

9. Thiamine, riboflavin, niacin, vitamin B₆, pantothenic acid, and biotin

9.1 Introduction

The B-complex vitamins covered here are listed in Table 9.1 along with the physiological roles of the coenzyme forms and a brief description of clinical deficiency symptoms.

Rice and wheat are the staples for many populations of the world. Excessive refining and polishing of cereals removes considerable proportions of B vitamins contained in these cereals. Clinical manifestations of deficiency of some B vitamins—such as beriberi (cardiac and dry), peripheral neuropathies, pellagra, and oral and genital lesions (related to riboflavin deficiency)—were once major public health problems in some parts of the world. These manifestations have now declined, the decline being brought about not through programmes which distribute synthetic vitamins but through changes in the patterns of food availability and consequent changes in dietary practices.

Although many clinical manifestations of B-vitamin deficiencies have decreased, there is evidence of widespread subclinical deficiency of these vitamins (especially of riboflavin and pyridoxine). These subclinical deficiencies, although less dramatic in their manifestations, exert deleterious metabolic effects. Despite the progress in reduction of large-scale deficiency in the world, there are periodic reports of outbreaks of B-complex deficiencies which are linked to deficits of B vitamins in populations under various distress conditions.

Refugee and displaced population groups (20 million people by current United Nations estimates) are at risk for B-complex deficiency because most cereal foods used under emergency situations are not fortified with micronutrients (1). Recent reports have implicated the low B-complex content of diets as a factor in the outbreak of peripheral neuropathy and visual loss observed in the adult population of Cuba (2–4). This deficiency in Cuba resulted from the consequences of an economic blockade (4).

Because of the extensive literature pertaining to the study of the B-complex vitamins, the references cited here have been limited to those published after

TABLE 9.1

Physiologic roles and deficiency signs of B-complex vitamins

Vitamin	Physiologic roles	Clinical signs of deficiency
Thiamin (B ₁)	Coenzyme functions in metabolism of carbohydrates and branched-chain amino acids	Beriberi, polyneuritis, and Wernicke-Korsakoff syndrome
Riboflavin (B ₂)	Coenzyme functions in numerous oxidation and reduction reactions	Growth, cheilosis, angular stomatitis, and dermatitis
Niacin (nicotinic acid and nicotinamide)	Cosubstrate/coenzyme for hydrogen transfer with numerous dehydrogenases	Pellagra with diarrhoea, dermatitis, and dementia
Vitamin B ₆ (pyridoxine, pyridoxamine, and pyridoxal)	Coenzyme functions in metabolism of amino acids, glycogen, and sphingoid bases	Nasolateral seborrhoea, glossitis, and peripheral neuropathy (epileptiform convulsions in infants)
Pantothenic acid	Constituent of coenzyme A and phosphopantetheine involved in fatty acid metabolism	Fatigue, sleep disturbances, impaired coordination, and nausea
Biotin	Coenzyme functions in bicarbonate-dependent carboxylations	Fatigue, depression, nausea, dermatitis, and muscular pains

the publication of the 1974 edition of the FAO/WHO *Handbook on human nutritional requirements* (5). Greater weight has been given to studies which used larger numbers of subjects over longer periods, more thoroughly assessed dietary intake, varied the level of the specific vitamin being investigated, and used multiple indicators, including those considered functional in the assessment of status. These indicators have been the main basis for ascertaining requirements. Although extensive, the bibliographic search of recently published reports presented in this chapter most likely underestimates the extent of B-complex deficiency given that many cases are not reported in the medical literature. Moreover, outbreaks of vitamin deficiencies in populations are usually not publicized because governments may consider the existence of these conditions to be politically sensitive information. Additional references are listed in the publication by the Food and Nutrition Board of the Institute of Medicine of the United States National Academy of Sciences (6).

9.2 Thiamine

9.2.1 Background

Deficiency

Thiamine (vitamin B₁, aneurin) deficiency results in the disease called beriberi, which has been classically considered to exist in dry (paralytic) and wet (oede-

matous) forms (7, 8). Beriberi occurs in human-milk-fed infants whose nursing mothers are deficient. It also occurs in adults with high carbohydrate intakes (mainly from milled rice) and with intakes of anti-thiamine factors, such as the bacterial thiaminases that are in certain ingested raw fish (7). Beriberi is still endemic in Asia. In relatively industrialized nations, the neurologic manifestations of Wernicke-Korsakoff syndrome are frequently associated with chronic alcoholism in conjunction with limited food consumption (9). Some cases of thiamine deficiency have been observed with patients who are hypermetabolic, are on parenteral nutrition, are undergoing chronic renal dialysis, or have undergone a gastrectomy. Thiamine deficiency has also been observed in Nigerians who ate silk worms, Russian schoolchildren (Moscow), Thai rural elderly, Cubans, Japanese elderly, Brazilian Xavante Indians, French Guyanese, south-east Asian schoolchildren who were infected with hookworm, Malaysian detention inmates, and people with chronic alcoholism.

Toxicity

Thiamine toxicity is not a problem because renal clearance of the vitamin is rapid.

Role in human metabolic processes

Thiamine functions as the coenzyme thiamine pyrophosphate (TPP) in the metabolism of carbohydrates and branched-chain amino acids. Specifically the Mg^{2+} -coordinated TPP participates in the formation of α -ketols (e.g. among hexose and pentose phosphates) as catalysed by transketolase and in the oxidation of α -keto acids (e.g. pyruvate, α -ketoglutarate, and branched-chain α -keto acids) by dehydrogenase complexes (10, 11). Hence, when there is insufficient thiamine, the overall decrease in carbohydrate metabolism and its interconnection with amino acid metabolism (via α -keto acids) has severe consequences, such as a decrease in the formation of acetylcholine for neural function.

9.2.2 Biochemical indicators

Indicators used to estimate thiamine requirements are urinary excretion, erythrocyte transketolase activity coefficient, erythrocyte thiamine, blood pyruvate and lactate, and neurologic changes. The excretion rate of the vitamin and its metabolites reflects intake, and the validity of the assessment of thiamine nutriture is improved with load test. Erythrocyte transketolase activity coefficient reflects TPP levels and can indicate rare genetic defects. Erythrocyte thiamine is mainly a direct measure of TPP but when combined with high

performance liquid chromatography (HPLC) separation can also provide a measure of thiamine and thiamine monophosphate.

Thiamine status has been assessed by measuring urinary thiamine excretion under basal conditions or after thiamine loading; transketolase activity; and free and phosphorylated forms in blood or serum (6, 9). Although overlap with baseline values for urinary thiamine was found with oral doses below 1 mg, a correlation of 0.86 between oral and excreted amounts was found by Bayliss et al. (12). The erythrocyte transketolase assay, in which an activity coefficient based on a TPP stimulation of the basal level is given, continues to be a main functional indicator (9), but some problems have been encountered. Gans and Harper (13) found a wide range of TPP effects when thiamine intakes were adequate (i.e. above 1.5 mg/day over a 3-day period). In some cases, the activity coefficient may appear normal after prolonged deficiency (14). This measure seemed poorly correlated with dietary intakes estimated for a group of English adolescents (15). Certainly, there are both interindividual and genetic factors affecting the transketolase (16). Baines and Davies (17) suggested that it is useful to determine erythrocyte TPP directly because the coenzyme is less susceptible to factors that influence enzyme activity; there are also methods for determining thiamine and its phosphate esters in whole blood (18).

