in breast milk (<1.05 µmol/l or 30 µg/dl) (*89,90*).

According to WHO criteria (*5*), a greater than 1% prevalence of night blindness in children aged 24–71 months, or the presence of serum retinol concentrations of less than 0.70 µmol/l in 10% or more of children aged 6–71 months indicates a public health problem **(Table 3.5)**. It has been suggested recently that a prevalence of night blindness of more than 5% in pregnant women should be added to the list of criteria that signify a public health problem (*88*).

3.2.2 Risk factors for deficiency

Usually vitamin A deficiency develops in an environment of ecological, social and economical deprivation, in which the key risk factors for vitamin A

TABLE 3.4

Indicators for assessing vitamin A status at the population level

Indicator	Sample	Population group	Cut-off to define deficiency		Comments
			Mild	Severe	
Prevalence of night blindness (%)	Clinical examination	Children 6–71 months	>1%	>5%	
		Pregnant women	>5%	Not defined	Night blindness prevalence is assessed by interview about reported occurrence during last pregnancy.
Retinol	Serum or plasma[a]	Preschool-age children	0.35–0.7 µmol/l	<0.35 µmol/l	Good indicator of vitamin A status at population level. Also depressed by infection.
Retinol	Breast milk	Lactating women	<1.05 µmol/l (<87 µg/g milk fat)	Not defined	Directly related to the vitamin A status of the mother. Provides information about the vitamin A status of both the mother and her breast-fed infant. Should be measured after the first month postpartum, i.e. once the milk composition has become stable.

[a] Ethylene diamine tetraacetic acid (EDTA) should not be used as the anticoagulant.

Sources: references (*5,91*).

TABLE 3.5
Criteria for assessing the public health severity of vitamin A deficiency

Indicator	Population group	Prevalence indicating a public health problem (% of the population)
Night blindness	Pregnant women	>5
Night blindness	Children 24–71 months	>1
Bitot's spots	Children 24–71 months	>0.5
Serum retinol <0.7 µmol/l (<20 µg/dl)	Children 6–71 months	≥10

Sources: references (*5,88*).

deficiency are a diet low in sources of vitamin A (i.e. dairy products, eggs, fruits and vegetables), poor nutritional status, and a high rate of infections, in particular, measles and diarrhoeal diseases (see **Table 1.2**).

The best sources of vitamin A are animal source foods, in particular, liver, eggs and dairy products, which contain vitamin A in the form of retinol, i.e in a form that can be readily used by the body. It is not surprising then that the risk of vitamin A deficiency is strongly inversely related to intakes of vitamin A from animal source foods. In fact, it is difficult for children to meet their requirements for vitamin A if their diet is low in animal source foods (*92*), especially if their diet is also low in fat. Fruits and vegetables contain vitamin A in the form of carotenoids, the most important of which is β-carotene. In a mixed diet, the conversion rate of β-carotene to retinol is approximately 12:1 (higher, i.e. less efficient than previously believed). The conversion of the other provitamin-A carotenoids to retinol is less efficient, the corresponding conversion rate being of the order of 24:1 (*91,93*). Various food preparation techniques, such as cooking, grinding and the addition of oil, can improve the absorption of food carotenoids (*94–96*). Synthetic β-carotene in oil, which is widely used in vitamin A supplements, has a conversion rate to retinol of 2:1, and the synthetic forms of β-carotene that are commonly used to fortify foods, a conversion rate of 6:1 (*93*).

3.2.3 Health consequences of deficiency and benefits of intervention

Vitamin A deficiency is the leading cause of preventable severe visual impairment and blindness in children, and significantly increases their risk of severe illness and death. An estimated 250 000–500 000 vitamin A-deficient children become blind every year, approximately half of which die within a year of becoming blind. Subclinical vitamin A deficiency is also associated with an increased risk of child mortality, especially from diarrhoea and measles. A meta-analysis demonstrated that high dose vitamin A supplementation can reduce mortality from measles by as much as 50%. Another analysis found that

improvement of vitamin A status, whether by supplementation or fortification, decreased all-cause mortality in children aged between 6 months and 5 years by 23% (*12*).

In addition to causing night blindness, vitamin A deficiency is probably an important contributor to maternal mortality and other poor outcomes in pregnancy and lactation. According to the results of one study, in which vitamin A-deficient pregnant women received vitamin A or β-carotene supplements at doses equivalent to their weekly requirement for the vitamin, maternal mortality was reduced by 40% and 49%, respectively, relative to a control group (*97*). Other studies have shown night blindness to be a risk factor for maternal mortality and morbidity: in Nepal, for example, the death rate from infections was about five times higher among unsupplemented pregnant women who reported night blindness compared with those who did not (*98*). Vitamin A deficiency also increases vulnerability to other disorders, such as iron deficiency (*see section 3.1.3*). Providing an iron supplement with vitamin A to pregnant women in Indonesia increased haemoglobin concentrations by approximately 10 g/l more than did supplementation with iron alone (*99*).

3.3 Iodine

Iodine is present in the body in minute amounts, mainly in the thyroid gland. Its only confirmed role is in the synthesis of thyroid hormones. Iodine deficiency is a major public health problem for populations throughout the world, but particularly for young children and pregnant women, and in some settings represents a significant threat to national social and economic development. The most devastating outcome of iodine deficiency is mental retardation: it is currently one of the world's main causes of preventable cognitive impairment. This is the primary motivation behind the current worldwide drive to eliminate iodine deficiency disorders (IDD).

3.3.1 Prevalence of deficiency

The recommended indicators for assessing the extent of iodine deficiency within a population are median urinary iodine and total goitre prevalence (**Table 3.6**). According to generally accepted criteria, iodine deficiency is a public health problem in populations where the median urinary iodine concentration is below 100 µg/l, or in areas where goitre is endemic, that is to say, where more than 5% of children aged 6–12 years have goitre (**Table 3.7**).

As the median urinary iodine concentration reflects current iodine intake and responds relatively rapidly to the correction of iodine deficiency, it is usually the preferred indicator for monitoring the impact of interventions for IDD control. An expanded set of indicators for assessing national progress towards the goal of the sustainable elimination of IDDs is given in Annex A. This indicator set,

TABLE 3.6

Indicators for assessing iodine status at the population level

Indicator	Sample	Population group	Cut-off to define deficiency		Comments
			Mild	Severe	
Iodine	Urine	Children 6–12 years	Median <100 µg/l	Median <20 µg/l	Recommended indicator for monitoring or evaluating iodine status at the population level. As urinary iodine distribution is not normal, cut-off is defined on the basis of median values.
Total goiter prevalence	Clinical examination	Children 6–12 years	>5%	>30%	Reflects past or current thyroid dysfunction and can be measured by clinical examination or by ultrasonography. Not recommended for monitoring the impact of interventions as goitre response to iodine status correction is delayed.

Source: reference (*6*).

TABLE 3.7
Criteria for assessing the public health severity of iodine deficiency

Severity of public health problem	Indicator	
	Median urinary iodine (µg/l)	Total goitre prevalence (%)
Mild	50–99	5.0–19.9
Moderate	20–49	20–29.9
Severe	<20	>30

Source: reference (*6*).

which has been recommended by WHO, relates not just to the population's iodine status (as measured by urinary concentrations) but includes various programmatic indicators which measure the sustainability of the salt iodization programme itself.

According to recent WHO estimates, some 1989 million people have inadequate iodine nutrition (*2*). The WHO regions, ranked by the absolute number of people affected are, in decreasing order of magnitude, South-East Asia, Europe, the Western Pacific, Africa, the Eastern Mediterranean and the Americas (**see Table 1.1**). In some parts of the world, for example, in parts of eastern and western Europe, iodine deficiency, in its subclinical form, is re-emerging, having previously been eliminated. This underscores the need to sustain efforts to control iodine deficiency on a global scale.

3.3.2 Risk factors for deficiency

The main factor responsible for the development of iodine deficiency is a low dietary supply of iodine (*100*). This tends to occur in populations living in areas where the soil has been deprived of iodine as the result of past glaciation, and subsequently, because of the leaching effects of snow, water and heavy rainfall.

Iodine deficiency is exacerbated by a high consumption of natural goitrogens that are present in some staple foods such as cassava. The antithyroid action of goitrogens is related to the presence of thiocyanate which inhibits thyroid iodide transport and, at higher doses, competes with iodide in the synthesis of thyroid hormones (*101*). Goitrogenicity is determined by the balance between the dietary supply of iodine and thiocyanate: goitre develops when the urinary iodine (µg): thiocyanate (mg) ratio falls below 3.

3.3.3 Health consequences of deficiency and benefits of intervention

Iodine deficiency is associated with a large range of abnormalities, grouped under the heading of "iodine deficiency disorders", that reflect thyroid

TABLE 3.8
The spectrum of iodine deficiency disorders

Fetus	Abortions
	Stillbirths
	Congenital abnormalities
Neonate	Increased infant mortality
	Cognitive impairment and neurological disorders, including endemic cretinism and endemic mental retardation
	Hypothyroidism
	Increased susceptibility of the thyroid gland to nuclear radiation
Child, adolescent and adult	Hypothyroidism
	Goitre
	Retarded physical development in child and adolescent
	Impaired mental function
	Decreased fertility
	Iodine-induced hyperthyroidism in adults
	Increased susceptibility of the thyroid gland to nuclear radiation
	Spontaneous hyperthyroidism in the elderly
	Goitre with its complications

Source: adapted from reference (*9*).

dysfunction (*9*). Goitre and cretinism are the most visible manifestations of iodine deficiency; others include hypothyroidism, decreased fertility rate, increased perinatal death and infant mortality (**Table 3.8**).

When iodine intake is abnormally low, an adequate production of thyroid hormones may still be achieved by increased secretion of thyroid stimulating hormone (TSH). However, a prolonged stimulation of the thyroid gland by TSH will result in goitre. This condition is indicative of thyroid hyperplasia, which occurs because of the thyroid's inability to synthesize sufficient thyroid hormones.

Irreversible mental retardation is the most serious disorder induced by iodine deficiency (*9,102,103*). A deficit in iodine resulting in thyroid failure during the critical period of brain development, that is, from fetal life up to the third month after birth, will result in irreversible alterations in brain function (*104,105*). In areas of severe endemic iodine deficiency, cretinism may affect up to 5–15% of the population. Some individuals living in regions of mild or moderate iodine deficiency exhibit neurological and intellectual deficits that are similar to, but less marked, than those found in overt cretins. A meta-analysis of 19 studies conducted in regions of severe deficiency showed that iodine deficiency is responsible for a mean IQ loss of 13.5 points among affected populations (*104*).

