

## 2. Vitamin A

### 2.1 Role of vitamin A in human metabolic processes

Vitamin A (retinol) is an essential nutrient needed in small amounts by humans for the normal functioning of the visual system; growth and development; and maintenance of epithelial cellular integrity, immune function, and reproduction. These dietary needs for vitamin A are normally provided for as preformed retinol (mainly as retinyl ester) and provitamin A carotenoids.

#### 2.1.1 Overview of vitamin A metabolism

Preformed vitamin A in animal foods occurs as retinyl esters of fatty acids in association with membrane-bound cellular lipid and fat-containing storage cells. Provitamin A carotenoids in foods of vegetable origin are also associated with cellular lipids but are embedded in complex cellular structures such as the cellulose-containing matrix of chloroplasts or the pigment-containing portion of chromoplasts. Normal digestive processes free vitamin A and carotenoids from food matrices, which is a more efficient process from animal than from vegetable tissues. Retinyl esters are hydrolysed and the retinol and freed carotenoids are incorporated into lipid-containing, water-miscible micellar solutions. Products of fat digestion (e.g. fatty acids, monoglycerides, cholesterol, and phospholipids) and secretions in bile (e.g. bile salts and hydrolytic enzymes) are essential for the efficient solubilization of retinol and especially for solubilization of the very lipophilic carotenoids (e.g.  $\alpha$ - and  $\beta$ -carotene,  $\beta$ -cryptoxanthin, and lycopene) in the aqueous intestinal milieu. Micellar solubilization is a prerequisite to their efficient passage into the lipid-rich membrane of intestinal mucosal cells (i.e. enterocytes) (1–3). Diets critically low in dietary fat (under about 5–10g daily) (4) or disease conditions that interfere with normal digestion and absorption leading to steatorrhea (e.g. pancreatic and liver diseases and frequent gastroenteritis) can therefore impede the efficient absorption of retinol and carotenoids. Retinol and some carotenoids enter the intestinal mucosal brush border by diffusion in accord with the concentration gradient between the micelle and plasma membrane of

enterocytes. Some carotenoids pass into the enterocyte and are solubilized into chylomicrons without further change whereas some of the provitamin A carotenoids are converted to retinol by a cleavage enzyme in the brush border (3). Retinol is trapped intracellularly by re-esterification or binding to specific intracellular binding proteins. Retinyl esters and unconverted carotenoids together with other lipids are incorporated into chylomicrons, excreted into intestinal lymphatic channels, and delivered to the blood through the thoracic duct (2).

Tissues extract most lipids and some carotenoids from circulating chylomicrons, but most retinyl esters are stripped from the chylomicron remnant, hydrolysed, and taken up primarily by parenchymal liver cells. If not immediately needed, retinol is re-esterified and retained in the fat-storing cells of the liver (variously called adipocytes, stellate cells, or Ito cells). The liver parenchymal cells also take in substantial amounts of carotenoids. Whereas most of the body's vitamin A reserve remains in the liver, carotenoids are also deposited elsewhere in fatty tissues throughout the body (1). Usually, turnover of carotenoids in tissues is relatively slow, but in times of low dietary carotenoid intake, stored carotenoids are mobilized. A recent study in one subject using stable isotopes suggests that retinol can be derived not only from conversion of dietary provitamin carotenoids in enterocytes—the major site of bioconversion—but also from hepatic conversion of circulating provitamin carotenoids (5). The quantitative contribution to vitamin A requirements of carotenoid converted to retinoids beyond the enterocyte is unknown.

Following hydrolysis of stored retinyl esters, retinol combines with a plasma-specific transport protein, retinol-binding protein (RBP). This process, including synthesis of the unoccupied RBP (apo-RBP), occurs to the greatest extent within liver cells but it may also occur in some peripheral tissues. The RBP-retinol complex (holo-RBP) is secreted into the blood where it associates with another hepatically synthesized and excreted larger protein, transthyretin. The transthyretin-RBP-retinol complex circulates in the blood, delivering the lipophilic retinol to tissues; its large size prevents its loss through kidney filtration (1). Dietary restriction in energy, proteins, and some micronutrients can limit hepatic synthesis of proteins specific to mobilization and transport of vitamin A. Altered kidney functions or fever associated with infections (e.g. respiratory infections (6) or diarrhoea [7]) can increase urinary vitamin A loss.

Holo-RBP transiently associates with target tissue membranes, and specific intracellular binding proteins then extract the retinol. Some of the transiently sequestered retinol is released into the blood unchanged and is recycled (i.e. conserved) (1, 8). A limited reserve of intracellular retinyl esters is formed

that subsequently can provide functionally active retinol and its oxidation products (i.e. isomers of retinoic acid) as needed intracellularly. These biologically active forms of vitamin A are associated with specific cellular proteins which bind with retinoids within cells during metabolism and with nuclear receptors that mediate retinoid action on the genome (9). Retinoids modulate the transcription of several hundreds of genes (10–12). In addition to the latter role of retinoic acid, retinol is the form required for functions in the visual (13) and reproductive systems (14) and during embryonic development (15).

Holo-RBP is filtered into the glomerulus but recovered from the kidney tubule and recycled. Normally vitamin A leaves the body in urine only as inactive metabolites resulting from tissue utilization and in bile secretions as potentially recyclable active glucuronide conjugates of retinol (8). No single urinary metabolite has been identified which accurately reflects tissue levels of vitamin A or its rate of utilization. Hence, at this time urine is not a useful biological fluid for assessment of vitamin A nutriture.