9.2.3 Factors affecting requirements

Because thiamine facilitates energy utilization, its requirements have traditionally been expressed on the basis of energy intake, which can vary depending on activity levels. However, Fogelholm et al. (19) found no difference in activation coefficients for erythrocyte transketolase between a small group of skiers and a less physically active group of control subjects. Also, a study with thiamine-restricted Dutch males whose intake averaged 0.43 mg/day for 11 weeks did not reveal an association between short bouts of intense exercise and decreases in indicators of thiamine status (20). Alcohol consumption may interfere with thiamine absorption as well (9).

9.2.4 Evidence used to derive recommended intakes

Recommendations for infants are based on adequate food intake. Mean thiamine content of human milk is 0.21 mg/l (0.62 μ mol/l) (21), which corresponds to 0.16 mg (0.49 μ mol) thiamine per 0.75 l of secreted milk per day. The blood concentration for total thiamine averages 210 ± 53 nmol/l for infants up to 6 months but decreases over the first 12–18 months of life (22).

A study of 13–14-year-old children related dietary intake of thiamine to several indicators of thiamine status (15). Sauberlich et al. (23) concluded from

a carefully controlled depletion–repletion study of seven healthy young men that 0.3 mg thiamine per 4184 kJ met their requirements. Intakes below this amount lead to irritability and other symptoms and signs of deficiency (24). Anderson et al. (25) reported thiamine intakes of 1.0 and 1.2 mg/day as minimal for women and men, respectively. Hoorn et al. (26) reported that 23% of 153 patients aged 65–93 years were deemed deficient based on a transketolase activation coefficient greater than 1.27, which was normalized after thiamine administration. Nichols and Basu (27) found that only 57% of 60 adults aged 65–74 years had TPP effects of less than 14% and suggested that ageing may increase thiamine requirements.

An average total energy cost of 230 MJ has been estimated for pregnancy (28). With an intake of 0.4 mg thiamine/4184 kJ, this amounts to a total of 22 mg thiamine needed during pregnancy, or 0.12 mg/day when the additional thiamine need for the second and third trimesters (180 days) is included. Taking into account the increased need for thiamine because of an increased growth in maternal and fetal compartments and a small increase in energy utilization, an overall additional requirement of 0.3 mg/day is considered adequate (6).

It is estimated that lactating women transfer 0.2 mg thiamine to their infants through their milk each day. Therefore, an additional 0.1 mg is estimated as the need for the increased energy cost of about 2092 kJ/day associated with lactation (6).

9.2.5 Recommended nutrient intakes for thiamine

The recommendations for thiamine are given in Table 9.2.

TABLE 9.2
**Recommended nutrient intakes for thiamine,
by group**

Group	Recommended nutrient intake (mg/day)
<i>Infants and children</i>	
0–6 months	0.2
7–12 months	0.3
1–3 years	0.5
4–6 years	0.6
7–9 years	0.9
<i>Adolescents</i>	
Females, 10–18 years	1.1
Males, 10–18 years	1.2
<i>Adults</i>	
Females, 19+ years	1.1
Males, 19+ years	1.2
<i>Pregnant women</i>	1.4
<i>Lactating women</i>	1.5

9.3 Riboflavin

9.3.1 Background

Deficiency

Riboflavin (vitamin B₂) deficiency results in the condition of hypo- or ariboflavinosis, with sore throat; hyperaemia; oedema of the pharyngeal and oral mucous membranes; cheilosis; angular stomatitis; glossitis; seborrheic dermatitis; and normochromic, normocytic anaemia associated with pure red cell cytoplasia of the bone marrow (8, 29). As riboflavin deficiency almost invariably occurs in combination with a deficiency of other B-complex vitamins, some of the symptoms (e.g. glossitis and dermatitis) may result from other complicating deficiencies. The major cause of hyporiboflavinosis is inadequate dietary intake as a result of limited food supply, which is sometimes exacerbated by poor food storage or processing. Children in developing countries will commonly demonstrate clinical signs of riboflavin deficiency during periods of the year when gastrointestinal infections are prevalent. Decreased assimilation of riboflavin also results from abnormal digestion, such as that which occurs with lactose intolerance. This condition is highest in African and Asian populations and can lead to a decreased intake of milk, as well as an abnormal absorption of the vitamin. Absorption of riboflavin is also affected in some other conditions, for example, tropical sprue, celiac disease, malignancy and resection of the small bowel, and decreased gastrointestinal passage time. In relatively rare cases, the cause of deficiency is inborn errors in which the genetic defect is in the formation of a flavoprotein (e.g. acyl-coenzyme A [coA] dehydrogenases). Also at risk are infants receiving phototherapy for neonatal jaundice and perhaps those with inadequate thyroid hormone. Some cases of riboflavin deficiency have been observed in Russian schoolchildren (Moscow) and south-east Asian schoolchildren (infected with hookworm).

Toxicity

Riboflavin toxicity is not a problem because of limited intestinal absorption.

Role in human metabolic processes

Conversion of riboflavin to flavin mononucleotide (FMN) and then to the predominant flavin, flavin adenine dinucleotide (FAD), occurs before these flavins form complexes with numerous flavoprotein dehydrogenases and oxidases. The flavocoenzymes (FMN and FAD) participate in oxidation-reduction reactions in metabolic pathways and in energy production via the respiratory chain (10, 11).

9.3.2 Biochemical indicators

Indicators used to estimate riboflavin requirements are urinary flavin excretion, erythrocyte glutathione reductase activity coefficient, and erythrocyte flavin. The urinary flavin excretion rate of the vitamin and its metabolites reflects intake; validity of assessment of riboflavin adequacy is improved with load test. Erythrocyte glutathione reductase activity coefficient reflects FAD levels; results are confounded by such genetic defects as glucose-6-phosphate dehydrogenase deficiency and heterozygous β -thalassemia. Erythrocyte flavin is largely a measure of FMN and riboflavin after hydrolysis of labile FAD and HPLC separation.

Riboflavin status has been assessed by measuring urinary excretion of the vitamin in fasting, random, and 24-hour specimens or by load return tests (amounts measured after a specific amount of riboflavin is given orally); measuring erythrocyte glutathione reductase activity coefficient; or erythrocyte flavin concentration (6, 9, 29). The HPLC method with fluorometry gives lower values for urinary riboflavin than do fluorometric methods, which measure the additive fluorescence of similar flavin metabolites (30). The metabolites can comprise as much as one third of total urinary flavin (31, 32) and in some cases may depress assays dependent on a biological response because certain catabolites can inhibit cellular uptake (33). Under conditions of adequate riboflavin intake (approximately 1.3 mg/day for adults), an estimated 120 μ g (320 nmol) total riboflavin or 80 μ g/g of creatinine is excreted daily (32).