Correction of iodine deficiency, when carried out at the right time, reduces or eliminates all consequences of iodine deficiency. The validity of this statement is borne out by the sharp reduction in the incidence of IDD that is consistently observed when iodine is added to the diet (*see section 1.3*), and the recurrence of IDD when an effective IDD control programme is interrupted in a previously iodine-deficient population (*106*).

CHAPTER 4

Zinc, folate, vitamin B_{12} and other B vitamins, vitamin C, vitamin D, calcium, selenium and fluoride

4.1 Zinc

Zinc is an essential component of a large number of enzymes, and plays a central role in cellular growth and differentiation in tissues that have a rapid differentiation and turnover, including those of the immune system and those in the gastrointestinal tract. The positive impact of zinc supplementation on the growth of some stunted children, and on the prevalence of selected childhood diseases such as diarrhoea, suggests that zinc deficiency is likely to be a significant public health problem, especially in developing countries. However, the extent of zinc deficiency worldwide is not well documented. All population age groups are at risk of zinc deficiency, but infants and young children are probably the most vulnerable. Pregnant and lactating women are also likely to be very susceptible to zinc deficiency, and there is an urgent need for more information on the implications of low zinc status in these particular population groups (*107,108*).

4.1.1 Prevalence of deficiency

The lack of reliable and widely accepted indicators of zinc status of adequate sensitivity means that the global prevalence of zinc deficiency is uncertain. Those indicators that are available, such as zinc concentration in plasma and hair (see **Table 4.1**), detect changes in zinc status only in cases of severe deficiency, and may fail to detect marginal deficiency.

As suggested above, there are, however, several good reasons to suspect that zinc deficiency is common, especially in infants and children. Firstly, a high prevalence of low plasma zinc, which is a reasonable indicator of relatively severe depletion, has been observed in some population groups. Secondly, several randomized control trials have demonstrated that stunted children, and/or those with low plasma zinc, respond positively to zinc supplementation, a finding that suggests that zinc deficiency was a limiting factor in their growth. Growth stunting affects about a third of children in less wealthy regions of the world and is very common in settings where diets are of poor quality. This is not too say that zinc deficiency affects up to one third of children in the developing world since zinc deficiency is only but one of several possible causes of growth stunting.

TABLE 4.1

Indicators for assessing zinc status at the population level

Indicator	Sample	Population group	Cut-off to define deficiency	Comments
Zinc	Serum or plasma	Applies to all population groups	<70 µg/dl	No universally agreed cut-offs. Plasma zinc is homeostatically regulated and therefore may not detect marginal deficiency. Values change diurnally. Plasma zinc is decreased by pregnancy, hypoalbuminemia (PEM) and infection.
Zinc	Erythrocytes (RBC)	Applies to all population groups	No universally agreed cut-offs at this time	May be used as a secondary supportive indicator.
Zinc	Hair	Applies to all population groups	No universally agreed cut-offs at this time	Needs further research before this can be used as a supportive indicator. Not widely used as an indicator in population surveys.

PEM, protein energy malnutrition; RBC, red blood cell.

Sources: references (*91,93*).

Using estimates of zinc intake and bioavailability derived from FAO's food balance data, it has been calculated that about 20% of the world's population could be at risk of zinc deficiency. The geographical regions most affected are believed to be, in descending order of severity, south Asia (in particular, Bangladesh and India), Africa and the western Pacific (*109*). It is probable that the occurrence of zinc deficiency is strongly associated with that of iron deficiency, because both iron and zinc are found in the same foods (i.e. meat, poultry and fish) and, in both cases, their absorption from foods is inhibited by the presence of phytates. The minerals differ in that zinc is not as affected by blood loss as is iron.

4.1.2 Risk factors for deficiency

The central role of zinc in cell division, protein synthesis and growth means that an adequate supply is especially important for infants, and pregnant and lactating women. Principal risk factors for zinc deficiency include diets low in zinc or high in phytates, malabsorption disorders (including the presence of intestinal parasites and diarrhoea), impaired utilization of zinc and genetic diseases (e.g. acrodermatitis enteropathica, sickle-cell anaemia) (**Table 1.2**).

The bioavailability of zinc is dependent on dietary composition, in particular, on the proportion of high-phytate foods in the diet (i.e. selected cereals and legumes). The molar ratio of phytate:zinc in meals or diets provides a useful measure of zinc bioavailability. At high ratios (i.e. above 15 : 1), zinc absorption from food is low, that is to say, less than 15% (*110,111*). The inclusion of animal proteins can improve the total zinc intake and the efficiency of zinc absorption from a phytate-containing diet (*112*). For instance, the addition of animal source foods to a diet based on rice and wheat approximately doubled the amount of zinc that was absorbed by young Chinese women (*113*). Using data obtained from experimental zinc absorption studies, various criteria have been developed to differentiate between diets likely to have high, moderate and low zinc bioavailability; these are summarized in **Table 4.2**.

The extent to which the presence of phytates inhibits the absorption of zinc is not precisely known at the present time. It is interesting to note that several studies have shown that zinc absorption from some legume-based diets is comparable to that from a diet based on animal products, despite the relatively high phytate content of the former (*112,114*), and that in adult women, approximately 30% of dietary zinc is absorbed across a wide range of different diets (*93*). In a controlled experiment, infants absorbed nearly 45% of the zinc from a wheat-soy complementary food, regardless of whether it contained 0.77% or 0.3% phytic acid (*115*). In Malawi, 24% of the zinc was absorbed from high-phytate maize meals consumed by children, again a relatively high proportion given the phytate content (*116*).

Competitive interactions can occur between zinc and other minerals that have similar physical and chemical properties, such as iron and copper. When present in large amounts (e.g. in the form of supplements) or in aqueous solution, these minerals reduce zinc absorption. However, at the levels present in the usual diet and in fortified foods, zinc absorption is not generally affected (*93*). On the other hand, high levels of dietary calcium (i.e. >1 g per day), which might be consumed by some individuals, can inhibit zinc absorption, especially in the presence of phytates. The degree of impairment varies depending on the type of diet and the source of the calcium (*93*). Unlike iron, zinc absorption is neither inhibited by phenolic compounds, nor enhanced by vitamin C.

TABLE 4.2

Classification of usual diets according to the potential bioavailability of their zinc content

Bioavailability[a]	Main dietary characteristics
High	Refined diets low in cereal fibre, low in phytic acid content, and with a phytate : zinc molar ratio <5; adequate protein content principally from non-vegetable sources, such as meats and fish. Includes semi-synthetic formula diets based on animal protein.
Moderate	Mixed diets containing animal or fish protein. Lacto-ovo, ovovegetarian or vegan diets not based primarily on unrefined cereal grains or high-extraction-rate flours. Phytate : zinc molar ratio of total diet within the range 5–15 or not in excess of 10 if more than 50% of energy intake is from unfermented unrefined cereal grains and flours, and the diet is fortified with inorganic calcium salts (>1 g Ca^{2+}/day). Bioavailability of zinc improves when the diet includes animal protein sources (including milk).
Low	Diets high in unrefined, unfermented and ungerminated cereal grains[b], especially when fortified with inorganic calcium salts and when intake of animal protein is negligible. Phytate: zinc molar ratio of total diet exceeds 15[c]. High-phytate soy protein products constitute the primary protein source. Diets in which, singly or collectively, approximately 50% of the energy intake is from the following high-phytate foods: high-extraction-rate (≥90%) wheat, rice, maize, grains and flours, oatmeal, and millet; chapatti flours and *tanok*; and sorghum, cowpeas, pigeon peas, grams, kidney beans, blackeyed beans, and groundnut flours. High intakes of inorganic calcium salts (>1 g Ca^{2+}/day), either as supplements or as adventitious contaminants (e.g. from calcareous geophagia), potentiate the inhibitory effects; low intakes of animal protein exacerbate these effects.

[a] At intakes adequate to meet the average normative requirements for absorbed zinc the three bioavailability levels correspond to 50%, 30% and 15% absorption. With higher zinc intakes, the fractional absorption is lower.

[b] Germination of such grains or fermentation of many flours can reduce antagonistic potency; if cereal grains have been germinated then the diet should then be classified as having moderate zinc bioavailability.

[c] Vegetable diets with phytate:zinc ratios >30 are not unknown; for such diets, an assumption of 10% bioavailability of zinc or less may be justified, especially if the intake of protein is low, or the intake of inorganic calcium salts is excessive, or both.

Source: reference (*93*).

The influence of all of the above-mentioned risk factors for zinc deficiency is difficult to integrate in any coherent way. In particular, further research is needed to evaluate the bioavailability of zinc from usual diets in developing countries and to better understand the relationship between dietary patterns and zinc supply.

4.1.3 Health consequences of deficiency and benefits of intervention

Zinc deficiency is often hard to identify as its clinical manifestations are largely non-specific (**Table 1.2**). The symptoms of severe deficiency include dermatitis, retarded growth, diarrhoea, mental disturbances and recurrent infections. Moderate and mild deficiencies are even more difficult to diagnose, not only because they are characterized by a diversity of symptoms, but also on account of the fact that there are no suitable biomarkers of zinc deficiency (*117*).

In children, impaired growth (stunting) is one of the possible consequences of zinc deficiency. Zinc supplementation trials conducted over the last few decades in children from developing countries have clearly demonstrated the positive benefits of improved zinc status, including improved growth rates and reductions in the incidence of various infectious diseases (*17,18,118*). For example, a meta-analysis of randomized controlled supplementation trials reported an 18% decrease in diarrhoea incidence, a 25% reduction in diarrhoea prevalence, and a 41% fall in the incidence of pneumonia (*18*). Zinc supplementation also led to fewer episodes of malaria and fewer clinic visits due to complications of malaria in Papua New Guinea (*118*), but not in Burkina Faso (*119*).

The effect of maternal zinc status on pregnancy outcomes is unclear at the present time (*120*). Although severe zinc deficiency has been associated with poor maternal pregnancy outcomes (*121*), studies involving moderate deficiency have proved inconclusive (*122*). Maternal zinc supplementation in Peru improved fetal neurobehavioral development (*123*), but had no effect on size at birth or pregnancy duration (*124*). In India, zinc supplements helped to reduce mortality among low-birth-weight infants (*125*). Interestingly, the zinc content of breast milk has not been shown to correlate with maternal zinc intake and appears to be unaffected by supplementation (*126,127*).