### 2.1.2 Biochemical mechanisms for vitamin A functions

Vitamin A functions at two levels in the body: the first is in the visual cycle in the retina of the eye; the second is in all body tissues where it systemically maintains the growth and soundness of cells. In the visual system, carrier-bound retinol is transported to ocular tissue and to the retina by intracellular binding and transport proteins. Rhodopsin, the visual pigment critical to dim-light vision, is formed in rod cells after conversion of all-*trans*-retinol to retinaldehyde, isomerization to the 11-*cis*-form, and binding to opsin. Alteration of rhodopsin through a cascade of photochemical reactions results in the ability to see objects in dim light (13). The speed at which rhodopsin is regenerated is related to the availability of retinol. Night blindness is usually an indicator of inadequate available retinol, but it can also be due to a deficit of other nutrients that are critical to the regeneration of rhodopsin, such as protein and zinc, and to some inherited diseases, such as retinitis pigmentosa.

The growth and differentiation of epithelial cells throughout the body are especially affected by vitamin A deficiency (VAD). In addition, goblet cell numbers are reduced in epithelial tissues and as a consequence, mucous secretions (with their antimicrobial components) diminish. Cells lining protective tissue surfaces fail to regenerate and differentiate, hence they flatten and accumulate keratin. Both factors—the decline in mucous secretions and loss of cellular integrity—reduce the body's ability to resist invasion from potentially pathogenic organisms. Pathogens can also compromise the immune system by directly interfering with the production of some types of protective secre-

tions and cells (11). Classical symptoms of xerosis (drying or non-wetability) and desquamation of dead surface cells as seen in ocular tissue (i.e. xerophthalmia) are the external evidence of the changes also occurring to various degrees in internal epithelial tissues.

Current understanding of the mechanism of vitamin A action within cells outside the visual cycle is that cellular functions are mediated through specific nuclear receptors. Binding with specific isomers of retinoic acid (i.e. all-*trans*- and 9-*cis*-retinoic acid) activates these receptors. Activated receptors bind to DNA response elements located upstream of specific genes to regulate the level of expression of those genes (12). These retinoid-activated genes regulate the synthesis of a large number of proteins vital to maintaining normal physiologic functions. There may, however, be other mechanisms of action that are as yet undiscovered (10).

## **2.2 Populations at risk for, and consequences of, vitamin A deficiency**

### **2.2.1 Definition of vitamin A deficiency**

VAD is not easily defined. WHO defines it as tissue concentrations of vitamin A low enough to have adverse health consequences even if there is no evidence of clinical xerophthalmia (16). In addition to the specific signs and symptoms of xerophthalmia and the risk of irreversible blindness, non-specific symptoms include increased morbidity and mortality, poor reproductive health, increased risk of anaemia, and contributions to slowed growth and development. However, these nonspecific adverse effects may be caused by other nutrient deficits as well, making it difficult to attribute non-ocular symptoms specifically to VAD in the absence of biochemical measurements reflective of vitamin A status.

### **2.2.2 Geographic distribution and magnitude**

In 1995, WHO estimated the global distribution of VAD (Table 2.1) and categorized countries according to the seriousness of VAD as a public health problem on the basis of both clinical and moderate and severe subclinical (prevalence of low blood levels of retinol) indicators of deficiency (16, 17). It was estimated that about 3 million children have some form of xerophthalmia and, on the basis of blood levels, another 250 million are subclinically deficient (17). The magnitude of the subclinical estimate is currently being re-evaluated to establish quantitatively a benchmark for measuring prevalence trends. The actual number of subclinical deficiencies based on the prevalence of low serum levels of retinol, however, remains uncertain because

TABLE 2.1  
**Estimates of clinical and subclinical vitamin A deficiency in preschool children, by WHO region<sup>a</sup>**

Region	Clinical (millions)	Subclinical (severe and moderate) (millions)	Prevalence (%)
Africa	1.04	52	49
The Americas	0.06	16	20
South-East Asia	1.45	125	69
Europe	NA	NA	NA
Eastern Mediterranean	0.12	16	22
Western Pacific	0.13	42	27
<b>Subtotal</b>	2.80	251	
<b>Total</b>		254	

NA, not applicable.

<sup>a</sup> Based on a projection for 1994 from those countries in each region where data were available.

Source: adapted from reference (17).

of the confounding and poorly quantified role of infections (see section 2.2.5).

Epidemiological studies repeatedly report clustering of VAD, presumably resulting from concurrent occurrences of several risk factors. This clustering may occur among both neighbourhoods and households (18).

### 2.2.3 Age and sex

VAD can occur in individuals of any age. However, it is a disabling and potentially fatal public health problem for children under 6 years of age. VAD-related blindness is most prevalent in children under 3 years of age (19). This period of life is characterized by high requirements for vitamin A to support rapid growth, and the transition from breastfeeding to dependence on other dietary sources of the vitamin. In addition, adequate intake of vitamin A reduces the risk of catching respiratory and gastrointestinal infections. The increased mortality risk from concurrent infections extends at least to 6 years of age and is associated with both clinical and subclinical VAD (20). There is little information regarding the health consequences of VAD in school-age children. The prevalence of Bitot's spots (i.e. white foamy patches on the conjunctiva) may be highest in this age group but their occurrence may reflect past more than current history of VAD (21). Women of reproductive age are also thought to be vulnerable to VAD during pregnancy and lactation because they often report night blindness (22, 23) and because their breast milk is fre-

quently low in vitamin A (24, 25). Not all night blindness in pregnant women, however, responds to vitamin A treatment (23).

There is no consistent, clear indication in humans of a sex differential in vitamin A requirements during childhood. Growth rates, and presumably the need for vitamin A, from birth to 10 years for boys are consistently higher than those for girls (26). In the context of varied cultural and community settings, however, variations in gender-specific child-feeding and care practices are likely to subsume a small sex differential in requirements to account for reported sex differences in the prevalence of xerophthalmia. Pregnant and lactating women require additional vitamin A to support maternal and fetal tissue growth and lactation losses, additional vitamin A which is not needed by other post-adolescent adults (27).