The erythrocyte glutathione reductase assay, with an activity coefficient (AC) expressing the ratio of activities in the presence and absence of added FAD, continues to be used as a main functional indicator of riboflavin status, but some limitations in the technique have been noted. The reductase in erythrocytes from individuals with glucose-6-phosphate dehydrogenase deficiency (often present in blacks) has an increased avidity for FAD, which makes this test invalid (34). Sadowski (35) has set an upper limit of normality for the AC at 1.34 based on the mean value plus 2 standard deviations from several hundred apparently healthy individuals aged 60 years and over. Suggested guidelines for the interpretation of such enzyme ACs are as follows: less than 1.2, acceptable; 1.2–1.4, low; greater than 1.4, deficient (9). In general agreement with earlier findings on erythrocyte flavin, Ramsay et al. (36) found a correlation between cord blood and maternal erythrocyte deficiencies and suggested that values greater than 40 nmol/l could be considered adequate.

9.3.3 Factors affecting requirements

Several studies reported modest effects of physical activity on the erythrocyte glutathione reductase AC (37–41). A slight increase in the AC and decrease in urinary flavin of weight-reducing women (39) and older women undergoing exercise training (41) were “normalized” with 20% additional riboflavin. However, riboflavin supplementation did not lead to an increase in work performance when such subjects were not clinically deficient (42–45).

Bioavailability of riboflavin in foods, mostly as digestible flavocoenzymes, is excellent at nearly 95% (6), but absorption of the free vitamin is limited to about 27 mg per single meal or dose in an adult (46). No more than about 7% of food flavin is found as 8- α -FAD covalently attached to certain flavoprotein enzymes. Although some portions of the 8- α -(amino acid)-riboflavins are released by proteolysis of these flavoproteins, they do not have vitamin activity (47).

A lower fat–carbohydrate ratio may decrease the riboflavin requirements of the elderly (48). Riboflavin interrelates with other B vitamins, notably niacin, which requires FAD for its formation from tryptophan, and vitamin B₆, which requires FMN for conversion of the phosphates of pyridoxine and pyridoxamine to the coenzyme pyridoxal 5'-phosphate (PLP) (49). Contrary to earlier reports, no difference was seen in riboflavin status of women taking oral contraceptives when dietary intake was controlled by providing a single basic daily menu and meal pattern after 0.6 mg riboflavin/4184 kJ was given in a 2-week acclimation period (50).

9.3.4 Evidence used to derive recommended intakes

As reviewed by Thomas et al. (51), early estimates of riboflavin content in human milk showed changes during the postpartum period; more recent investigations of flavin composition of both human (52) and cow (53) milk have helped clarify the nature of the flavins present and provide better estimates of riboflavin equivalence. For human milk consumed by infants up to age 6 months, the riboflavin equivalence averages 0.35 mg/l (931 nmol/l) or 0.26 mg/0.75 l of milk/day (691 nmol/0.75 l of milk/day) (6). For low-income Indian women with erythrocyte glutathione reductase activity ratios averaging 1.80 and a milk riboflavin content of 0.22 mg/l, their breast-fed infants averaged AC ratios near 1.36 (54). Hence, a deficiency sufficient to reduce human-milk riboflavin content by one third can lead to a mild subclinical deficiency in infants.

Studies of riboflavin status in adults include those by Belko et al. (38, 39) in modestly obese young women on low-energy diets, by Bates et al. (55) on deficient Gambians, and by Kuizon et al. (56) on Filipino women. Most of a 1.7-mg dose of riboflavin given to healthy adults consuming at least this

amount was largely excreted in the urine (32). Such findings corroborate earlier work indicating a relative saturation of tissue with intakes above 1.1 mg/day. Studies by Alexander et al. (57) on riboflavin status in the elderly show that doubling the estimated riboflavin intakes of 1.7 mg/day for women aged 70 years and over, with a reductase AC of 1.8, led to a doubling of urinary riboflavin from 1.6 µg to 3.4 µg/mg (4.2 to 9.0 nmol/mg) creatinine and a decrease in AC to 1.25. Boisvert et al. (48) obtained normalization of the glutathione reductase AC in elderly Guatemalans with approximately 1.3 mg/day of riboflavin, with a sharp increase in urinary riboflavin occurring at intakes above 1.0–1.1 mg/day.

Pregnant women have an increased erythrocyte glutathione reductase AC (58, 59). Kuizon et al. (56) found that riboflavin at 0.7 mg/4184 kJ was needed to lower the AC of four of eight pregnant women to 1.3 within 20 days, whereas only 0.41 mg/4184 kJ was needed for five of seven non-pregnant women. Maternal riboflavin intake was positively associated with fetal growth in a study of 372 pregnant women (60). The additional riboflavin requirement of 0.3 mg/day for pregnancy is an estimate based on increased growth in maternal and fetal compartments. For lactating women, an estimated 0.3 mg riboflavin is transferred in milk daily and, because utilization for milk production is assumed to be 70% efficient, the value is adjusted upward to 0.4 mg/day.

9.3.5 Recommended nutrient intakes for riboflavin

The recommendations for riboflavin are given in Table 9.3.

TABLE 9.3

Recommended nutrient intakes for riboflavin, by group

Group	Recommended nutrient intake (mg/day)
<i>Infants and children</i>	
0–6 months	0.3
7–12 months	0.4
1–3 years	0.5
4–6 years	0.6
7–9 years	0.9
<i>Adolescents</i>	
Females, 10–18 years	1.0
Males, 10–18 years	1.3
<i>Adults</i>	
Females, 19+ years	1.1
Males, 19+ years	1.3
<i>Pregnant women</i>	1.4
<i>Lactating women</i>	1.6

9.4 Niacin

9.4.1 Background

Deficiency

Niacin (nicotinic acid) deficiency classically results in pellagra, which is a chronic wasting disease associated with a characteristic erythematous dermatitis that is bilateral and symmetrical, a dementia after mental changes including insomnia and apathy preceding an overt encephalopathy, and diarrhoea resulting from inflammation of the intestinal mucous surfaces (8, 9, 61). At present, pellagra occurs endemically in poorer areas of Africa, China, and India. Its cause has been mainly attributed to a deficiency of niacin; however, its biochemical interrelationship with riboflavin and vitamin B₆, which are needed for the conversion of L-tryptophan to niacin equivalents (NEs), suggests that insufficiencies of these vitamins may also contribute to pellagra (62). Pellagra-like syndromes occurring in the absence of a dietary niacin deficiency are also attributable to disturbances in tryptophan metabolism (e.g. Hartnup disease with impaired absorption of the amino acid and carcinoid syndrome where the major catabolic pathway routes to 5-hydroxytryptophan are blocked) (61). Pellagra also occurs in people with chronic alcoholism (61). Cases of niacin deficiency have been found in people suffering from Crohn disease (61).