4.2 Folate

Folate (vitamin B_9) plays a central role in the synthesis and methylation of nucleotides that intervene in cell multiplication and tissue growth. Its role in protein synthesis and metabolism is closely interrelated to that of vitamin B_{12}. The combination of severe folate deficiency and vitamin B_{12} deficiency can result in megaloblastic anaemia. Low intakes of folate are also associated with a higher risk of giving birth to infants with neural tube defects and possibly other birth defects, and with an increased risk of cardiovascular diseases, cancer and impaired cognitive function in adults.

4.2.1 Prevalence of deficiency

Serum folate is a good indicator of recent dietary folate intake, and the most widely used method of assessing folate status (*128*). Erythrocyte folate is,

however, the better indicator of long-term status and of tissue folate stores. Elevated plasma homocysteine concentrations are a strong predictor of inadequate folate status. However, other vitamin deficiencies (e.g. vitamins B_2, B_6 and B_{12}) also increase homocysteine values. Indicators of folate status are summarized in **Table 4.3** (*93,128,129*).

The global prevalence of folate deficiency is uncertain, owing to a lack of data (*130*). Only a few countries have national or even regional biochemical data on folate status. Furthermore, efforts to compare usual dietary intakes with estimated requirements (an alternative means of assessing the likely prevalence of deficiency in a population) are hampered by difficulties in measuring the folate content of foods.

TABLE 4.3

Indicators for assessing folate (vitamin B_9) status at the population level

Indicator	Sample	Population group	Cut-off to define deficiency	Comments
Folate	Serum	Applies to all population groups	<10 nmol/l (4.4 µg/l)	Serum folate is the most widely used indicator of folate status. It is considered to be a sensitive indicator of recent intake, but a less valid indicator of body stores.
Folate	Erythrocytes (RBC)	Applies to all population groups	<305 nmol/l (140 µg/l)	Erythrocyte folate concentrations reflect long-term folate status and tissue folate stores.
Total homocysteine (free and bound)	Plasma	Applies to all population groups	12–16 µmol/l (1.62–2.2 mg/l)	Total plasma homocysteine is a good predictor of folate status: it is increased in cases of inadequate folate status. Not specific because also increased by vitamin B_2, B_6 and B_{12} deficiencies and influenced by gender, race and renal insufficiency.

RBC, red blood cell.

Sources: references (*93,128,129*).

Folate deficiency tends to be more prevalent in populations that have a high intake of refined cereals (which are low in folate) and a low intake of leafy greens and fruits (which are high in folate). Dietary surveys in India show that people eating predominantly cereal-based diets only consume about 75 µg folate per day (*131*). Prior to the introduction of mandatory wheat flour fortification with folic acid in 1998, about 15% of adult women in the United States were believed to have low serum and/or erythrocyte folate levels. Similarly, in Chile, where the consumption of white wheat flour is high, low serum and erythrocyte folate concentrations were common before the fortification of flour with folic acid (*132*). In contrast, low plasma values are rare in countries such as Guatemala, Mexico and Thailand (*77*) where diets typically contain a higher proportion of fruits and vegetables. For instance, few whole blood samples from the Mexican National Nutrition Survey were low in folate, with the exception of those of children under 4 years of age, in which the prevalence of low blood folate was about 10% (*133*). Because of the high folate content of certain legumes, fruits and vegetables relative to refined cereals, it is possible that populations in some developing countries consume more folate than those in industrialized countries. Similarly, a study of pregnant women in Germany found that those who are lacto-ovo vegetarians (i.e. milk and egg consumers) or low meat consumers had higher levels of erythrocyte folate than the non-vegetarians; this was attributed to the fact that the lacto-ovo vegetarians were consuming proportionately more folate-rich vegetables than their non-vegetarian counterparts (*134*).

4.2.2 Risk factors for deficiency

The main sources of dietary folate are leafy green vegetables, fruits, yeast and liver. A low intake of these foods combined with a relatively high intake of refined cereals thus increases the risk for folate deficiency. Malabsorption conditions, infection with *Giardia lamblia,* bacterial overgrowth, genetic disorders (of folic acid metabolism) and chronic alcoholism are also risk factors for folate deficiency (see **Table 1.2**).

4.2.3 Health consequences of deficiency and benefits of intervention

Possible health consequences of a low folate status, which include megaloblastic anaemia, are summarized in Table 1.2. Folic acid has long been included in iron supplements provided to pregnant women in developing countries, despite rather limited evidence from Africa and India that folic acid reduces the risk of megaloblastic anaemia. In fact, there is little evidence to suggest that giving folic acid with iron is any better at preventing anaemia than providing iron alone (*77,135*).

Randomized trials conducted in China (*136*), the United States (*137*) and in various other locations have consistently shown that folic acid supplements taken

before and during the first 28 days after conception reduce the risk of women giving birth to an infant with a neural tube defect (*138*). Neural tube defects are serious malformations resulting in death or major lifelong disability in survivors; worldwide, an estimated 300 000 or more neonates are affected each year (*139*). Studies have also demonstrated that folic acid supplementation benefits some women who have an abnormal folate metabolism because of a genetic defect that affects their ability to utilize folate (*140*). Moreover, an analysis of data from different trials in which micronutrients were provided during pregnancy found folic acid to be the only micronutrient that was associated with a reduced risk of preterm delivery (*141*).

Several intervention trials have demonstrated that folic acid fortification lowers plasma homocysteine, even in populations with a relatively low prevalence of folate deficiency (*49*). Several lines of evidence indicate that even moderately elevated plasma homocysteine is an independent risk factor for cardiovascular disease (*142*) and stroke (*143*), both leading causes of death in many countries. While there is still some controversy concerning the direction of causality (*144*), a comparison of the results of genetic and prospective epidemiological studies, which would be expected to have different biases, strongly points to a direct causal pathway leading from elevated homocysteine to cardiovascular disease (*145*). Higher plasma homocysteine levels are also associated in industrialized countries with a higher risk of impaired cognitive function in adults (*146*), and many abnormal pregnancy outcomes, including eclampsia and premature delivery, and other birth defects such as orofacial cleft palate and heart defects. However, the evidence for the benefits of supplementation for these conditions is not as strong than that linking supplementation to prevention of neural tube defects (*147*).

The addition of folic acid to enriched grain products in the United States, a practice which, as mentioned above, was introduced in 1998, has since produced a substantial increase in average blood folate levels among women of childbearing age (*148*). This has resulted in the virtual elimination of low serum folate (*149*) and the lowering of plasma homocysteine in the population at large (*49*). The level of folic acid added (140 µg/100 g flour) is unlikely to bring total folate intakes above the Tolerable Upper Intake Level (UL) of 1 000 µg per day in any life stage or gender group (*128*), or to exacerbate or obscure problems caused by vitamin B_{12} deficiency (see section 4.3).

4.3 Vitamin B_{12}

Vitamin B_{12} (cobalamin) is a cofactor in the synthesis of an essential amino acid, methionine. Its metabolic role is closely linked to that of folate in that one of the vitamin B_{12}-dependent enzymes, methionine synthase, is vital to the functioning of the methylation cycle in which 5-methyltetrahydrofolate acts as a source of

methyl donor groups which are necessary for cell metabolism and survival. Deficiency of this vitamin can thus impair the utilization of folate and causes neurological deterioration, megaloblastic anaemia, elevated plasma homocysteine and possibly, impaired immune function. In infants and young children it can cause severe developmental delays.

4.3.1 Prevalence of deficiency

Vitamin B_{12} status is usually assessed by measuring concentrations in plasma or serum (**Table 4.4**) (*93,128,129*) Although elevated urinary and plasma methylmalonic acid (MMA) levels are more specific, and often more sensitive, indicators of vitamin B_{12} deficiency, MMA concentrations are more difficult and expensive to measure than those of vitamin B_{12}. Elevated homocysteine is a good predictor of vitamin B_{12} status.

TABLE 4.4

Indicators for assessing vitamin B_{12} (cobalamin) status at the population level

Indicator	Sample	Population group	Cut-off to define deficiency	Comments
Vitamin B_{12}	Serum or plasma	Applies to all population groups	<150 pmol/l (<203 mg/l)	Reflects both recent intake and body stores. Values above the cut-off do not necessarily indicate adequate status. If values are marginal, analysis of serum methylmalonic acid is indicated.
Methylmalonic acid (MMA)	Serum or plasma	Applies to all population groups	>271 nmol/l	Increased when supply of vitamin B_{12} is low. Preferred indicator since increased levels are highly specific to vitamin B_{12} deficiency.
Total homocysteine (free and bound)	Plasma	Applies to all population groups	12–16 mmol/l (1.62–2.2 mg/l)	Total plasma homocysteine is a good predictor of vitamin B_{12} status: it is increased in cases of inadequate folate status. Not specific because also increased by vitamin B_2, B_6 and B_{12} deficiencies and influenced by gender, race and renal insufficiency.

Sources: references (*93,128,129*).

Variability in the plasma levels used to define vitamin B_{12} deficiency (see **Table 4.4**) make the results of the few studies of its prevalence difficult to generalize. Moreover, there is no clear evidence that vitamin B_{12} deficiency varies with countries or regions. In countries where vitamin B_{12} deficiency has been assessed at the national level, low serum vitamin B_{12} concentrations were prevalent, i.e. in Venezuela (11–12% in preschool and school-aged children), Germany (15% in women of reproductive age), the United Kingdom (31% of the elderly) and New Zealand (12% of the elderly). The prevalence was lower in the United States (0–3% in preschool and school-aged children, adults and the elderly) and in Costa Rica (5.3% in lactating women). In smaller studies, a high proportion of low plasma vitamin B_{12} concentrations were found in Kenya (40% in school-aged children), Zimbabwe (24% of the elderly), Israel (21% in adults), and India (46% in adults), while in other countries such as Botswana (preschool-aged children), Thailand (school-aged children) and Japan (adults), <1% of plasma vitamin B_{12} concentrations were low (*130,150–152*).

4.3.2 Risk factors for deficiency

Vitamin B_{12} is synthesized by microorganisms in the gut of animals and is subsequently absorbed and incorporated into animal tissues. Products from herbivorous animals (i.e. meat, eggs, milk) are thus the only source of the vitamin for humans. Consequently, intakes are very low or close to zero in many population groups that are economically disadvantaged, or among those who avoid animal products for religious or other reasons. There is a high risk of deficiency in strict vegetarians and even lacto-ovo vegetarians (i.e. milk and egg consumers) have lower plasma concentrations of the vitamin compared with meat-consumers (*153*). Low maternal intake and/or status in the lactating mother will lead to inadequate amounts of vitamin B_{12} in breast milk, and subsequently, deficiency in the infant. Malabsorption syndromes and some inborn errors of metabolism are also risk factors for vitamin B_{12} deficiency.