#### **2.2.4 Risk factors**

VAD is most common in populations consuming most of their vitamin A needs from provitamin carotenoid sources and where minimal dietary fat is available (28). About 90% of ingested preformed vitamin A is absorbed, whereas the absorption efficiency of provitamin A carotenoids varies widely, depending on the type of plant source and the fat content of the accompanying meal (29). Where possible, an increased intake of dietary fat is likely to improve the absorption of vitamin A in the body.

In areas with endemic VAD, fluctuations in the incidence of VAD throughout the year reflect the balance between intake and need. Periods of general food shortage (and specific shortages in vitamin A-rich foods) coincide with peak incidence of VAD and common childhood infectious diseases (e.g. diarrhoea, respiratory infections, and measles). Seasonal food availability influences VAD prevalence directly by influencing access to provitamin A sources; for example, the scarcity of mangoes in hot arid months followed by the glutting of the market with mangoes during harvest seasons (30). Seasonal growth spurts in children, which frequently follow seasonal post-harvest increases in energy and macronutrient intakes, can also affect the balance. These increases are usually obtained from staple grains (e.g. rice) and tubers (e.g. light-coloured yams) that are not, however, good sources of some micronutrients (e.g. vitamin A) to support the growth spurt (31).

Food habits and taboos often restrict consumption of potentially good food sources of vitamin A (e.g. mangoes and green leafy vegetables). Culture-specific factors for feeding children, adolescents, and pregnant and lactating women are common (28, 32–34). Illness- and childbirth-related proscriptions of the use of specific foods pervade many traditional cultures (35). Such influences alter short- and long-term food distribution within families. However,

some cultural practices can be protective of vitamin A status and they need to be identified and reinforced.

### 2.2.5 Morbidity and mortality

The consequences of VAD are manifested differently in different tissues. In the eye, the symptoms and signs, together referred to as xerophthalmia, have a long, well-recognized history and have until recently been the basis for estimating the global burden from the disease (19). Although ocular symptoms and signs are the most specific indicators of VAD, they occur only after other tissues have impaired functions that are less specific and less easily assessed.

The prevalence of ocular manifestations (i.e. xerophthalmia or clinical VAD) is now recognized to far underestimate the magnitude of the problem of functionally significant VAD. Many more preschool-age children, and perhaps older children and women who are pregnant or lactating, have their health compromised when they are subclinically deficient. In young children, subclinical deficiency, like clinical deficiency, increases the severity of some infections, particularly diarrhoea and measles, and increases the risk of death (20, 36). Moreover, the incidence (37) and prevalence (38) of diarrhoea may also increase with subclinical VAD. Meta-analyses conducted by three independent groups using data from several randomized trials provide convincing evidence that community-based improvement of the vitamin A status of deficient children aged 6 months to 6 years reduces their risk of dying by 20–30% on average (20, 39, 40). Mortality in children who are blind from keratomalacia or who have corneal disease is reported to be from 50% to 90% (19, 41), and measles mortality associated with VAD is increased by up to 50% (42). Limited data are available from controlled studies of the possible link between morbidity history and vitamin A status of pregnant and lactating women (43).

There are discrepancies in the link between incidence and severity of infectious morbidity of various etiologies and vitamin A status. A great deal of evidence supports an association of VAD with severity of an infection once acquired, except for respiratory diseases, which are non-responsive to treatment (16, 36–38, 44). The severity of pneumonia associated with measles, however, is an exception because it decreases with the treatment of vitamin A supplementation (42, 45).

Infectious diseases depress circulating retinol and contribute to vitamin A depletion. Enteric infections may alter the absorptive surface area, compete for absorption-binding sites, and increase urinary loss (7, 46, 47). Febrile systemic infections also increase urinary loss (6, 48) and metabolic utilization

rates and may reduce apparent retinol stores if fever occurs frequently (49). In the presence of latent deficiency, disease occurrence is often associated with precipitating ocular signs (50, 51). Measles virus infection is especially devastating to vitamin A metabolism, adversely interfering with both efficiencies of utilization and conservation (42, 51, 52). Severe protein–energy malnutrition affects many aspects of vitamin A metabolism, and even when some retinyl ester stores are still present, malnutrition—often coupled with infection—can prevent transport-protein synthesis, resulting in immobilization of existing vitamin A stores (53).

The compromised integrity of the epithelium, together with the possible alteration in hormonal balance at severe levels of deficiency, impairs normal reproductive functions in animals (9, 14, 15, 24, 54, 55). Controlled human studies are, of course, lacking. In animals and humans, congenital anomalies can result if the fetus is exposed to severe deficiency or large excesses of vitamin A at critical periods early in gestation (first trimester) when fetal organs are being formed (24, 56). Reproductive performance, as measured by infant outcomes, in one community-based clinical intervention trial, however, was not influenced by vitamin A status (43).

The growth of children may be impaired by VAD. Interventions with vitamin A only have not consistently demonstrated improved growth in community studies because VAD seldom occurs in isolation from other nutrient deficiencies that also affect growth and may be more limiting (57).

A lack of vitamin A can affect iron metabolism when deficiencies of both nutrients coexist and particularly in environments that favour frequent infections (58). Maximum haemoglobin response occurs when iron and vitamin A deficiencies are corrected together (59). VAD appears to influence the availability of storage iron for use by haematopoietic tissue (59, 60). However, additional research is needed to clarify the mechanisms of the apparent interaction.

### 2.3 Units of expression

In blood, tissues, and human milk, vitamin A levels are conventionally expressed in  $\mu\text{g}/\text{dl}$  or  $\mu\text{mol}/\text{l}$  of all-*trans*-retinol. Except for postprandial conditions, most of the circulating vitamin A is retinol whereas in most tissues (such as the liver), secretions (such as human milk), and other animal food sources, it exists mainly as retinyl esters, which are frequently hydrolysed before analytical detection.