Toxicity

Although therapeutically useful in lowering serum cholesterol, administration of chronic high oral doses of nicotinic acid can lead to hepatotoxicity as well as dermatologic manifestations. An upper limit (UL) of 35 mg/day as proposed by the United States Food and Nutrition Board (6) was adopted by this Consultation.

Role in human metabolic processes

Niacin is chemically synonymous with nicotinic acid although the term is also used for its amide (nicotinamide). Nicotinamide is the other form of the vitamin; it does not have the pharmacologic action of the acid that is administered at high doses to lower blood lipids, but exists within the redox-active coenzymes, nicotinamide adenine dinucleotide (NAD) and its phosphate (NADP), which function in dehydrogenase–reductase systems requiring transfer of a hydride ion (10, 11). NAD is also required for non-redox adenosine diphosphate–ribose transfer reactions involved in DNA repair (63) and calcium mobilization. NAD functions in intracellular respiration and with enzymes involved in the oxidation of fuel substrates such as glyceraldehyde-3-phosphate, lactate, alcohol, 3-hydroxybutyrate, and pyruvate. NADP func-

tions in reductive biosyntheses such as fatty acid and steroid syntheses and in the oxidation of glucose-6-phosphate to ribose-5-phosphate in the pentose phosphate pathway.

9.4.2 Biochemical indicators

Indicators used to estimate niacin requirements are urinary excretion, plasma concentrations of metabolites, and erythrocyte pyridine nucleotides. The excretion rate of metabolites—mainly *N'*-methyl-nicotinamide and its 2- and 4-pyridones—reflects intake of niacin and is usually expressed as a ratio of the pyridones to *N'*-methyl-nicotinamide. Concentrations of metabolites, especially 2-pyridone, are measured in plasma after a load test. Erythrocyte pyridine nucleotides measure NAD concentration changes.

Niacin status has been monitored by daily urinary excretion of methylated metabolites, especially the ratio of the 2-pyridone to *N'*-methyl-nicotinamide; erythrocyte pyridine nucleotides; oral dose uptake tests; erythrocyte NAD; and plasma 2-pyridone (6, 9). Shibata and Matsuo (64) found that the ratio of urinary 2-pyridone to *N'*-methyl-nicotinamide was as much a measure of protein adequacy as it was a measure of niacin status. Jacob et al. (65) found this ratio too insensitive to marginal niacin intake. The ratio of the 2-pyridone to *N'*-methyl-nicotinamide also appears to be associated with the clinical symptoms of pellagra, principally the dermatitic condition (66). In plasma, 2-pyridone levels change in reasonable proportion to niacin intake (65). As in the case of the erythrocyte pyridine nucleotides (nicotinamide coenzymes), NAD concentration decreased by 70% whereas NADP remained unchanged in adult males fed diets with only 6 or 10mg NEs/day (67). Erythrocyte NAD provided a marker that was at least as sensitive as urinary metabolites of niacin in this study (67) and in a niacin depletion study of elderly subjects (68).

9.4.3 Factors affecting requirements

The biosynthesis of niacin derivatives on the pathway to nicotinamide coenzymes stems from tryptophan, an essential amino acid found in protein, and as such, this source of NE increases niacin intake. There are several dietary, drug, and disease factors that reduce the conversion of tryptophan to niacin (61), such as the use of oral contraceptives (69). Although a 60-to-1 conversion factor represents the average for human utilization of tryptophan as an NE, there are substantial individual differences (70, 71). There is also an interdependence of enzymes within the tryptophan-to-niacin pathway where vitamin B₆ (as pyridoxal phosphate) and riboflavin (as FAD) are functional.

Further, riboflavin (as FMN) is required for the oxidase that forms coenzymic PLP from the alcohol and amine forms of phosphorylated vitamin B₆ (49).

9.4.4 Evidence used to derive recommended intakes

Niacin content of human milk is approximately 1.5 mg/l (12.3 μmol/l) and the tryptophan content is 210 mg/l (1.0 mmol/l) (21). Hence, the total content is approximately 5 mg NEs/l or 4 mg NEs/0.75 l secreted daily in human milk. Recent studies (64, 70) together with those reported in the 1950s suggest that 12.5 mg NEs, which corresponds to 5.6 mg NEs/4184 kJ, is minimally sufficient for niacin intake in adults.

For pregnant women, where 230 MJ is the estimated energy cost of pregnancy, calculated needs above those of non-pregnant women are 5.6 mg NEs/4186 kJ (1000 kcal) × 230 000 kJ (55 000 kcal), or 308 mg NEs for the entire pregnancy or 1.7 mg NEs/day (308 mg NEs/180 days) for the second and third trimester, which is about a 10% increase. In addition, about 2 mg NEs/day is required for growth in maternal and fetal compartments (6).

For lactating women, an estimated 1.4 mg preformed niacin is secreted daily, and an additional requirement of less than 1 mg is needed to support the energy expenditure of lactation. Hence, 2.4 mg NEs/day is the additional requirement for lactating women.

9.4.5 Recommended nutrient intakes for niacin

The recommendations for niacin are given in Table 9.4.

9.5 Vitamin B₆

9.5.1 Background

Deficiency

A deficiency of vitamin B₆ alone is uncommon because it usually occurs in association with a deficit in other B-complex vitamins (72). Early biochemical changes include decreased levels of plasma pyridoxal 5'-phosphate (PLP) and urinary 4-pyridoxic acid. These are followed by decreases in synthesis of transaminases (aminotransferases) and other enzymes of amino acid metabolism such that there is an increased presence of xanthurenate in the urine and a decreased glutamate conversion to the anti-neurotransmitter γ-aminobutyrate. Hypovitaminosis B₆ may often occur with riboflavin deficiency, because riboflavin is needed for the formation of the coenzyme PLP. Infants are especially susceptible to insufficient intakes, which can lead to epileptiform convulsions. Skin changes include dermatitis with cheilosis and glossitis. Moreover, there is usually a decrease in circulating lymphocytes and

TABLE 9.4

Recommended nutrient intakes for niacin, by group

Group	Recommended nutrient intake (mgNEs/day)
<i>Infants and children</i>	
0–6 months	2 ^a
7–12 months	4
1–3 years	6
4–6 years	8
7–9 years	12
<i>Adolescents</i>	
10–18 years	16
<i>Adults</i>	
Females, 19+ years	14
Males, 19+ years	16
<i>Pregnant women</i>	18
<i>Lactating women</i>	17

NEs, niacin equivalents.