Gastric atrophy, which occurs with ageing and following prolonged *Helicobacter pylori* infection, results in very poor absorption of vitamin B_{12} from food. However, the crystalline form of the vitamin that is used as a fortificant and in supplements can still be absorbed by most individuals. For this reason, Canada and the United States recommend that their elderly population, more than 20% of which is likely to have some level of vitamin B_{12} deficiency, should consume a substantial part of their recommended vitamin B_{12} intake as fortified foods and/or supplements (*128*). The prevalence of vitamin B_{12} deficiency due to gastric atrophy may be even higher in developing countries, due to a much earlier age of onset and a higher prevalence of *Helicobacter pylori* infection.

4.3.3 Health consequences of deficiency and benefits of intervention

Moderate to severe vitamin B_{12} deficiency results in megaloblastic anaemia and the demyelination of the central nervous system, and in turn, various neurological disorders. The latter are variably reversible after correction of the deficiency (*154*). When serum vitamin B_{12} concentrations fall below 150 pmol/l, abnormalities in the function of some enzymes may occur with the risk, at lower concentration, of potentially irreversible poor memory and cognitive function, impaired nerve conduction and megaloblastic anaemia in individuals of all ages. In a peri-urban area of Guatemala City, for example, schoolchildren with low plasma vitamin B_{12} performed less well on tests of perception and memory, were less accurate in a reasoning (oddity) task, and had poorer academic performance and adaptability (*155*). Infants fed with breast milk from vitamin B_{12}-deficient mothers exhibited a failure to thrive, poor brain development and, in some cases, mental retardation (*156*).

Several studies, mainly from industrialized nations, have demonstrated the benefits of vitamin B_{12} supplementation in susceptible population groups. For example, vitamin B_{12} supplementation of deficient infants born to strictly vegetarian mothers reduced the incidence of anaemia and tremors, and improved their general development (*156*). Among the elderly, vitamin B_{12} supplementation produced improved symptoms in those with clinical signs of deficiency (*157*). To date, few vitamin B_{12} intervention trials have been carried out in developing countries. A recent supplementation programme involving Kenyan schoolchildren has, however, reported significant reductions in the prevalence of vitamin B_{12} deficiency in those receiving supplements of meat or milk compared with placebo or energy-supplemented groups (*152*).

4.4 Other B vitamins (thiamine, riboflavin, niacin and vitamin B_6)

As the food sources of the various B-complex vitamins are similar, it is not surprising that diets inadequate in one B vitamin are more than likely to be deficient in the others. These water-soluble vitamins are readily destroyed during cooking in water and by heat (although niacin is stable to heat). More significantly, the milling and degerming of cereal grains removes almost all of the thiamine (vitamin B_1), riboflavin (vitamin B_2) and niacin (vitamin B_3), which is the reason why restoration of these particular nutrients to wheat and corn flour has been widely practised for the last 60 years. This strategy has certainly contributed to the virtual elimination of vitamin B deficiencies and their associated diseases (i.e. beriberi and pellagra) in the industrialized countries.

Historically, little attention has been paid to the assessment of thiamine, riboflavin, niacin and vitamin B_6 status. One of the reasons why these B-complex

vitamins have been neglected in the past is the lack of reliable information about the consequences of marginal or subclinical deficiencies (see **Table 1.2**). However, evidence is mounting that vitamin B deficiencies are highly prevalent in many developing countries, in particular where diets are low in animal products, fruits and vegetables, and where cereals are milled prior to consumption. Pregnant and lactating women, infants and children are at the highest risk of deficiency. Because the mother's intake and body stores of these vitamins affect the amount she secretes in breast milk, appropriate fortification can provide her with a steady supply during lactation and thereby improve the vitamin B status of her infants and young children.

4.4.1 Thiamine

Thiamine (vitamin B_1) is a cofactor for several key enzymes involved in carbohydrate metabolism and is also directly involved in neural function. It is likely that thiamine deficiency, in its subclinical form, is a significant public health problem in many parts of the world. Severe deficiency causes beriberi, a disease that was once commonplace among populations with a high carbohydrate intake, especially in the form of white rice. As mentioned above, beriberi has been largely eradicated in most industrialized countries, but the disease still occurs in some Asian countries where rice is the staple food. In addition, outbreaks of beriberi are regularly reported in regions suffering social and economic stress brought about by war, famine and other emergency situations.

4.4.1.1 Prevalence of deficiency

The most widely used biochemical indicators of thiamine status are urinary thiamine excretion (UTE), erythrocyte thiamine transketolase activity (ETKA) and the thiamine pyrophosphate effect (TPPE), which is increased in thiamine deficiency (see **Table 4.5**). UTE provides information about the adequacy of dietary intakes of thiamine, but not about the degree of depletion of tissue reserves. Nor is it a very sensitive indicator in cases of subclinical deficiency. Both ETKA and TPPE reflect tissue reserves of thiamine and provide a direct functional evaluation at the cellular level. ETKA is generally regarded as the best single test of thiamine status, despite some reports of poor correlations between this and other measures of thiamine status. Ideally, ETKA should be used in combination with TPPE in order to confirm a diagnosis of thiamine deficiency. In lactating women, the concentration of thiamine in breast milk can be used as an indicator of thiamine deficiency.

Although the lack of reliable biochemical data means that it is not known just how widespread a problem subclinical thiamine deficiency is, thiamine levels in breast milk coupled with infant mortality rates can provide valuable information on the likelihood of the existence of thiamine deficiency in a community. These

TABLE 4.5

Indicators for assessing thiamine (vitamin B_1) status at the population level

Indicator	Sample	Population group	Cut-off to define deficiency		Comments
			Mild	Severe	
Thiamine excretion (μg/g creatinine)	Urine	1–3 years	<175 μg/g	<120 μg/g	Reflects recent intakes.
		4–6 years	<120 μg/g	<85 μg/g	Cut-offs are substantially higher for children.
		7–9 years	<180 μg/g	<70 μg/g	Not a very sensitive indicator of mild deficiency.
		10–12 years	<180 μg/g	<60 μg/g	
		13–15 years	<150 μg/g	<50 μg/g	
		Adults	<65 μg/g	<27 μg/g	
		Pregnancy (second trimester)	<55 μg/g	<27 μg/g	
		Pregnancy (third trimester)	<50 μg/g	<21 μg/g	
Thiamine excretion (μg/24 hours) (UTE)	Urine	Adult	<100 μg/d	<40 μg/d	
Thiamine	Breast milk	Lactating women	<100 μg/l	<50 μg/l	Low levels of thiamine in breast milk combined with an increased infant mortality rate suggest the existence of thiamine deficiency in a community.
Thiamine transketolase activity coefficient (ETKA)	Erythrocytes (RBC)	Can apply to all population groups	≥1.20%	≥1.25%	Generally regarded as the best test of thiamine status, but some studies find poor correlation with other measures. Poor standardization of the test.
Thiamine pyrophosphate effect (TPPE)	Erythrocytes (RBC)	Can apply to all population groups	>15%	>25%	The assay is performed in the absence and in the presence of added thiamine and the result expressed as an activity coefficient, i.e. as the percentage increase in thiamine transketolase activity that is obtained after the addition of thiamine pyrophosphate to the erythrocyte.

RBC, red blood cell.

Source: references (*93, 128, 129, 158*).

TABLE 4.6

Proposed criteria for assessing the public health severity of thiamine deficiency

Indicator	Severity of public health problem (% of population below the cut-off value defining deficiency, unless otherwise stated)		
	Mild	Moderate	Severe
Clinical signs (clinical cases)	<1 (or ≥1 clinical case)	1–4	≥5
TPPE test >25%	5–19	20–49	≥50
Urinary thiamine (per g creatinine)	5–19	20–49	≥50
Breast milk thiamine <50 µg/l	5–19	20–49	≥50
Dietary intake <0.33 mg/1 000 kcal	5–19	20–49	≥50
Infant mortality between 2nd and 5th month	No decline in mortality rates	Slight peak in mortality rates	Marked peak in mortality rates

TPPE, thiamine pyrophosphate effect.

Source: references (*158*).

and other proposed criteria for classifying thiamine deficiency in relation to its public health severity are shown in **Table 4.6**.

Although far less prevalent than in the past, recent cases of severe thiamine deficiency or beriberi have been reported in Indonesia (*159*) and the Seychelles (*160*). The disease still appears in Japan and in north-eastern parts of Thailand where intakes of raw fish (which contain an anti-thiamine compound, thiaminase) and polished rice are high (*161,162*). Thiamine depletion is also a fairly regular occurrence among displaced populations and in refugees dependent on milled white cereals in countries such as Djibouti, Ethiopia, Guinea, Nepal and Thailand (*158*), which would suggest that refugees, displaced populations and those affected by famines are among those at especially high risk for thiamine deficiency. Sporadic outbreaks of thiamine deficiency have occurred in The Gambia, the number of cases peaking during the rainy season, i.e. a time of food shortages (*163*) and in Cuba during the 1992–1993 epidemic of neuropathy (*164*). Despite the concomitant nature of poor thiamine status and the outbreak of neuropathy in the Cuban outbreak, it is by no means certain that thiamine deficiency was responsible for the wide-scale neuropathy (*165*).

4.4.1.2 Risk factors for deficiency

The main sources of thiamine are wheat germ and yeast extracts, offal from most animals, legumes (i.e. pulses, groundnuts and beans) and green vegetables. A low intake of animal and diary products and legumes, and a high consumption of refined rice and cereals are thus the main risk factors for thiamine deficiency. A diet rich in foods that contain high levels of anti-thiamine compounds is an additional risk factor. The most common thiamine antagonist is

thiaminase which is naturally present in some raw fish (*166,167*) and sometimes as a bacterial food contaminant (*168*). Anti-thiamine compounds may also be found in tea, ferns and betel nuts (*169*). Chronic alcohol abuse and genetic disorders are also risk factors for thiamine deficiency (see **Table 1.2**).

4.4.1.3 Health consequences of deficiency and benefits of intervention

There are two distinct forms of severe thiamine deficiency: an oedematous form known as wet beriberi and a non-oedematous neurological form known as dry beriberi. The wet form is associated with potentially fatal heart failure, whereas the dry form tends to be chronic and results in peripheral neuropathy. Many cases of thiamine deficiency present with a mixture of symptoms and thus are properly termed "thiamine deficiency with cardiopathy and peripheral neuropathy" (*158*). Thiamine deficiency in infants is rarely seen today, and is largely confined to infants who are breastfed by thiamine-deficient mothers. In such cases, it is almost always an acute disease, involving oedema and cardiac failure with a high fatality rate.