To express the vitamin A activity of carotenoids in diets on a common basis, a Joint FAO/WHO Expert Group (61) in 1967 introduced the concept

of the retinol equivalent (RE) and established the following relationships among food sources of vitamin A:

1 $\mu\text{g}$ retinol	= 1 RE
1 $\mu\text{g}$ $\beta$ -carotene	= 0.167 $\mu\text{g}$ RE
1 $\mu\text{g}$ other provitamin A carotenoids	= 0.084 $\mu\text{g}$ RE.

These equivalencies were derived from balance studies to account for the less efficient absorption of carotenoids (at that time thought to be about one third that of retinol) and their bioconversion to vitamin A (one half for  $\beta$ -carotene and one fourth for other provitamin A carotenoids). It was recognized at the time that the recommended conversion factors (i.e. 1:6 for vitamin A: $\beta$ -carotene and 1:12 for vitamin A:all other provitamin carotenoids) were only best approximations for a mixed diet, which could under- or overestimate bioavailability depending not only on the quantity and source of carotenoids in the diet, but also on how the foods were processed and served (e.g. cooked or raw, whole or puréed, with or without fat). In 1988, a Joint FAO/WHO Expert Consultation (62) confirmed these conversion factors for operational application in evaluating mixed diets. In reaching its conclusion, the Consultation noted the controlled depletion–repletion studies in adult men using a dark adaptation endpoint that reported a 2:1 equivalency of supplemental  $\beta$ -carotene to retinol (63), and the range of factors that could alter the equivalency ratio when dietary carotenoids replaced supplements.

Recently there has been renewed interest in re-examining conventional conversion factors by using more quantitative stable isotope techniques for measuring whole-body stores in response to controlled intakes (64–66) and by following post-absorption carotenoids in the triacylglycerol-rich lipoprotein fraction (67–70). The data are inconsistent but suggest that revision toward lower absorbability of provitamin A carotenoids is warranted (64, 68, 69). These studies indicate that the conditions that limit carotenoids from entering enterocytes rather than conversion once in the enterocyte are more significant than previously thought (71).

Other evidence questions the validity of factors used earlier, which suggests that 6  $\mu\text{g}$  of food-sourced  $\beta$ -carotene is equivalent to 2  $\mu\text{g}$  pure  $\beta$ -carotene in oil, and equivalent to 1  $\mu\text{g}$  dietary retinol. Currently, however, only one study has used post-absorptive serum carotenoids to directly compare, in healthy, adequately nourished adult humans in Holland, the absorption of carotene in oil with that of dietary  $\beta$ -carotene from a mixed diet predominately containing vegetables (72). The investigators reported that

about 7 $\mu\text{g}$  of  $\beta$ -carotene from the mixed predominately vegetable diet is equivalent to 1 $\mu\text{g}$  pure  $\beta$ -carotene when it is provided in oil. Assuming that 2 $\mu\text{g}$   $\beta$ -carotene in the enterocyte is equivalent to 1 $\mu\text{g}$  retinol, the conversion factor would be 1:14 for  $\beta$ -carotene and 1:28 for other provitamin A carotenoids. Other researchers using a similar methodology have reported factors from a variety of specific food sources that fall within this range. Lowest bioavailability is reported for leafy green vegetables and raw carrots and highest for fruit/tuber diets (68, 73–75). In view of the data available to date, conversion factors from usual mixed vegetable diets of 1:14 for  $\beta$ -carotene and 1:28 for other provitamin A carotenoids as suggested by Van het Hof et al. (72) are recommended. Where green leafy vegetables or fruits are more prominent than in the usual diet in Holland, adjustment to higher or lower conversion factors could be considered. For example, in the United States of America where fruits constitute a larger portion of the diet, the Food and Nutrition Board of the Institute of Medicine suggests retinol activity equivalency (RAE) factors of 12:1 for  $\beta$ -carotene and 24:1 for other provitamin A carotenoids (76).

Retinol equivalents in a diet are calculated as the sum of the weight of the retinol portion of preformed vitamin A plus the weight of  $\beta$ -carotene divided by its conversion factor, plus the weight of other provitamin A carotenoids divided by their conversion factor (62). Most recent food composition tables report  $\beta$ -carotene and, sometimes, other provitamin A carotenoids as  $\mu\text{g/g}$  edible portion. However, older food composition tables frequently report vitamin A as international units (IUs). The following conversion factors can be used to calculate comparable values as  $\mu\text{g}$ :

$$\begin{aligned} 1 \text{ IU retinol} &= 0.3 \mu\text{g retinol} \\ 1 \text{ IU } \beta\text{-carotene} &= 0.6 \mu\text{g } \beta\text{-carotene} \\ 1 \text{ IU retinol} &= 3 \text{ IU } \beta\text{-carotene.} \end{aligned}$$

It is strongly recommended that weight or molar units replace the use of IUs to decrease confusion and overcome limitations in the non-equivalence of the IU values for retinol and  $\beta$ -carotene. For example, after converting all values from food composition tables to weight units, the vitamin A equivalency of a mixed diet should be determined by dividing the weight by the recommended weight equivalency value for preformed and specific provitamin A carotenoids. Hence, if a diet contained 150 $\mu\text{g}$  retinol, 1550 $\mu\text{g}$   $\beta$ -carotene, and 1200 $\mu\text{g}$  other provitamin A carotenoids, the vitamin A equivalency of the diet would be:

$$150 \mu\text{g} + (1550 \mu\text{g} \div 14) + (1200 \mu\text{g} \div 28) = 304 \mu\text{g retinol equivalency.}$$

## 2.4 Sources and supply patterns of vitamin A

### 2.4.1 Dietary sources

Preformed vitamin A is found almost exclusively in animal products, such as human milk, glandular meats, liver and fish liver oils (especially), egg yolk, whole milk, and other dairy products. Preformed vitamin A is also used to fortify processed foods, which may include sugar, cereals, condiments, fats, and oils (77). Provitamin A carotenoids are found in green leafy vegetables (e.g. spinach, amaranth, and young leaves from various sources), yellow vegetables (e.g. pumpkins, squash, and carrots), and yellow and orange non-citrus fruits (e.g. mangoes, apricots, and papayas). Red palm oil produced in several countries worldwide is especially rich in provitamin A (78). Some other indigenous plants also may be unusually rich sources of provitamin A. Such examples are the palm fruit known in Brazil as *buriti*, found in areas along the Amazon River (as well as elsewhere in Latin America) (79), and the fruit known as *gac* in Viet Nam, which is used to colour rice, particularly on ceremonial occasions (80). Foods containing provitamin A carotenoids tend to have less biologically available vitamin A but are more affordable than animal products. It is mainly for this reason that carotenoids provide most of the vitamin A activity in the diets of economically deprived populations.