^a Preformed.

sometimes a normocytic, microcytic, or sideroblastic anaemia as well (9). The sensitivity of such systems as sulfur amino acid metabolism to vitamin B₆ availability is reflected in homocysteinaemia. A decrease in the metabolism of glutamate in the brain, which is found in vitamin B₆ insufficiency, reflects a nervous system dysfunction. As is the case with other micronutrient deficiencies, vitamin B₆ deficiency results in an impairment of the immune system. Of current concern is the pandemic-like occurrence of low vitamin B₆ intakes in many people who eat poorly (e.g. people with eating disorders). Vitamin B₆ deficiency has also been observed in Russian schoolchildren (Moscow), south-east Asian schoolchildren (infected with hookworm), elderly Europeans (Dutch), and in some individuals with hyperhomocysteinaemia or who are on chronic haemodialysis. Several medical conditions can also affect vitamin B₆ metabolism and thus lead to deficiency symptoms.

Toxicity

Use of high doses of pyridoxine for the treatment of pre-menstrual syndrome, carpal tunnel syndrome, and some neurologic diseases has resulted in neurotoxicity. A UL of 100mg/day as proposed by the United States Food and Nutrition Board (6) was adopted by this Consultation.

Role in human metabolic processes

There are three natural vitamers (different forms of the vitamin) of vitamin B₆, namely pyridoxine, pyridoxamine, and pyridoxal. All three must be phosphorylated and the 5'-phosphates of the first two vitamers are oxidized to the functional PLP, which serves as a carbonyl-reactive coenzyme to a number of enzymes involved in the metabolism of amino acids. Such enzymes include aminotransferases, decarboxylases, and dehydratases; δ -aminolevulinate synthase in haem biosynthesis; and phosphorylase in glycogen breakdown and sphingoid base biosynthesis (10, 11).

9.5.2 Biochemical indicators

Indicators used to estimate vitamin B₆ requirements are PLP, urinary excretion, erythrocyte aminotransferases activity coefficients, tryptophan catabolites, erythrocyte and whole blood PLP, and plasma homocysteine. PLP is the major form of vitamin B₆ in all tissues and the plasma PLP concentration reflects liver PLP. Plasma PLP changes fairly slowly in response to vitamin intake. The excretion rate of vitamin B₆ and particularly its catabolite, 4-pyridoxate, reflects intake. Erythrocyte aminotransferases for aspartate and alanine reflect PLP levels and show large variations in activity coefficients. The urinary excretion of xanthurenate, a tryptophan catabolite, is typically used after a tryptophan load test.

Vitamin B₆ status is most appropriately evaluated by using a combination of the above indicators, including those considered as direct indicators (e.g. vitamer concentration in cells or fluids) and those considered to be indirect or functional indicators (e.g. erythrocyte aminotransferase saturation by PLP or tryptophan metabolites) (9). Plasma PLP may be the best single indicator because it appears to reflect tissue stores (73). Kretsch et al. (74) found that diets containing less than 0.05 mg vitamin B₆ given to 11 young women led to abnormal electroencephalograph patterns in two of the women and a plasma PLP concentration of approximately 9 nmol/l. Hence, a level of about 10 nmol/l is considered sub-optimal. A plasma PLP concentration of 20 nmol/l has been proposed as an index of adequacy (6) based on recent findings (73, 75). Plasma PLP levels have been reported to fall with age (6, 76). Urinary 4-pyridoxic acid level responds quickly to changes in vitamin B₆ intake (73) and is therefore of questionable value in assessing status. However, a value higher than 3 μ mol/day, achieved with an intake of approximately 1 mg/day, has been suggested to reflect adequate intake (77). Erythrocyte aminotransferases for aspartate and alanine are commonly measured before and after addition of PLP to ascertain amounts of apoenzymes, the proportion of which increases with vitamin B₆ depletion. Values of 1.5–1.6 for the aspartate aminotransferase

and approximately 1.2 for the alanine aminotransferase have been suggested as being adequate (9, 77). Catabolites from tryptophan and methionine have also been used to assess vitamin B₆ status. In a review of the relevant literature, Leklem (77) suggested that a 24-hour urinary excretion of less than 65 μmol xanthurenate after a 2-g oral dose of tryptophan indicates normal vitamin B₆ status.

9.5.3 Factors affecting requirements

A recent review by Gregory (78) confirms that bioavailability of vitamin B₆ in a mixed diet is about 75% (79), with approximately 8% of this total contributed by pyridoxine β -D-glucoside, which is about half as effectively utilized (78) as free B₆ vitamers or their phosphates. The amine and aldehyde forms of vitamin B₆ are probably about 10% less effective than pyridoxine (80). Despite the involvement of PLP with many enzymes affecting amino acid metabolism, there seems to be only a slight effect of dietary proteins on vitamin B₆ status (81). Several studies have reported decreases in indicators of vitamin B₆ status in women receiving oral contraceptives (82, 83), but this probably reflects hormonal stimulation of tryptophan catabolism rather than any deficiency of vitamin B₆ per se. Subjects with pre-eclampsia or eclampsia have plasma PLP levels lower than those of healthy pregnant women (84, 85).

9.5.4 Evidence used to derive recommended intakes

The average intake of vitamin B₆ for infants, based on human-milk content, is 0.13 mg/l/day (86) or 0.1 mg/0.75 l/day. With an average maternal dietary intake of vitamin B₆ of 1.4 mg/day, human milk was found to contain 0.12 mg/l, and plasma PLP of nursing infants averaged 54 nmol/l (87). Extrapolation on the basis of metabolic body size, weight, and growth suggests 0.3 mg/day as an adequate intake for infants 6–12 months of age (6). Information on vitamin B₆ requirements for children is limited, but Heiskanen et al. (88) found an age-related decrease in erythrocyte PLP and an increase in the aspartate aminotransferase activation. However, this age-related decrease in erythrocyte PLP may accompany normal growth and health rather than reflect real deficiency.

In a review of earlier studies of men with various protein intakes, Linkswiler (89) concluded that normalization of a tryptophan load test required 1.0–1.5 mg vitamin B₆. Miller et al. (90) found that 1.6 mg vitamin B₆ led to plasma PLP levels above 30 nmol/l for young men with various protein intakes. From several investigations of young women (91–94), a requirement closer to 1.0–1.2 mg vitamin B₆ could be estimated.

Limited studies of the elderly indicate that requirements may be somewhat higher, at least to maintain plasma PLP above the 20-nmol level (95, 96), which is the proposed index of adequacy.

During pregnancy, indicators of vitamin B₆ status decrease, especially in the third trimester (85, 97, 98). It is not clear, however, whether this is a normal physiological phenomenon. For a maternal body store of 169 mg and fetal plus placental accumulation of 25 mg vitamin B₆, about 0.1 mg/day is needed, on average, over gestation (6). With additional allowances for the increased metabolic need and weight of the mother and assuming about 75% bioavailability, an additional average requirement of 0.25 mg in pregnancy can be estimated. Because most of this need is in the latter stages of pregnancy and vitamin B₆ is not stored to any significant extent, an extra 0.5 mg/day of vitamin B₆ may be justified to err on the side of safety.

For lactation, it may be prudent to add 0.6 mg vitamin B₆ to the base requirement for women because low maternal intakes could lead to a compromised vitamin B₆ status in the infant (99).