The Wernicke–Korsakov syndrome is induced by thiamine deficiency and usually manifests as various neurological disorders that are typically associated with impaired cognitive function. It is only observed in chronic alcoholics or in those with a genetic abnormality in transketolase, a thiamine-dependent enzyme.

Several studies have indicated that supplementation can reverse the symptoms of thiamine deficiency. During an outbreak *of beriberi* in The Gambia, for example, the affected groups responded well to thiamine supplementation (*163*).

4.4.2 Riboflavin

Riboflavin (vitamin B_2) is a precursor of various nucleotides, most notably flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which act as coenzymes in various metabolic pathways and in energy production. Riboflavin deficiency rarely occurs in isolation, and is frequently associated with deficiencies in one or more of the other B-complex vitamins.

4.4.2.1 Prevalence of deficiency

The urinary excretion of riboflavin, which is reduced in case of deficiency, has been used in several studies to assess riboflavin status. Urinary riboflavin reflects recent intake of the vitamin, but it is not a particularly good indicator of body stores (**Table 4.7**). A more useful functional test in this respect is the erythrocyte glutathione reductase activity coefficient (EGRAC) (*170*). Erythrocyte flavin nucleotides (FMN + FAD) concentration is, however, probably the best measure of riboflavin status: not only is this less susceptible to short-term fluctuations, but it is also more stable than EGRAC values (*171*).

TABLE 4.7

Indicators for assessing riboflavin (vitamin B_2) status at the population level

Indicator	Sample	Population group	Cut-off to define deficiency		Comments
			Mild	Severe	
Flavin excretion nmol/g creatinine	Urine	Applies to all population groups	<72 nmol/g	<50 nmol/g	Reflects recent intakes. HPLC analysis gives the best determination.
Flavin nucleotides (FMN and FAD)	Erythrocytes (RBC)	Applies to all population groups	<400 nmol/l	<270 nmol/l	Probably the best measure of riboflavin status; less susceptible to short-term fluctuation and more stable than the erythrocyte glutathione reductase activity coefficient. Method involves hydrolysis of FAD to flavin nucleotide. HPLC analysis gives the best determination.
Erythrocyte glutathione reductase activity coefficient (EGRAC)	Erythrocytes (RBC)	Applies to all population groups	>1.2	>1.4	Functional assay that reflects body stores. Not specific as affected by G6PD deficiency and heterozygous β-thalassemia.

HPLC, high performance liquid chromatography; FMN, flavin mononucleotide; FAD, flavin adenine dinucleotide; RBC, red blood cell; G6PD, glucose 6-phosphate deshydrogenase.

Sources: references (*93,128,129*).

In the few studies in which riboflavin status has been assessed at the population level, the prevalence of deficiency is alarmingly high (*172*). Abnormal riboflavin-dependent enzyme function has been reported in almost all pregnant women in The Gambia (*173*); in 50% of elderly and 77% of lactating women in Guatemala (*174*); and in 87% of night-blind women in rural Nepal (*171*). Furthermore, in a survey in China, urinary riboflavin was low in more than 90% of adults (*175*).

4.4.2.2 Risk factors for deficiency

The main dietary sources of riboflavin are meat and dairy products; only small amounts are found in grains and seeds. Leafy green vegetables are also a fairly good source of riboflavin and in developing countries tend to be the main source of the vitamin. Deficiency is thus likely to be more prevalent among those whose intake of animal source foods is low. In common with several of the other B-complex vitamins, chronic alcoholism is also a risk factor.

4.4.2.3 Health consequences of deficiency and benefits of intervention

Symptoms of riboflavin deficiency are non-specific. Early symptoms may include weakness, fatigue, mouth pain, burning eyes and itching. More advanced deficiency is characterized by dermatitis with cheilosis and angular stomatitis, brain dysfunction and microcytic anaemia (**Table 1.2**). Riboflavin deficiency also reduces the absorption and utilization of iron for haemoglobin synthesis. It is possible that riboflavin deficiency is a contributory factor in the high prevalence of anaemia worldwide (see *section 3.1.1*), a suggestion which is supported by reports from The Gambia and Guatemala that riboflavin supplementation improved the haemoglobin response to iron supplementation in anaemic subjects (*176,177*). Almost nothing is known about the effects of milder deficiency, although depletion studies conducted in the United States found evidence of electroencephalogram abnormalities.

4.4.3 Niacin

Niacin (nicotinic acid or vitamin B_3), as a functional group of the coenzymes, nicotinamide adenine dinucleotide (NAD) and its phosphate (NADP), is essential for oxidative processes. Deficiency results in pellagra and is associated with a heavily cereal-based diet that is low in bioavailable niacin, tryptophan (an amino acid) and other micronutrients needed for the synthesis of niacin and tryptophan. Niacin is unique among the vitamins in that at least part of the body's requirement for it can be met through synthesis from an amino acid (tryptophan): the conversion of 60 mg tryptophan (via a niacin derivative) produces 1 mg of niacin.

4.4.3.1 Prevalence of deficiency

There are no direct indicators of niacin status (**Table 4.8**). Assessment is therefore based on the measurement of one or preferably more urinary metabolites of niacin, such as N'-methyl-nicotinamide (NMN) (which reflects recent dietary intake) or the ratio of 2-pyridone:NMN. Provisional criteria proposed by WHO for defining the severity of the public health problem based on these biomarkers are listed in **Table 4.9**.

At present, evaluation of the prevalence of niacin deficiency is almost entirely based on occurrence of clinical signs of deficiency, i.e. pellagra. There is very little biochemical information on niacin status, and thus on the prevalence of subclinical deficiency, from developing countries.

Pellagra was widespread in parts of southern Europe and in the United States during the 19th and early 20th centuries, but fortification of cereal grain products has since all but eradicated the condition from industrialized countries. It is, however, still common in India, and in parts of Africa and China, especially where populations are dependent on maize-based diets. More recently, pellagra has been reported in areas where diets are largely sorghum-based, and where there is a dependence on polished rice. The prevalence of pellagra is also high among displaced populations living in refugee camps based in south and eastern parts of Africa (*178*). For example, up to 6.4% of Mozambican refugees based in Malawi were affected by an outbreak of pellagra (*179*).

4.4.3.2 Risk factors for deficiency

Niacin is widely distributed in plant and animal foods. The main sources are baker's yeast, animal and dairy products, cereals, legumes and leafy green vegetables. Niacin depletion is a risk where diets rely heavily on refined grains or grain products and have little variety. Severe deficiency, pellagra, is predominantly found in people who consume diets that are deficient in bioavailable niacin and low in tryptophan, such as maize- or sorghum-based diets.

In maize, niacin is largely present in a bound form, only 30% of which bioavailable. However, the bioavailability of this bound form of niacin can be improved by hydrolysis with a mild alkali. The soaking of maize in lime water, as is traditionally done in the preparation of *tortillas* in some Latin American countries, releases niacin from niacytin, and thus increases the amount of niacin that can be absorbed. Bound niacin can be also be released by heat: the roasting of coffee beans, for instance, increases the bioavailability of the nicotinic acid content from 20 to 500 mg/kg (*167*). These practices possibly account, at least in part, for the absence of pellagra in Latin America. The regular consumption of milk and rice can also help prevent pellagra; although they are low in niacin, milk and rice are rich in tryptophan.

TABLE 4.8

Indicators for assessing niacin (nicotinic acid) status at the population level[a]

Indicator	Sample	Population group	Cut-off to define deficiency		Comments
			Mild	Severe	
N′-methyl-nicotinamide (NMN)	Urine	Adults	<1.6 mg/g creatinine (<17.5 μmol//24 h)	<0.5 mg/g creatinine (<5.8 μmol//24 h)	Reflects recent dietary intake of niacin.
		Pregnancy (second trimester)	<2.0 mg/g creatinine	<0.6 mg/g creatinine	
		Pregnancy (third trimester)	<2.5 mg/g creatinine	<0.8 mg/g creatinine	
Ratio of 2-pyridone: N′-methyl-nicotinamide	Urine	Applies to all population groups	<0.5	<0.5	Provides a measure of protein adequacy rather than niacin status.
Pyridine nucleotides	Erythrocytes (RBC)	Applies to all population groups	No universally agreed cut-offs at this time		A potentially sensitive indicator of niacin inadequacy.

RBC, red blood cell.

[a] As no direct indicator of niacin status is currently available, it is necessary to measure one or preferably more urinary metabolites of niacin.

Sources: references (*93,128,129,178*).

TABLE 4.9

Proposed criteria for assessing the public health severity of niacin deficiency

Indicator	Severity of public health problem (% of population below the cut-off value defining deficiency)		
	Mild	Moderate	Severe
Clinical signs (clinical cases)	<1	1–4	≥5
Urinary N′-methyl-nicotinamide ≥0.50 mg/g creatinine	5–19	20–49	≥50
Urinary ratio of 2-pyridone: N′-methyl-nicotinamide <1.0	5–19	20–49	≥50
Dietary intake <5 mg niacin equivalents/day	5–19	20–49	≥50

Source: reference (*178*).

4.4.3.3 Health consequences of deficiency and benefits of intervention

Clinical signs of niacin deficiency, pellagra, develop within 2 to 3 months of consuming a diet inadequate in niacin and/or tryptophan (**Table 1.2**). The most characteristic sign of pellagra is a symmetrically pigmented rash on areas of skin exposed to sunlight. Other manifestations include changes in the mucosa of the digestive tract, leading to oral lesions, vomiting and diarrhoea, and neurological symptoms such as depression, fatigue and loss of memory.

4.4.4 Vitamin B_6

Vitamin B_6 is in fact a group of three naturally-occurring compounds: pyridoxine (PN), pyridoxal (PL) and pyridoxamine (PM). The different forms of vitamin B_6 are phosphorylated and then oxidized to generate pyridoxal 5′-phosphate (PLP), which serves as a carbonyl-reactive coenzyme to various enzymes involved in the metabolism of amino acids. Vitamin B_6 deficiency alone is relatively uncommon, but occurs most often in association with deficiencies of the other B vitamins.

4.4.4.1 Prevalence of deficiency

Although there are several biochemical indicators of vitamin B_6 status (**Table 4.10**), all suffer from limitations of one kind or another. For this reason, vitamin B_6 status is best evaluated by using a combination of indicators. The absence of a suitable single indicator means that vitamin B_6 status has only rarely been assessed at the population level but according to a recent report from Indonesia, low intakes among children are likely to be common; among the children surveyed about 10% of those from urban areas and 40% of those from rural areas exhibited biochemical signs of deficiency (*180*). Moreover, about 40% of lactating mothers in Egypt had low concentrations of vitamin B_6 in breast milk, and both these women and their infants presented abnormal behaviours (*181*).