### 2.4.2 Dietary intake and patterns

Although vitamin A status cannot be assessed from dietary intake alone, dietary intake assessment can provide evidence of risk of an inadequate status. However, quantitative collection of dietary information is fraught with measurement problems. These problems arise both from obtaining representative quantitative dietary histories from individuals, communities, or both, and from interpreting these data while accounting for differences in bioavailability, preparation losses, and variations in food composition data among population groups (77). This is especially difficult in populations consuming most of their dietary vitamin A from provitamin carotenoid sources. Simplified guidelines have been developed recently in an effort to improve the collection of reliable dietary intake information from individuals and communities (69, 81).

### 2.4.3 World and regional supply and patterns

In theory, the world's food supply is sufficient to meet global requirements for vitamin A. Great differences exist, however, in the availability of sources (animal and vegetable) and in per capita consumption of the vitamin among different countries, age categories, and socioeconomic groups. VAD as a global public health problem is therefore largely due to inequitable food dis-

tribution among and within countries and households in relation to the need for ample bioavailable vitamin A sources (82, 83).

FAO global estimates for 1984 indicate that preformed vitamin A constituted about one third of total dietary vitamin A activity (62). World availability of vitamin A for human consumption at that time was approximately 220 µg of preformed retinol per capita per day and 560 µg RE from provitamin carotenoids (about 3400 µg carotenoids for a 1:6 conversion factor) per person per day, a total of about 790 µg RE. These values are based on supply estimates and not consumption estimates. Losses commonly occur during food storage and processing, both industrially and in the home (77).

The estimated available regional supply of vitamin A from a more recent global evaluation shown in Table 2.2 illustrates the variability in amounts and sources of vitamin A. This variability is linked to access to the available supply of foods containing vitamin A, which varies with household income, with poverty being a yardstick for risk of VAD. VAD is most prevalent in South-East Asia, Africa, and the Western Pacific (Table 2.1), where vegetable sources contribute nearly 80% or more of the available supply of retinol equivalents. Furthermore, in South-East Asia the total available supply is about half of that of most other regions and is particularly low in animal sources. In contrast, the Americas, Eastern Mediterranean, and Europe have a supply ranging from 700 to 1000 µg RE/day, one third of which comes from animal sources. Based on national data from the United States Continuing Survey of Food Consumption (84) and the third National Health and Nutrition Examination Survey (85) mean dietary intakes of children aged 0–6 years were estimated to be  $864 \pm 497$  and  $921 \pm 444$  µg RE per day, respectively. In the Dietary and Nutritional Survey of British Adults (86), the median intake of men and women aged 35–49 years was 1118 µg RE and 926 µg RE, respectively, which corresponded to serum retinol concentrations of 2.3 µmol/l and 1.8 µmol/l, respectively. In a smaller scale survey in the United Kingdom, median intakes for non-pregnant women who did not consume liver or liver products during the survey week were reported to be 686 µg RE per day (87).

The available world supply figures in Table 2.2 were recently recalculated using a bioavailability ratio of 1:30 for retinol to other provitamin A carotenoids (88). This conversion factor was justified on the basis of one published controlled intervention study conducted in Indonesia (89) and a limited number of other studies not yet published in full. Applying the unconfirmed conversion factor to the values in Table 2.2 would lead to the conclusion that regional and country needs for vitamin A could not be met from predominantly vegetarian diets. However, this is inconsistent with the preponderance of epidemiological evidence. Most studies report a positive response when

TABLE 2.2  
**Available supply of vitamin A, by WHO region**

Region	Animal sources ( $\mu\text{g RE/day}$ )	Vegetable sources ( $\mu\text{g RE/day}$ )	Total ( $\mu\text{g RE/day}$ )
Africa	122	654 (84) <sup>a</sup>	776
The Americas	295	519 (64)	814
South-East Asia	53	378 (90)	431
Europe	271	467 (63)	738
Eastern Mediterranean	345	591 (63)	936
Western Pacific	216	781 (78)	997
<b>Total</b>	212	565 (72)	777

<sup>a</sup> Numbers in parentheses indicate the percentage of total retinol equivalents from carotenoid food sources.

Source: reference (20).

vegetable sources of provitamin A are given under controlled conditions to deficient subjects freed of confounding parasite loads and provided with sufficient dietary fat (90, 91). Emerging data are likely to justify a lower biological activity for provitamin A carotenoids because of the mix of total carotenoids found in food sources in a usual meal (67–69). The present Consultation concluded that the 1:6 bioconversion factor originally derived on the basis of balance studies should be retained until there is firm confirmation of more precise methodologies from ongoing studies.

## 2.5 Indicators of vitamin A deficiency

### 2.5.1 Clinical indicators of vitamin A deficiency

Ocular signs of VAD are assessed by clinical examination and history, and are quite specific in preschool-age children. However, these are rare occurrences that require examination of large populations in order to obtain incidence and prevalence data. Subclinical VAD being the more prevalent requires smaller sample sizes for valid prevalence estimates (16).