9.5.5 Recommended nutrient intakes for vitamin B₆

The recommendations for vitamin B₆ are given in Table 9.5.

TABLE 9.5
**Recommended nutrient intakes for vitamin B₆,
by group**

Group	Recommended nutrient intake (mg/day)
<i>Infants and children</i>	
0–6 months	0.1
7–12 months	0.3
1–3 years	0.5
4–6 years	0.6
7–9 years	1.0
<i>Adolescents</i>	
Females, 10–18 years	1.2
Males, 10–18 years	1.3
<i>Adults</i>	
Females, 19–50 years	1.3
Males, 19–50 years	1.3
Females, 51+ years	1.5
Males, 51+ years	1.7
<i>Pregnant women</i>	1.9
<i>Lactating women</i>	2.0

9.6 Pantothenate

9.6.1 Background

Deficiency

The widespread occurrence of releasable pantothenic acid in food makes a dietary deficiency unlikely (8, 9, 100, 101). If a deficiency occurs, it is usually accompanied by deficits of other nutrients. The use of experimental animals, an antagonistic analogue (ω -methylpantothenate) given to humans, and more recently, the feeding of semi-synthetic diets virtually free of pantothenate (102), have all helped to define signs and symptoms of deficiency. Subjects become irascible; develop postural hypotension; have rapid heart rate on exertion; suffer epigastric distress with anorexia and constipation; experience numbness and tingling of the hands and feet (“burning feet” syndrome); and have hyperactive deep tendon reflexes and weakness of finger extensor muscles. Some cases of pantothenate deficiency have been observed in patients with acne and other dermatitic conditions.

Toxicity

Toxicity is not a problem with pantothenate, as no adverse effects have been observed (6).

Role in human metabolic processes

Pantothenic acid is a component of CoA, a cofactor that carries acyl groups for many enzymatic processes, and of phosphopantetheine within acyl carrier proteins, a component of the fatty acid synthase complex (10, 11). The compounds containing pantothenate are most especially involved in fatty acid metabolism and the pantothenate-containing prosthetic group additionally facilitates binding with appropriate enzymes.

9.6.2 Biochemical indicators

Indicators used to estimate pantothenate requirements are urinary excretion and blood levels. Excretion rate reflects intake. Whole blood, which contains the vitamin itself and pantothenate-containing metabolites, has a general correlation with intake; erythrocyte levels, however, seem more meaningful than plasma or serum levels.

Relative correspondence to pantothenate status has been reported for urinary excretion and for blood content of both whole blood and erythrocytes (6, 9). Fry et al. (102) reported a decline in urinary pantothenate levels from approximately 3 to 0.8 mg/day (13.7–3.6 μ mol/day) in young men fed a deficient diet for 84 days. Urinary excretion for a typical American diet was found to be 2.6 mg/day (12 μ mol/day) (79). Pantothenate intake estimated for

adolescents was significantly correlated with pantothenate in urine (103). Whole-blood pantothenate fell from 1.95 to 1.41 µg/ml (8.8 to 6.4 µmol/l) when six adult males were fed a pantothenate-free diet (102). Whole-blood content corresponded to intake (103), and the range in whole blood was reported to be 1.57–2.66 µg/ml (7.2–12.1 µmol/l) (104). There is an excellent correlation of whole-blood concentrations of pantothenate with the erythrocyte concentration, with an average value being 334 ng/ml (1.5 µmol/l) (103). The lack of sufficient population data, however, suggests the current use of an adequate intake rather than a recommended intake as a suitable basis for recommendations.

9.6.3 Factors affecting requirements

A measurement of urinary excretion of pantothenate after feeding a formula diet containing both bound and free vitamin indicates that approximately 50% of the pantothenate present in natural foods may be bioavailable (79).

9.6.4 Evidence used to derive recommended intakes

Infant requirements are based on an estimation of the pantothenic acid content of human milk, which according to reported values is at least 2.2 mg/l (21, 105). For a reported average human-milk intake of 0.75 l/day (106–108) these values suggest that 1.7 mg/day is an adequate intake by younger (0–6 months) infants. Taking into consideration growth and body size, 1.8 mg/day may be extrapolated for older (7–12 months) infants (105).

The studies of Eissenstat et al. (103) of adolescents suggest that intakes of less than 4 mg/day were sufficient to maintain blood and urinary pantothenate. Kathman and Kies (109) found a range of pantothenate intake of 4 mg/day to approximately 8 mg/day in 12 adolescents who were 11–16 years old. The usual pantothenate intake for American adults has been reported to be 4–7 mg/day (102, 109–111). Hence, around 5 mg/day is apparently adequate.

For pregnancy, there is only one relatively recent study that found lower blood pantothenate levels but no difference in urinary excretion in pregnant women compared with non-pregnant controls (112).

During lactation, blood pantothenate concentrations were found to be significantly lower at 3 months postpartum (112). Given a loss of 1.7 mg/day (7.8 µmol/day) through milk supply and lower maternal blood concentrations corresponding to intakes of about 5–6 mg/day, the recommended intake for a lactating woman may be increased to 7 mg/day.

9.6.5 Recommended nutrient intakes for pantothenic acid

The recommendations for pantothenate are given in Table 9.6.

TABLE 9.6

Recommended nutrient intakes for pantothenate, by group

Group	Recommended nutrient intake (mg/day)
<i>Infants and children</i>	
0–6 months	1.7
7–12 months	1.8
1–3 years	2.0
4–6 years	3.0
7–9 years	4.0
<i>Adolescents</i>	
10–18 years	5.0
<i>Adults</i>	
Females, 19+ years	5.0
Males, 19+ years	5.0
<i>Pregnant women</i>	6.0
<i>Lactating women</i>	7.0

9.7 Biotin

9.7.1 Background

Deficiency

Biotin deficiency in humans has been clearly documented with prolonged consumption of raw egg whites, which contain biotin-binding avidin. Biotin deficiency has also been observed in cases of parenteral nutrition with solutions lacking biotin given to patients with short-gut syndrome and other causes of malabsorption (9, 113, 114). Some cases of biotin deficiency have been noted in infants with intractable nappy dermatitis and in those fed special formulas. Dietary deficiency in otherwise normal people is probably rare. Some patients have multiple carboxylase deficiencies and there are occasional biotinidase deficiencies. Clinical signs of deficiency include dermatitis of an erythematous and seborrheic type; conjunctivitis; alopecia; and central nervous system abnormalities such as hypotonia, lethargy, and developmental delay in infants, and depression, hallucinations, and paresthesia of the extremities in adults.

Toxicity

Toxicity is not a problem because of the limited intestinal absorption of biotin.

Role in human metabolic processes

Biotin functions as a coenzyme within several carboxylases after its carboxyl functional group becomes amide linked to the ϵ -amino of specific lysyl residues of the apoenzymes (10, 11). In humans and other mammals, biotin operates within four carboxylases. Three of the four biotin-dependent carboxylases are mitochondrial (pyruvate carboxylase, methylcrotonyl-CoA carboxylase, and propionyl-CoA carboxylase) whereas the fourth (acetyl-CoA carboxylase) is found in both mitochondria and the cytosol. In all these cases, biotin serves as a carrier for the transfer of active bicarbonate into a substrate to generate a carboxyl product.