TABLE 4.10

Indicators for assessing vitamin B_6 (pyridoxine) status at the population level[a]

Indicator	Sample	Population group	Cut-off to define deficiency		Comments
			Mild	Severe	
Pyridoxal 5′-phosphate (PLP)	Plasma	Adults	<20 nmol/l	<10 nmol/l	Probably the best indicator of vitamin B_6 status. Reflects tissue stores. Concentration reported to fall with age.
	Urine	Adults	<3 mmol/day	No universally agreed cut-offs at this time	Reflects recent dietary intake.
Aspartate aminotransferase apoenzyme form: total enzyme	Erythrocytes (RBC)	Adults	>1.6	No universally agreed cut-offs at this time	Measured before and after addition of pyridoxal 5′-phosphate (PLP) to ascertain amounts of apoenzyme. The ratio is increased in cases of vitamin B_6 deficiency. Reflects long-term vitamin B_6 status.
Alanine aminotransferase apoenzyme form: total enzyme	Erythrocytes (RBC)	Adults	>1.25	No universally agreed cut-offs at this time	Measured before and after addition of pyridoxal 5′-phosphate (PLP) to ascertain amounts of apoenzyme. The ratio is increased in cases of vitamin B_6 deficiency. Reflects long-term vitamin B_6 status.
Total homocysteine (free and bond)	Plasma	Adults	12–16 μmol/l	No universally agreed cut-offs at this time	Influenced by vitamin B_6, B_{12}, folate status, gender, race and renal insufficiency.

RBC, red blood cell.

[a] No direct indicator of vitamin B_6 status is currently available; in order to assess vitamin B_6 status it is therefore necessary to measure a combination of indicators.

Sources: references (*93,128,129*).

4.4.4.2 Risk factors for deficiency

Vitamin B_6 is widely distributed in foods, but meats, wholegrain products, vegetables and nuts are especially good sources of the vitamin. Cooking and storage losses range from a few percent to nearly half of the vitamin B_6 originally present. Plants generally contain pyridoxine (PN), the most stable form, while animal products contain the less stable pyridoxal (PL) and the functional PLP form. In common with several of the other B vitamins, low intakes of animal products and a high consumption of refined cereals are the main risk factors for vitamin B_6 deficiency. Similarly, chronic alcoholism is an additional risk factor for deficiency.

4.4.4.3 Health consequences of deficiency and benefits of intervention

Symptoms of severe vitamin B_6 deficiency are non-specific (**Table 1.2**) and include neurological disorders (i.e. epileptic convulsions), skin changes (i.e. dermatitis, glossitis, cheilosis) and possibly anaemia. Vitamin B_6 deficiency is a risk factor for elevated plasma homocysteine (*182*). In trials, vitamin B_6 supplements increased secretion of the vitamin in the breast milk of lactating women (*183*).

4.5 Vitamin C

Vitamin C is a redox system comprised of ascorbic acid and dehydroascorbic acid, and as such acts as an electron donor. Its main metabolic function is the maintenance of collagen formation. It is also an important antioxidant. Although severe vitamin C deficiency (scurvy) is now relatively rare, the prevalence of milder or marginal deficiency is probably quite high.

4.5.1 Prevalence of deficiency

Concentrations of ascorbic acid in blood plasma or serum reflect recent intakes of vitamin C, and in this respect, are more reliable indicators of vitamin C status than ascorbic acid concentrations in erythrocytes (**Table 4.11**). White blood cell (leukocyte) ascorbic acid concentrations are more closely related to tissue stores and probably provide the most sensitive indicator of vitamin C status, but being technically more difficult to measure, are impractical for routine and large-scale population surveys. Criteria for defining the public health significance of vitamin C deficiency, as proposed by WHO, are given in **Table 4.12**.

Despite its near eradication, severe vitamin C deficiency (scurvy) still occurs periodically in displaced populations maintained for long periods of time (i.e. 3–6 months) on food aid and without access to fresh fruit and vegetables (*184*). Outbreaks have been repeatedly reported from refugee camps in the Horn of Africa (i.e. Ethiopia, Kenya, Somalia, Sudan) and Nepal. In the mid-1980s, the prevalence of scurvy in refugee camps in north-west Somalia varied between

TABLE 4.11

Indicators for assessing vitamin C status at the population level

Indicator	Sample	Population group	Cut-off to define deficiency		Comments
			Mild	Severe	
Ascorbic acid	Serum/plasma	Applies to all population groups	<0.3 mg/100 ml	<0.2 mg/100 ml	Reflects recent intake.
Ascorbic acid	Erythrocytes (RBC)	Applies to all population groups	<0.5 mg/100 ml	<0.3 mg/100 ml	Reflects recent intake, but less reliable than serum/plasma ascorbic acid concentration.
Ascorbic acid	Leukocytes	Applies to all population groups	<114 nmol/10^8 cells	<57 nmol/10^8 cells	Reflects body stores. Considered to be the most sensitive indicator of vitamin C status, but as technically complex to measure and interpretation is limited by the absence of standardized reporting procedures, not widely used for population surveys.

RBC, red blood cell.

Sources: references (*129,184,190*).

TABLE 4.12
Proposed criteria for assessing the public health severity of vitamin C deficiency

Indicator	Severity of public health problem (% of population)		
	Mild	Moderate	Severe
Clinical signs (clinical cases)	<1	1–4	≥5
Serum ascorbic acid:			
<0.2 mg/100 ml	10–29	30–49	≥50
<0.3 mg/100 ml	30–49	50–69	≥70

Sources: adapted from references (*184,190*).

7% and 44% (*185*); in eastern Sudan the prevalence rate was 22% (*186*), and in Kassala, Sudan, 15% (*187*). Scurvy has also been observed in selected population groups, such as infants, and in some communities of mine labourers (*188*).

In contrast, the prevalence of mild vitamin C deficiency worldwide is probably fairly high. In the United States, data from the third National Health and Nutrition Examination Survey (NHANES III 1988–1994) have indicated that the prevalence of marginal vitamin C deficiency (defined as less than 0.3 mg ascorbic acid per 100 ml serum) is about 9% in women and 13% in men (*189*).

4.5.2 Risk factors for deficiency

Vitamin C is widely available in foods of both plant and animal origin, but the best sources are fresh fruits and vegetables, and offal. As germination increases vitamin C content, germinated grains and pulses also contain high levels of vitamin C. However, because vitamin C is unstable when exposed to an alkaline environment or to oxygen, light and heat, losses may be substantial during storage and cooking.

Deficiency is usually a result of a low consumption of fresh fruits and vegetables, caused by any one or a combination of factors such as seasonal unavailability, transportation difficulties and/or unaffordable cost. Displaced populations who rely on cooked, fortified rations and who do not have access to fresh fruits and vegetables are at a high risk for deficiency. For these population groups, vitamin C supplementation is recommended, at least until they are able to obtain a more normal diet. Chronic alcoholics, institutionalized elderly and people living on a restricted diet containing little or no fruits and vegetables, are also at risk of vitamin C deficiency. As the vitamin C content of cow's milk is low, infants represent a further subgroup that is potentially high-risk for vitamin C deficiency. There have been a number of reports – across several world regions – of scurvy in infants fed on evaporated cow's milk (*191,192*).

4.5.3 Health consequences of deficiency and benefits of intervention

The clinical symptoms of scurvy include follicular hyperkeratosis, haemorrhagic manifestations, swollen joints, swollen bleeding gums and peripheral oedema, and even death. These symptoms appear within 3–4 months of consuming diets with a very low vitamin C content (<2 mg per day). In infants, manifestations of scurvy include a haemorrhagic syndrome, signs of general irritability, tenderness of the legs and pseudoparalysis involving the lower extremities (see **Table 1.2**). The adverse effects of mild deficiency are uncertain, but may include poor bone mineralization (due to slower production of collagen), lassitude, fatigue, anorexia, muscular weakness and increased susceptibility to infections.

As vitamin C increases the absorption of non-haem iron from foods, a low intake of vitamin C will exacerbate any iron deficiency problems, especially in individuals who consume only small amounts of meat, fish or poultry. Indeed, anaemia is a frequent manifestation of scurvy. The addition of vitamin C to iron-fortified foods greatly improves the absorption of the iron. In Chile, for example, it was necessary to also add vitamin C to iron-fortified dried milk consumed by young children before any significant improvements in iron status could be detected (*40*) (see also *section 5.1.2.1*).

4.6 Vitamin D

Vitamin D is one of the most important regulators of calcium and phosphorus homeostasis. It also plays many roles in cell differentiation and in the secretion and metabolism of hormones, including parathyroid hormone and insulin. Vitamin D (calciferol) is synthesized in the skin of most animals, including humans, from its precursor, 7-dehydrocholesterol, by the action of sunlight. This produces a naturally-occurring form of the vitamin known as vitamin D_3. Vitamin D can also be obtained from the diet, either as vitamin D_3 or as a closely-related molecule of plant origin known as vitamin D_2. Since both forms are metabolized by humans in much the same way, from a nutritional perspective, vitamin D_3 and vitamin D_2 can be considered to be equivalent. Vitamin D_3 is metabolized first in the liver to 25-hydroxyvitamin D (25-OH-D_3), and then in the kidney to 1,25-dihydroxyvitamin D (1,25-$(OH)_2$-D_3), which is the biologically active form of the vitamin.

Severe vitamin D deficiency produces the bone disease called rickets in infants and children, and osteomalacia in adults, conditions which are characterized by the failure of the organic matrix of bone to calcify. The global prevalence of vitamin D deficiency is uncertain, but it is likely to be fairly common worldwide, and especially among infants and young children, the elderly and those living at high latitudes where daylight hours are limited in the winter months.

4.6.1 Prevalence of deficiency

In infants and young children, a concentration of 25-OH-D in serum below about 27.5 nmol/l (11 ng/ml) is indicative of a low vitamin D status (**Table 4.13**). An elevated serum concentration of alkaline phosphatase can also indicate vitamin D deficiency; alkaline phosphatase is increased in patients with rickets or osteomalacia but is not specific to either of these conditions. In adults, the combination of low plasma 25-OH-D and elevated parathyroid hormone (PTH) is probably the most reliable indicator of vitamin D deficiency (*193*). In the absence of biochemical data, the existence of rickets in infants and children, and a high fracture risk among the elderly population, would suggest that vitamin D deficiency might be a public health problem.