A full description of clinical indicators of VAD, with coloured illustrations for each, can be found in the WHO field guide (19). The most frequently occurring is night-blindness, which is the earliest manifestation of xerophthalmia. In its mild form it is generally noticeable after stress from a bright light that bleaches the rhodopsin (visual purple) found in the retina. VAD prolongs the time to regenerate rhodopsin, and thus delays adaptation time in dark environments. Night-blind young children tend to stumble when going from bright to dimly-lit areas and they, as well as night-blind mothers, tend to remain inactive at dusk and at night (92).

No field-applicable objective tool is currently available for measuring night-blindness in children under about 3 years of age. However, it can be measured

by history in certain cultures (93). In areas where night-blindness is prevalent, many cultures coin a word descriptive of the characteristic symptom that they can reliably recall on questioning, making this a useful tool for assessing the prevalence of VAD (94). It must be noted that questioning for night-blindness is not always a reliable assessment measure where a local term is absent. In addition, there is no clearly defined blood retinol level that is directly associated with occurrence of the symptom, such that could be used in conjunction with questioning. Vitamin A-related night-blindness, however, responds rapidly (usually within 1–2 days) to administration of vitamin A.

### 2.5.2 Subclinical indicators of vitamin A deficiency

Direct measurement of concentrations of vitamin A in the liver (where it is stored) or in the total body pool relative to known specific vitamin A-related conditions (e.g. night-blindness) would be the indicator of choice for determining requirements. This cannot be done with the methodology currently available for population use. There are several more practical biochemical methods for estimating subclinical vitamin A status but all have limitations (16, 93, 95, 96). Each method is useful for identifying deficient populations, but not one of these indicators is definitive or directly related quantitatively to disease occurrence. The indicators of choice are listed in Table 2.3. These indicators are less specific to VAD than clinical signs of the eye and less sensitive than direct measurements for evaluating subclinical vitamin A status. WHO recommends that where feasible at least two subclinical biochemical indicators, or one biochemical and a composite of non-biochemical risk factors, should be measured and that both types of indicators should point to deficiency in order to identify populations at high risk of VAD (16). Cut-off points given in Table 2.3 represent the consensus gained from practical experience in comparing populations with some evidence of VAD with those without VAD. There are no field studies that quantitatively relate the prevalence of adverse health symptoms (e.g. incidence or prevalence of severe diarrhoeal disease) and relative levels of biologic indicator cut-off values. Furthermore, each of the biochemical indicators listed is subject to confounding factors which may be unrelated to vitamin A status (e.g. infections).

Although all biochemical indicators currently available have limitations, the preferred biochemical indicator for population assessment is the distribution of serum levels of vitamin A (serum retinol). Only at very low blood levels ( $<0.35\ \mu\text{mol/l}$ ) is there an association with corneal disease prevalence (97). Blood levels between  $0.35$  and  $0.70\ \mu\text{mol/l}$  are likely to characterize subclinical deficiency (98), but subclinical deficiency may still be present at levels

TABLE 2.3

**Indicators of subclinical VAD in mothers and in children aged 6–71 months**

Indicator	Cut-off to indicate deficiency
Night-blindness (24–71 months)	≥1% report a history of night-blindness
Biochemical	
Breast-milk retinol	≤1.05 μmol/l (≤8 μg/g milk fat)
Serum retinol	≤0.70 μmol/l
Relative dose response	≥20%
Modified relative dose response	Ratio ≥0.06

Source: adapted from reference (16).

between 0.70 and 1.05 μmol/l and occasionally above 1.05 μmol/l (99). The prevalence of values below 0.70 μmol/l is a generally accepted population cut-off for preschool-age children to indicate risk of inadequate vitamin A status (16) and above 1.05 μmol/l to indicate an adequate status (100, 101). As noted elsewhere, clinical and subclinical infections can lower serum levels of vitamin A on average by as much as 25%, independently of vitamin A intake (102, 103). Therefore, at levels between about 0.5 and 1.05 μmol/l, the relative dose response or the modified relative dose response test on a subsample of the population can be useful for identifying the prevalence of critically depleted body stores when interpreting the left portion of serum retinol distribution curves.

## 2.6 Evidence used for making recommendations

Requirements and safe levels of intake for vitamin A recommended in this report do not differ significantly from those proposed by the 1988 Joint FAO/WHO Expert Consultation (62) except to the extent that they have been adapted to the age, pregnancy, and lactation categories defined by the present Expert Consultation. The term “safe level of intake” used in the 1988 report is retained because the intake levels do not strictly correspond to the definition of a recommended nutrient intake recommended here (see section 1.2).

The mean requirement for an individual is defined as the minimum daily intake of vitamin A, expressed as μg retinol equivalents (μg RE), to prevent xerophthalmia in the absence of clinical or subclinical infection. This intake should account for the proportionate bioavailability of preformed vitamin A (about 90%) and provitamin A carotenoids from a diet that contains sufficient fat (e.g. at least 10g daily). The required level of intake is set to prevent clinical signs of deficiency, allow for normal growth, and reduce the risk of

vitamin A-related severe morbidity and mortality within any given population. It does not allow for frequent or prolonged periods of infections or other stresses.

The safe level of intake for an individual is defined as the average continuing intake of vitamin A required to permit adequate growth and other vitamin A-dependent functions and to maintain an acceptable total body reserve of the vitamin. This reserve helps offset periods of low intake or increased need resulting from infections and other stresses. Useful indicators include a plasma retinol concentration above  $0.70\mu\text{mol/l}$ , which is associated with a relative dose response below 20%, or a modified relative dose response below 0.06. For lactating women, breast-milk retinol levels above  $1.05\mu\text{mol/l}$  (or above  $8\mu\text{g/g}$  milk fat) are considered to reflect minimal maternal stores because levels above  $1.05\mu\text{mol/l}$  are common in populations known to be healthy and without evidence of insufficient dietary vitamin A (24, 25).