9.7.2 Biochemical indicators

Indicators used to estimate biotin requirements are urinary excretion of biotin and excretion of 3-hydroxyisovalerate. The excretion rate of the vitamin and its metabolites in urine is assessed by avidin-based radioimmunoassay with HPLC. Excretion of 3-hydroxyisovalerate inversely reflects the activity of β -methylcrotonyl-CoA carboxylase, which is involved in leucine metabolism.

Both indicators, urinary excretion of biotin as assessed with an avidin-based radioimmunoassay with HPLC, and 3-hydroxyisovalerate excretion have been used to assess status (115). The isolation and chemical identification of more than a dozen metabolites of biotin established the main features of its function in microbes and mammals (116, 117). Zemleni et al. have quantified the major biotin metabolites (118). Both biotin and *bis*-norbiotin excretions were found to decline in parallel in individuals on a diet containing raw egg whites (115). In these individuals the levels of urinary 3-hydroxyisovalerate, which increase as a result of decreased activity of β -methylcrotonyl-CoA carboxylase and altered leucine metabolism, rose from a normal mean of 112 to 272 $\mu\text{mol}/24$ hours. Decreased excretion of biotin, abnormally increased excretion of 3-hydroxyisovalerate, or both have been associated with overt cases of biotin deficiency (119–124). The lack of sufficient population data, however, suggests the current use of an adequate intake rather than a recommended intake as a suitable basis for recommendations.

9.7.3 Evidence used to derive recommended intakes

The biotin content of human milk is estimated to be approximately 6 $\mu\text{g}/\text{l}$ (24 nmol/l) based on several studies (125–127) that report values ranging from about 4 to 7 $\mu\text{g}/\text{l}$ (16.4–28.9 nmol/l). Hence, the estimated intake of biotin for an infant consuming 0.75 l of human milk per day is 5 $\mu\text{g}/\text{day}$ during the first half-year and for older infants (7–12 months of age) is perhaps 6 $\mu\text{g}/\text{day}$.

Requirements for children and adults have been extrapolated as follows (6):

$$\text{Adequate intake for child or adult} = (\text{adequate intake young infant}) \times (\text{weight adult or child/weight infant})^{0.75}$$

For pregnancy, there are at present insufficient data to justify an increase in the adequate intake, although Mock et al. (128) reported decreased urinary biotin and 3-hydroxyisovalerate in a large fraction of seemingly healthy pregnant women.

For lactating women, the intake of biotin may need to be increased by an additional 5 µg/day to cover the losses due to breastfeeding.

9.7.4 Recommended nutrient intakes for biotin

The recommendations for biotin are given in Table 9.7.

9.8 General considerations for B-complex vitamins

9.8.1 Notes on suggested recommendations

For the six B-complex vitamins considered here, recommendations for infants are based largely on the composition and quantity of human milk consumed, and are thus considered to be adequate intakes. Younger infants (0–6 months) are considered to derive adequate intake from milk alone; recommendations for older infants (7–12 months) are adjusted by metabolic scaling such that a factor—weight of 7–12 month-old infant/weight of 0–6 month-old infant)^{0.75}—is multiplied by the recommendation for the younger infant (6). Recommendations have been given to use the higher (7–12 months) level of B-vitamin requirements for all infants in the first year of life.

TABLE 9.7

Recommended nutrient intakes for biotin, by group

Group	Recommended nutrient intake (µg/day)
<i>Infants and children</i>	
0–6 months	5
7–12 months	6
1–3 years	8
4–6 years	12
7–9 years	20
<i>Adolescents</i>	
10–18 years	25
<i>Adults</i>	
Females, 19+ years	30
Males, 19+ years	30
<i>Pregnant women</i>	30
<i>Lactating women</i>	35

For most of the B vitamins, there is little or no direct information that can be used to estimate the amounts required by children and adolescents. Hence, an extrapolation from the adult level is used where a factor—(weight of child/weight of adult)^{0.75} × (1 + growth factor)—is multiplied by the adult recommendation (6).

For all but one of the B-complex vitamins covered here, data are not sufficient to justify altering recommendations for the elderly. Only vitamin B₆ has altered recommendations for the elderly. However, for pregnancy and lactation, increased maternal needs related to increases in energy and replacement of secretion losses are considered.

9.8.2 Dietary sources of B-complex vitamins

A listing of some food sources that provide good and moderate amounts of the vitamins considered in this chapter is given in Table 9.8.

9.9 Recommendations for future research

In view of the issues raised in this chapter on B-complex vitamins, the following recommendations are given:

- Actual requirements of B-complex vitamins are least certain for children, adolescents, pregnant and lactating women, and the elderly, and as such, deserve further study.
- Studies need to include graded levels of the vitamin above and below current recommendations and should consider or establish clearly defined cut-off values for clinical adequacy and inadequacy and be conducted for periods of time sufficient for ascertaining equilibrium dynamics.

TABLE 9.8
Dietary sources of water-soluble B vitamins^a

Vitamin	Good-to-moderate dietary sources
Thiamine (B ₁)	Pork, organ meats, whole grains, and legumes
Riboflavin (B ₂)	Milk and dairy products, meats, and green vegetables
Niacin (nicotinic acid and nicotinamide)	Liver, lean meats, grains, and legumes (can be formed from tryptophan)
Vitamin B ₆ (pyridoxine, pyridoxamine, and pyridoxal)	Meats, vegetables, and whole-grain cereals
Pantothenic acid	Animal tissues, whole-grain cereals, and legumes (widely distributed)
Biotin	Liver, yeast, egg, yolk, soy flour, and cereals

^a Not including vitamin B₁₂.

- For status indicators, additional functional tests would be useful for riboflavin (e.g. the activity of FMN-dependent pyridoxine [pyridoxamine] 5'-phosphate oxidase in erythrocytes), niacin (e.g. sensitive blood measures, especially of NAD), and perhaps pantothenate.
- The food content and bioavailability of pantothenate and biotin need further investigation to establish the available and preferred food sources reasonable for different populations.

Primary efforts should now be in the arena of public health and nutrition education with emphasis on directing people and their governments to available and healthful foods; the care necessary for their storage and preparation; and achievable means for adjusting intake with respect to age, sex, and health status.