Breast-fed infants who are not exposed to sunlight are unlikely to obtain enough vitamin D from breast milk beyond the first few months of life, especially if their mother's stores of the vitamin are low. Vitamin D deficiency in infants as a result of low maternal stores and/or infant exposure to sunlight (especially during winter months) has been reported in countries as diverse as China (*194*) and France (*195*). Infants and children on macrobiotic diets tend to have a high prevalence of rickets, due to the low vitamin D content of maternal milk and the absence of fortified cow's milk in their diets (*196*).

Children living in the far northerly latitudes, whose exposure to ultraviolet light is low especially during the winter months, are at high risk for rickets (*197*). Vitamin D deficiency is also common in adults living at higher latitudes: for

TABLE 4.13

Indicators for assessing vitamin D status at the population level

Indicator	Sample	Population group	Cut-off to define deficiency	Comments
25-hydroxyvitamin D (25-OH-D)	Serum	Applies to all population groups	<27.5 nmol/l (<11 ng/ml)	Serum 25-hydroxyvitamin D in combination with parathyroid hormone is a valuable indicator of vitamin D status.
Parathyroid hormone (PTH)	Serum	Applies to all population groups	No universally agreed cut-offs at this time	Serum parathyroid hormone is inversely correlated with serum 25-hydroxyvitamin D and may be a valuable indicator of vitamin D status.
Alkaline phosphatase	Serum	Applies to all population groups	No universally agreed cut- offs at this time	Increased in cases of osteomalacia or rickets.

Sources: references (*93, 129, 193*).

instance, surveys carried out in China after winter in populations living at about 41°N found that 13–48% of adults were deficient in this vitamin, with the highest prevalence occurring in older men (*198*). In Beijing, 45% of adolescent girls were found to be deficient (*199*).

4.6.2 Risk factors for deficiency

Most (about 80%) of the vitamin D in the body is produced in the skin. This process usually supplies all of the vitamin D needed by infants, children and adults. However, above and below latitudes 40°N and 40°S, the intensity of ultraviolet radiation in sunlight is not sufficient to produce adequate amounts of vitamin D in exposed skin during the 3–4 winter months. At the very high latitudes, synthesis can be inadequate for as long as 6 months of the year. Inadequate synthesis in winter is seen as far south as Turkey and Israel; low serum levels of vitamin D are also highly prevalent in the winter in Delhi, India (29°N) (*200*). Vitamin D synthesis in the skin will also be inadequate if the body is consistently covered by clothing, a probable factor in the high prevalence of deficiency among veiled women (e.g. Kuwaiti women) and their breast-fed infants and children (*201*).

In the elderly, dietary requirements for vitamin D are increased because the ability of the skin to synthesize this vitamin decreases with age; at age 65 years, vitamin D synthesis in the skin is about 75% slower than that in younger adults. Dark-skinned individuals synthesize less vitamin D when exposed to ultraviolet light, and are therefore more vulnerable to deficiency at low levels of exposure to ultraviolet light. In the United States, cases of rickets have been reported among black breast-fed children (*202*), and according to the results of a recent national survey, 42% of African-American women had low plasma vitamin D concentrations (*56*).

Being naturally present in relatively few foods, dietary sources of vitamin D usually supply only a small fraction of the daily requirements for the vitamin. Salt-water fish such as herring, salmon, sardines and fish liver oil are the main dietary sources. Small quantities of vitamin D are found in other animal products (e.g. beef, butter), and if hens are fed vitamin D, eggs can provide substantial amounts of the vitamin. Because the consumption of these foods tends to be relatively low, in industrialized countries most dietary vitamin D comes from fortified milk and margarine. Milk only provides small amounts of vitamin D unless it is fortified.

Several studies have shown that the effects of poor vitamin D status are exacerbated by low calcium intakes. This has been demonstrated in adults from India (*200*) and in children from Nigeria (*203*). The Nigerian children with nutritional rickets responded better to calcium, with or without vitamin D, than to vitamin D alone (*203*).

4.6.3 Health consequences of deficiency and benefits of intervention

The clinical features of rickets include bone deformities and changes in the costochondral joints. The lesions are reversible after correction of vitamin D deficiency. In osteomalacia, in which the loss of calcium and phosphorus from bone causes it to lose strength, the main symptoms are muscular weakness and bone pain, but little bone deformity. Osteomalacia contributes to osteoporosis, a condition in which the bone becomes more brittle and porous due to the loss of bone tissue. Vitamin D supplementation reduced seasonal loss of bone tissue in North American women (*204*), and prevented fractures associated with osteoporosis in the elderly.

In many locations, the addition of vitamin D to selected foods has proved to be a prudent public health measure. The vitamin has been added to milk in Canada and the United States since the 1920s, a policy that has been largely responsible for the elimination of vitamin D deficiency rickets in children. However, low intakes of fortified dairy products by some elderly individuals, and by some black populations, are still associated with a much higher risk of vitamin D deficiency among these groups.

4.7 Calcium

Calcium is the most abundant mineral in the body. Most (>99%) of the body's 1 000–1 200 g of calcium is located in the skeleton where it exists as hydroxyapatite. In addition to its role in maintaining the rigidity and strength of the skeleton, calcium is involved in a large number of metabolic processes, including blood clotting, cell adhesion, muscle contraction, hormone and neurotransmitter release, glycogen metabolism, and cell proliferation and differentiation.

Osteoporosis, a disease characterized by reduced bone mass and thus increased skeletal fragility and susceptibility to fractures, is the most significant consequence of a low calcium status. Although an adequacy of calcium is important during the whole life span, it is especially important during childhood and adolescence (as these are periods of rapid skeletal growth), and for postmenopausal women and the elderly whose rate of bone loss is high.

4.7.1 Prevalence of deficiency

Unfortunately there are no practical population level indicators of calcium status (**Table 4.14**). Serum calcium, for example, is regulated by a complex homeostatic mechanism, which makes it an unreliable indicator of calcium status. For this reason, in most countries the prevalence of deficiency is not known. In the absence of reliable biochemical indicators, the best indication of calcium adequacy at present, especially for developing countries, is probably provided by comparing dietary intakes with recommended nutrient intakes (RNIs), despite

TABLE 4.14
Indicators for assessing calcium at the population level

Indicator	Sample	Population group	Cut-off to define deficiency	Comments
Calcium	Serum	Applies to all population groups	No universally agreed cut-offs at this time	Tightly homeostatically regulated and therefore does not reflect calcium status.
Calcium	Dietary intake	Applies to all population groups	No universally agreed cut-offs at this time	Probably the best indicator of calcium adequacy.

[a] At present there are no good biochemical measures for assessing calcium status.

Sources: references (*93,193*).

the variability and uncertainty in the currently recommended intakes for calcium (*93,193*). On the basis of the fact that intakes of dairy products are low, it is thus highly likely that low or very low calcium intakes are very common in developing countries.

Measurements of bone mineral density (BMD) and bone mineral content (BMC) have provided an alternative means of assessing the likely extent of calcium deficiency in some countries. In the United States, for example, it has been estimated that 5–6 million older women and 1–2 million older men have osteoporosis. Other approaches include measuring markers of bone resorption in urine or plasma, which tend to be higher in calcium deficient individuals. Such methods are, however, relatively expensive. All of the above measures are affected by, among many other factors, vitamin D status, level of physical activity and hormone levels, which further complicates the assessment of calcium adequacy at the population level.

4.7.2 Risk factors for deficiency

Intakes of calcium will almost certainly fall below the recommended levels where dairy product intake is low. Dairy products supply 50–80% of dietary calcium in most industrialized countries, while foods of plant origin supply about 25%. The calcium content of, and contribution from, most other foods is usually relatively small. Calcium absorption efficiency is increased by a low calcium status and by a low dietary calcium content. Absorption is homeostatically controlled through regulation by vitamin D. The strongest known inhibitor of calcium absorption is dietary oxalate, followed by the presence of phytates (*193*). Oxalate is not an important factor in most diets (although it is high in spinach, sweet potatoes and beans) but phytates are often consumed in large amounts, for instance, in legumes and wholegrain cereals.

4.7.3 Health consequences of deficiency and benefits of fortification

The numerous metabolic roles of calcium are sustained even when intakes are low, because calcium is withdrawn from the bone should homeostatic mechanisms fail to maintain an adequate calcium status in the extracellular fluid. Thus inadequate calcium intakes lead to decreased bone mineralization and subsequently an increased risk for osteoporosis in adults (**Table 1.2**).

In healthy individuals, bone mineral density increases until about 30 years of age, and thereafter begins to decline. Low intakes during childhood and adolescence can reduce peak bone density and thus increase the risk of osteoporosis in adulthood. The age of onset and severity of osteoporosis depends not only on the duration of inadequate calcium intakes, but also on a number of other factors, such as estrogen levels, vitamin D status and level of physical activity.

Although rickets is usually associated with vitamin D deficiency (see *section 4.6*), rickets has been observed in vitamin D-replete infants who also had low calcium intakes (*203*). In Chinese children aged 5 years from China, Hong Kong Special Administrative Region (Hong Kong SAR), intakes of <250 mg calcium per day were associated with a 14% lower bone mineral content and a 4% reduction in height relative to those consuming twice as much calcium (*205*). Supplementation of Gambian children with 1 000 mg calcium per day improved their bone mineralization (*206*). It has been suggested that calcium may confer other benefits, including the prevention of cancer and hypertension, but the role played by calcium in such diseases is unclear at the present time.

4.8 Selenium

Selenium is an essential element and a key constituent of at least 13 selenoproteins. These can be grouped into a number of distinct families, the glutathione peroxidases and the thioredoxin reductases, which are part of the antioxidant defence system of cells, and iodothyronine deiodinase, an enzyme which converts the inactive precursor of thyroxine, tetraiodothyronine (T_4) into the active form, tri-iodothyronine (T_3). In humans, the biological roles of selenium include the protection of tissues against oxidative stress, the maintenance of the body's defence systems against infection, and the modulation of growth and development. Severe deficiency can result in Keshan or Kaschin-Beck disease, which are endemic in several world regions.

4.8.1 Prevalence of deficiency

There are several reliable indicators of selenium status, such as the concentration of selenium in plasma, urine, hair or nails. However, the measurement of selenium in human samples presents a number of technical difficulties, a factor that limits the usefulness of such measures as indicators of status (**Table 4.15**). Indeed, the lack of simple assay techniques for selenium means that currently

TABLE 4.15

Indicators for assessing selenium status at the population level[a]

Indicator	Sample	Population group	Cut-off to define deficiency	Comments
Selenium	Plasma, urine	Applies to all population groups	0.8–1.1 µmol/l	Might reflect recent intake in low selenium environments but levels depend on the chemical form of the ingested selenium. Not appropriate for use in population surveys as technically difficult to measure.
Selenium	Erythrocytes (RBC)	Applies to all population groups	No universally agreed cut-offs at this time	Reflects stores but not appropriate for use in population surveys as technically difficult to measure.
Selenium	Hair, nails	Applies to all population groups	No universally agreed cut-offs at this time	Correlations do exist between dietary intake and hair and nail concentrations. Concentrations are affected by several factors such as frequency of hair washing (shampoos are high in selenium) and hair colour.