### 2.6.1 Infants and children

Vitamin A requirements for infants are calculated from the vitamin A provided in human milk. During at least the first 6 months of life, exclusive breastfeeding can provide sufficient vitamin A to maintain health, permit normal growth, and maintain sufficient stores in the liver (104).

Reported retinol concentrations in human milk vary widely from country to country ( $0.70\text{--}2.45\mu\text{mol/l}$ ). In some developing countries, the vitamin A intake of breast-fed infants who grow well and do not show signs of deficiency ranges from 120 to  $170\mu\text{g RE/day}$  (25, 104). Such intakes are considered adequate to cover infant requirements if the infant's weight is assumed to be at least at the 10th percentile according to WHO standards (62). However, this intake is unlikely to build adequate body stores, given that xerophthalmia is common in preschool-age children in the same communities with somewhat lower intakes. Because of the need for vitamin A to support the growth rate of infancy, which can vary considerably, a requirement estimate of  $180\mu\text{g RE/day}$  seems appropriate.

The safe level for infants up to 6 months of age is based on observations of breast-fed infants in communities in which good nutrition is the norm. Average consumption of human milk by such infants is about  $750\text{ml/day}$  during the first 6 months (104). Assuming an average concentration of vitamin A in human milk of about  $1.75\mu\text{mol/l}$ , the mean daily intake would be about  $375\mu\text{g RE}$ , which is therefore the recommended safe level. From 7–12 months, human milk intake averages  $650\text{ml/day}$ , which would provide  $325\mu\text{g}$  of vitamin A daily. Because breast-fed infants in endemic vitamin A-deficient

populations are at increased risk of death from 6 months onward, the requirement and recommended safe intake levels are increased to 190 µg RE/day and 400 µg RE/day, respectively.

The requirement (with allowance for variability) and the recommended safe intake for older children may be estimated from those derived for late infancy (i.e. 20 and 39 µg RE/kg body weight/day) (62). On this basis, and including allowances for storage requirements and variability, requirements for preschool-age children would be in the range of 200–400 µg RE daily. In poor communities where children 1–6 years old are reported to have intakes of about 100–200 µg RE/day, signs of VAD do occur; in southern India these signs were relieved and risk of mortality was reduced when the equivalent of 350–400 µg RE/day was given to children weekly (105). In the United States, most preschool-age children maintain serum retinol levels of 0.70 µmol/l or higher while consuming diets providing 300–400 µg RE/day (from the data-bank for the third National Health and Nutrition Examination Survey [<http://www.cdc.gov/nchs/nhanes.htm>]).

### 2.6.2 Adults

Estimates for the requirements and recommended safe intakes for adults are also extrapolated from those derived for late infancy, i.e. 4.8 and 9.3 µg RE/kg body weight/day (62). Detailed account of how the requirement for vitamin A is arrived at is provided in the FAO/WHO report of 1988 (62) and is not repeated here because no new studies have been published that indicate a need to revise the assumptions on which those calculations were based. The safe intakes recommended are consistent with the per capita vitamin A content in the food supply of countries that show adequate vitamin A status in all sectors of the population. Additional evidence that the existing safe level of intake is adequate for adults on a population basis is provided by an analysis of dietary data from the 1990 survey of British adults in whom there was no evidence of VAD (86). In another survey in the United Kingdom, the median intake of vitamin A among non-pregnant women who did not consume liver or liver products during the survey week was 686 µg RE/day (87). This value is substantially above the estimated mean requirement for pregnant women and falls quite short of the amount at which teratology risk is reported (106–108). About one third of the calculated retinol equivalents consumed by the British women came from provitamin A sources (20% from carrots).

### 2.6.3 Pregnant women

During pregnancy, women need additional vitamin A to sustain the growth of the fetus and to provide a limited reserve in the fetal liver, as

well as to maintain their own tissue growth. Currently, there are no reliable figures available for the specific vitamin A requirements for these processes (27).

Newborn infants need around 100 $\mu$ g of retinol daily to meet their needs for growth. During the third trimester the fetus grows rapidly and, although obviously smaller in size than the infant born full term, the fetus presumably has similar needs. Incremental maternal needs associated with pregnancy are assumed to be provided from maternal reserves in populations of adequately nourished healthy mothers. In populations consuming vitamin A at the basal requirement, an additional increment of 100 $\mu$ g/day during the full gestation period should enhance maternal storage during early pregnancy and allow for adequate amounts of vitamin A to be available for the rapidly growing fetus in late pregnancy. However, this increment may be minimal for women who normally ingest only the basal requirement of vitamin A, inasmuch as the needs and growth rate of the fetus will not be affected by the mother's initial vitamin A reserves.

A recent study in Nepal (43), where night-blindness is prevalent in pregnant women, provided 7000 $\mu$ g RE (about 23 300IU) weekly to pregnant and lactating women (equivalent to 1000 $\mu$ g RE/day). This level of intake normalized serum levels of vitamin A and was associated with a decrease in prevalence of night-blindness and a decrease in maternal mortality. However, the findings of this study need to be confirmed. In the interim period it seems prudent, recognizing that a large portion of the world's population of pregnant women live under conditions of deprivation, to increase by 200 $\mu$ g RE the recommended safe level to ensure adequacy of intake during pregnancy. Because therapeutic levels of vitamin A are generally higher than preventive levels, the safe intake level recommended during pregnancy is 800 $\mu$ g RE/day. Women who are or who might become pregnant should carefully limit their total daily vitamin A intake to a maximum of 3000 $\mu$ g RE (10000IU) to minimize risk of fetal toxicity (109).

#### **2.6.4 Lactating women**

If the amount of vitamin A recommended for infants is supplied by human milk, mothers who are breastfeeding should intake at least as much vitamin A in their diets as is needed to replace the amount lost through breastfeeding. Thus, the increments in basal and safe recommended intakes during lactation are 180 $\mu$ g RE and 350 $\mu$ g RE, respectively. After the infant reaches the age of 6 months or when solid foods are introduced, the mother's need for additional amounts of vitamin A lessens.

### 2.6.5 Elderly

There is no indication that the vitamin A requirements of healthy elderly individuals differ from those of other adults. It should be remembered, however, that diseases that impede vitamin A absorption, storage, and transport might be more common in the elderly than in other age groups.

## 2.7 Recommendations for vitamin A requirements

Table 2.4 summarizes the estimated mean requirements for vitamin A and the recommended safe intakes, taking into account the age and sex differences in mean body weights. For most values the true mean and variance are not known. It should be noted that there are no adequate data available to derive mean requirements for any group and, therefore, a recommended nutrient intake cannot be calculated. However, information is available on cures achieved in a few vitamin A-deficient adult men and on the vitamin A status of groups receiving intakes that are low but nevertheless adequate to prevent the appearance of deficiency-related syndromes. The figures for mean dietary requirements are derived from these, with the understanding that the curative dose is higher than the preventive dose. They are at the upper limits of the range so as to cover the mean dietary requirements of 97.5% of the population (62).

TABLE 2.4

### Estimated mean requirement and safe level of intake for vitamin A, by group

Group	Mean requirement ( $\mu\text{g RE/day}$ )	Recommended safe intake ( $\mu\text{g RE/day}$ )
<i>Infants and children</i>		
0–6 months	180	375
7–12 months	190	400
1–3 years	200	400
4–6 years	200	450
7–9 years	250	500
<i>Adolescents,</i>		
10–18 years	330–400	600
<i>Adults</i>		
<i>Females,</i>		
19–65 years	270	500
65+ years	300	600
<i>Males,</i>		
19–65 years	300	600
65+ years	300	600
<i>Pregnant women</i>	370	800
<i>Lactating women</i>	450	850

Source: adapted from reference (62).

In calculating the safe intake, a normative storage requirement was calculated as a mean for adults equivalent to 434 µg RE/day, and the recommended safe intake was derived in part by using this value plus 2 standard deviations. It is doubtful that this value can be applied to growing children. The safe intake for children was compared with the distribution of intakes and comparable serum vitamin A levels reported for children 0–6 years of age from the United States and with distributions of serum levels of vitamin A of children aged 9–62 months in Australia (110), where evidence of VAD is rare.

## 2.8 Toxicity

Because vitamin A is fat soluble and can be stored, primarily in the liver, routine consumption of large amounts of vitamin A over a period of time can result in toxic symptoms, including liver damage, bone abnormalities and joint pain, alopecia, headaches, vomiting, and skin desquamation. Hypervitaminosis A appears to be due to abnormal transport and distribution of vitamin A and retinoids caused by overloading of the plasma transport mechanisms (111).

The smallest daily supplement associated with liver cirrhosis that has been reported is 7500 µg taken for 6 years (107, 108). Very high single doses can also cause transient acute toxic symptoms that may include bulging fontanelles in infants; headaches in older children and adults; and vomiting, diarrhoea, loss of appetite, and irritability in all age groups. Rarely does toxicity occur from ingestion of food sources of preformed vitamin A. When this occurs, it usually results from very frequent consumption of liver products. Toxicity from food sources of provitamin A carotenoids is not reported, except for the cosmetic yellowing of skin.

Infants, including neonates (112), administered single doses equivalent to 15 000–30 000 µg retinol (50 000–100 000 IU) in oil generally show no adverse symptoms. However, daily prophylactic or therapeutic doses should not exceed 900 µg, which is well above the mean requirement of about 200 µg/day for infants. An increase in bulging fontanelles occurred in infants under 6 months of age in one endemically deficient population given two or more doses of 7500 µg or 15 000 µg preformed vitamin A in oil (113, 114), but other large-scale controlled clinical trials have not reported increased bulging after three doses of 7500 µg given with diphtheria-pertussis-tetanus immunizations at about 6, 10, and 14 weeks of age (115). No effects were detected at 3 years of age that related to transient vitamin A-induced bulging that had occurred before 6 months of age (112, 116).

Most children aged 1–6 years tolerate single oral doses of 60 000 µg (200 000 IU) vitamin A in oil at intervals of 4–6 months without adverse

symptoms (107). Occasionally diarrhoea or vomiting is reported but these symptoms are transient with no lasting sequelae. Older children seldom experience toxic symptoms unless they habitually ingest vitamin A in excess of 7500 µg (25 000 IU) for prolonged periods of time (107).

When women take vitamin A at daily levels of more than 7500 µg (25 000 IU) during the early stages of gestation, fetal anomalies and poor reproductive outcomes are reported (108). One report suggests an increased risk of teratogenicity at intakes as low as 3000 µg (10 000 IU), but this is not confirmed by other studies (108). Women who are pregnant or might become pregnant should avoid taking excessive amounts of vitamin A. A careful review of the latest available information by a WHO Expert Group recommended that daily intakes in excess of 3000 µg (10 000 IU), or weekly intakes in excess of 7500 µg (25 000 IU) should not be taken at any period during gestation (109). High doses of vitamin A (60 000 µg, or 200 000 IU) can be safely given to breastfeeding mothers for up to 2 months postpartum and up to 6 weeks to mothers who are not breastfeeding.

## 2.9 Recommendations for future research

Further research is needed in the following areas:

- the interaction of vitamin A and iron with infections, as they relate to serum levels and disease incidence and prevalence;
- the relationship between vitamin A, iron, and zinc and their roles in the severity of infections;
- the nutritional role of 9-*cis* retinoic acid and the mechanism which regulates its endogenous production;
- the bioavailability of provitamin A carotenoids from different classes of leafy and other green and orange vegetables, tubers, and fruits as typically provided in diets (e.g. relative to the level of fat in the diet or meal);
- identification of a reliable indicator of vitamin A status for use in direct quantification of mean requirements and for relating status to functions.

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