RBC, red blood cell.

[a] Selenium status is probably best assessed by means of a combination of indicators.

Sources: references (*93,208*).

there are no suitable biochemical indicators of selenium status that are appropriate for use in population surveys. Information regarding the prevalence of selenium deficiency is thus largely based on clinical observations and limited to the more severe forms, i.e. Keshan or Kaschin-Beck disease.

Selenium deficiency is endemic in some regions of China (*207*), where Keshan disease was first described, and also in parts of Japan, Korea, Scandinavia and Siberia. Endemic deficiency tends to occur in regions characterized by low soil selenium. For example, the distribution of Keshan disease and Kaschin-Beck disease in China reflects the distribution of soils from which selenium is poorly available to rice, maize, wheat and pasture grasses. Fortification of salt and/or fertilizers with selenium is crucial in these parts of the world.

4.8.2 Risk factors for deficiency

Usual diets in most countries satisfy selenium requirements. As indicated in the previous section, deficiency occurs only where the soil, and consequently the foods produced on those soils, is low in available selenium. Worldwide, the selenium content of animal products and that of cereals and plants, varies widely (at least 10-fold) depending on soil selenium content (*209*). The selenium content of foods of plant origin ranges from less than 0.1 µg/g to more than 0.8 µg/g, while the amount in animal products ranges from 0.1 to 1.5 µg/g (*210*). Where animal feeds are enriched with selenium, such as in the United States, the selenium content of animal products may be much higher. Concentrations of less than 10 ng/g in the case of grain and less than 3 ng/g in the case of water-soluble soil selenium have been proposed as indexes to define selenium-deficient areas (*93*).

In industrialized countries, meat provides about half of the dietary selenium. It is also a good source in areas of low soil selenium because animals absorb more of this nutrient when their intake is low. A low intake of animal source foods is thus likely to increase the risk of selenium deficiency. It is generally assumed that the bioavailability of selenium from the diet is high.

4.8.3 Health consequences of deficiency and benefits of intervention

Keshan disease is a cardiomyopathy associated with a low selenium intake and low levels of selenium in blood and hair. Reports of its occurrence across a wide zone of mainland China first appeared in the mainstream scientific literature in the 1930s. It has since also been observed in some areas of the southern Siberia. Symptoms include cardiac insufficiency and arrhythmias, congestive heart failure and heart enlargement (*211*), which are responsive to supplementation with sodium selenite. Because some features of Keshan disease cannot be explained by selenium deficiency alone, other contributing factors have been suggested, in particular, infection with the cocksackie virus (*212*).

The selenium deficiency syndrome known as Kaschin-Beck or Urov disease is found in parts of China and Siberia, and in Japan and Korea. This is a disease of cartilage tissue that occurs in pre-adolescent and adolescent children, causing osteoarthropathy, joint problems and growth stunting. Like Keshan disease, additional causal factors have been proposed to account for the etiology of Keschin-Beck disease, including exposure to mycotoxins from *Fusarium* mould (*213*), mineral imbalances and iodine deficiency (*214*).

Low intakes of selenium have been linked to a reduced conversion of the thyroid hormone, T_4 to T_3. The metabolic interrelations between selenium and iodine are such that deficiencies in one can sometimes exacerbate problems with the other. In the Democratic Republic of Congo, for instance, combined selenium and iodine deficiencies were shown to contribute to endemic myxoedematous cretinism. Administration of selenium alone appeared to aggravate this disease; by restoring selenium-dependent deiodinase activity, the synthesis and use of thyroxine (T_4) and iodine is increased, thereby exacerbating the iodine deficiency (*215*). Low selenium intakes have also been associated by some researchers with an increased incidence of cancer, in particular, oesophageal cancer and also with cardiovascular disease (*216*).

In areas of endemic selenium deficiency, fortification with selenium has been shown to rapidly increase plasma glutathione peroxidase levels and urinary selenium. For example, when selenium was added to fertilizers in Finland in 1984, plasma selenium levels doubled by 1991 and glutathione peroxidase activity was normalized (*217*). In addition, according to the results of large-scale survey (over 1 million people) selenium fortification of table salt has significantly reduced the prevalence of Keshan disease in China (*218*).

4.9 Fluoride

Unlike the other micronutrients considered in these guidelines, fluoride is not generally considered to be an essential nutrient according to the strict definition of the term (see Chapter 2: *section 2.1.1*). Nevertheless, fluoride is undoubtedly protective against tooth decay.

4.9.1 Prevalence of dental caries

There are no universally agreed methods for assessing fluoride status and no generally accepted criteria with which to define deficiency. However, concentrations in urine have sometimes been used as an indicator of fluoride status (**Table 4.16**).

The prevalence of dental caries is 40–60% lower in those areas of the United States where water is fluoridated compared with those where it is not. However, the increased use of fluoridated toothpaste and supplements by infants and

TABLE 4.16
Indicators for assessing fluoride status at the population level[a]

Indicator	Sample	Population group	Cut-off to define deficiency	Comments
Fluoride	Urine	Applies to all population groups	<0.5 mg/l	No universally agreed criteria for defining deficiency. The following cut-offs for urinary fluoridine are, however, sometimes used: adequate, 0.5–1.0 mg/l; deficient, <0.5 mg/l; excessive >1.5 mg/l.

[a] At present there are no universally agreed methods for assessing fluoride status.

Source: reference (*193*).

young children has made it difficult to differentiate between the beneficial effects of a fluoridated water supply and that of other sources of the mineral.

4.9.2 Risk factors for low intakes

Fluoride intake from most natural water supplies will be relatively low; a low fluoride content of water is thus the main risk factor for a low intake of this mineral. In Canada and the United States, for instance, water sources typically contain less than 0.4 mg/l, which compares with concentrations of 0.7–1.2 mg/l in fluoridated supplies. Moreover, the fluoride content of breast milk is low and foods contain well below 0.05 mg per 100 g, with exception of those prepared with fluoridated water and infant formulas.

4.9.3 Health consequences of low intakes and benefits of intervention

If ingested in water or foods, fluoride will become incorporated into the mineral of growing teeth and thus make them more resistant to decay. Continued exposure of the tooth surfaces to fluoride throughout life is also beneficial because it reduces the ability of bacteria to cause decay and promotes the remineralization of decayed areas. For these reasons, the addition of fluoride to public water supplies, or to salt or milk, can be an effective public health strategy for dental caries prevention (*219*). This practice does not increase the risk of osteoporosis for older individuals in the population (*220*), and according to the results of some studies, might even lower the risk (*221,222*).

Excessive fluoride intake carries a risk of enamel fluorosis, especially during the first 8 years of life. In severe cases of this condition, the enamel of the tooth becomes stained and pitted; in milder forms the enamel acquires opaque lines or patches. Enamel fluorosis does not occur at fluoride intakes ≤0.10 mg/kg body weight per day (*193*). In adults, excessive fluoride intake can result in skeletal

fluorosis, with symptoms that include bone pain, and in more severe cases, muscle calcification and crippling. Mild skeletal fluorosis only occurs at fluoride intakes that are in excess of 10 mg/day for more than 10 years. Symptoms of skeletal fluorosis are rarely seen in communities where the fluoride content of water supplies is below 20 ppm (20 mg/l).

4.10 Multiple micronutrient deficiencies

4.10.1 Prevalence and risk factors

Based on what is known about the prevalence of deficiencies in individual micronutrients, it is probable that multiple micronutrient deficiencies are common in several parts of the world and in certain population groups. Micronutrient deficiencies are more likely to coexist in individuals who consume diets that are poor in nutritional quality, or who have higher nutrient requirements due to high growth rates and/or the presence of bacterial infections or parasites. In particular, a diet that is low in animal source foods typically results in low intakes of bioavailable iron and zinc, calcium, retinol (pre-formed vitamin A), vitamin B_2 (riboflavin), vitamin B_6 and vitamin B_{12}. Often, poor quality diets also lack fresh fruits and vegetables, which means that intakes of vitamin C (ascorbic acid), β-carotene (provitamin A) and folate will also be inadequate. The milling of cereals removes several nutrients, notably, iron and zinc, various B vitamins (i.e. thiamine, riboflavin, niacin) and folate. Individuals who rely heavily on refined cereals are thus at increased risk of deficiency of all of these micronutrients. The breast milk of undernourished lactating women consuming a limited range of foods and with multiple micronutrient deficiencies, is most likely to be low in concentrations of vitamin A (retinol), the B vitamins, iodine and selenium. If the micronutrient content of breast milk is inadequate for optimal infant development, maternal supplementation may be required until adequate fortification programmes can be launched.

4.10.2 Health consequences and benefits of intervention

As several previous subsections have indicated, a deficiency in one micronutrient can impair the utilization of another. Conversely, improving an individual's status in one micronutrient, or even several micronutrients simultaneously in the case of multiple deficiencies, can have wider benefits. For example, iron deficiency may cause vitamin A to be trapped in the liver; several studies have shown that iron supplementation alone can increase serum retinol concentrations markedly (*85*). Goitre is more resistant to improvement by iodine supplementation in the presence of iron deficiency, and iron supplementation of deficient children improves their rate of goitre response to iodine supplements or iodine fortified salt (*87*). Similarly, the addition of vitamin A to iron supplements

increases blood haemoglobin by a substantial amount in vitamin A-depleted, anaemic populations (*99*) and can help to further increase iron stores (*223*). Deficiencies of vitamin B_{12}, folate, vitamin B_2 (riboflavin) and several other micronutrients can also contribute to anaemia (*77*). As vitamin C (from foods or added as a fortificant) improves the absorption of non-haem iron from food and many iron fortificants, it too is frequently added as well as iron as a fortificant.

In the past, interventions have targeted deficiencies in iron, vitamin A and iodine, in part because these can be detected more easily and more is known about their adverse effects. Typically, separate programmes were developed for each nutrient. In more recent years, it has become increasingly apparent that there are many reasons why multiple micronutrient fortification may be more appropriate and should be considered. In addition to treating and preventing iron, vitamin A and iodine deficiencies, fortification affords a good opportunity to control other micronutrient deficiencies that are likely to coexist in many populations.