References

1. *Report on the nutrition situation of refugees and displaced populations.* Geneva, United Nations Administrative Committee on Coordination, Subcommittee on Nutrition, 1998 (Refugee Nutrition Information System, 25).
2. Sadun A et al. Epidemic optic neuropathy in Cuba: eye findings. *Archives of Ophthalmology*, 1994, 112:691–699.
3. Ordunez-Garcia O et al. Cuban epidemic neuropathy, 1991–1994: history repeats itself a century after the “amblyopia of the blockade”. *American Journal of Public Health*, 1996, 86:738–743.
4. Hedges R et al. Epidemic optic and peripheral neuropathy in Cuba: a unique geopolitical public health problem. *Survey of Ophthalmology*, 1997, 41:341–353.
5. Passmore R, Nicol BM, Narayana Rao M. *Handbook on human nutritional requirements.* Geneva, World Health Organization, 1974 (WHO Monograph Series, No. 61).
6. Food and Nutrition Board. *Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B₆, folate, vitamin B₁₂, pantothenic acid, biotin, and choline.* Washington, DC, National Academy Press, 1998.
7. McCormick DB. Thiamin. In: Shils ME, Young VR, eds. *Modern nutrition in health and disease*, 6th ed. Philadelphia, PA, Lea & Febiger, 1988:355–361.
8. McCormick DB. Vitamin, Structure and function of. In: Meyers RA, ed. *Encyclopedia of molecular biology and molecular medicine*, Vol. 6. Weinheim, VCH (Verlag Chemie), 1997:244–252.
9. McCormick DB, Greene HL. Vitamins. In: Burtis VA, Ashwood ER, eds. *Tietz textbook of clinical chemistry*, 2nd ed. Philadelphia, PA, WB Saunders, 1994:1275–1316.
10. McCormick DB. Coenzymes, Biochemistry of. In: Meyers RA, ed. *Encyclopedia of molecular biology and molecular medicine*, Vol. 1. Weinheim, VCH (Verlag Chemie), 1996:396–406.
11. McCormick DB. Coenzymes, Biochemistry. In: Dulbecco R, ed. *Encyclopedia of human biology*, 2nd ed. San Diego, CA, Academic Press, 1997:847–864.
12. Bayliss RM et al. Urinary thiamine excretion after oral physiological doses of

- the vitamin. *International Journal of Vitamin and Nutrition Research*, 1984, 54:161–164.
13. Gans DA, Harper AE. Thiamin status of incarcerated and nonincarcerated adolescent males: dietary intake and thiamin pyrophosphate response. *American Journal of Clinical Nutrition*, 1991, 53:1471–1475.
 14. Schrijver J. Biochemical markers for micronutrient status and their interpretation. In: Pietrzik K, ed. *Modern lifestyles, lower energy intake and micronutrient status*. Heidelberg, Springer-Verlag, 1991:55–85.
 15. Bailey AL et al. Thiamin intake, erythrocyte transketolase (EC 2.2.1.1) activity and total erythrocyte thiamin in adolescents. *British Journal of Nutrition*, 1994, 72:111–125.
 16. Singleton CK et al. The thiamin-dependent hysteretic behavior of human transketolase: implications for thiamine deficiency. *Journal of Nutrition*, 1995, 125:189–194.
 17. Baines M, Davies G. The evaluation of erythrocyte thiamin diphosphate as an indicator of thiamin status in man, and its comparison with erythrocyte transketolase activity measurements. *Annals of Clinical Biochemistry*, 1988, 25:698–705.
 18. Gerrits J et al. Determination of thiamin and thiamin phosphates in whole blood by reversed-phase liquid chromatography with precolumn derivatization. In: McCormick DB, Suttie JW, Wagner C, eds. *Methods in enzymology. Vitamins and coenzymes*. San Diego, CA, Academic Press, 1997, 279:74–82.
 19. Fogelholm M et al. Dietary intake and thiamin, iron, and zinc status in elite Nordic skiers during different training periods. *International Journal of Sport Nutrition*, 1992, 2:351–365.
 20. van der Beek EJ et al. Thiamin, riboflavin and vitamin B₆: impact of restricted intake on physical performance in man. *Journal of American College of Nutrition*, 1994, 13:629–640.
 21. Committee on Nutrition. Composition of human milk: normative data. In: Forbes GB, Woodruff CW, eds. *Pediatric nutrition handbook*, 2nd ed. Elk Grove Village, IL, American Academy of Pediatrics, 1985:363–368.
 22. Wyatt DT, Nelson D, Hillman RE. Age-dependent changes in thiamin concentrations in whole blood and cerebrospinal fluid in infants and children. *American Journal of Clinical Nutrition*, 1991, 53:530–536.
 23. Sauberlich HE et al. Thiamin requirement of the adult human. *American Journal of Clinical Nutrition*, 1979, 32:2237–2248.
 24. Wood B et al. A study of partial thiamin restriction in human volunteers. *American Journal of Clinical Nutrition*, 1980, 33:848–861.
 25. Anderson SH, Charles TJ, Nicol AD. Thiamine deficiency at a district general hospital: report of five cases. *Quarterly Journal of Medicine*, 1985, 55:15–32.
 26. Hoorn RK, Flikweert JP, Westerink D. Vitamin B-1, B-2 and B-6 deficiencies in geriatric patients, measured by coenzyme stimulation of enzyme activities. *Clinica Chemica Acta*, 1975, 61:151–162.
 27. Nichols HK, Basu TK. Thiamin status of the elderly: dietary intake and thiamin pyrophosphate response. *Journal of American College of Nutrition*, 1994, 13:57–61.
 28. Food and Nutrition Board. *Nutrition during pregnancy. Part I. Weight gain. Part II. Nutrient supplements*. Washington, DC, National Academy Press, 1990.

29. McCormick DB. Riboflavin. In: Shils ME, Olson JA, Shike M, eds. *Modern nutrition in health and disease*, 8th ed. Philadelphia, PA, Lea & Febiger, 1994:366–375.
30. Smith MD. Rapid method for determination of riboflavin in urine by high-performance liquid chromatography. *Journal of Chromatography*, 1980, 182:285–291.
31. Chastain JL, McCormick DB. Flavin catabolites: identification and quantitation in human urine. *American Journal of Clinical Nutrition*, 1987, 46:830–834.
32. Roughead ZK, McCormick DB. Urinary riboflavin and its metabolites: effects of riboflavin supplementation in healthy residents of rural Georgia (USA). *European Journal of Clinical Nutrition*, 1991, 45:299–307.
33. Aw T-Y, Jones DP, McCormick DB. Uptake of riboflavin by isolated rat liver cells. *Journal of Nutrition*, 1983, 113:1249–1254.
34. Nichoalds GE. Riboflavin. Symposium in laboratory medicine. In: Labbae RF, ed. *Symposium on Laboratory Assessment of Nutritional Status. Clinics in Laboratory Medicine Series, Vol. 1*. Philadelphia, PA, WB Saunders, 1981, 1:685–698.
35. Sadowski JA. Riboflavin. In: Hartz SC, Russell RM, Rosenberg IH, eds. *Nutrition in the elderly. The Boston Nutritional Status Survey*. London, Smith-Gordon, 1992:119–125.
36. Ramsay VP et al. Vitamin cofactor saturation indices for riboflavin, thiamine, and pyridoxine in placental tissue of Kenyan women. *American Journal of Clinical Nutrition*, 1983, 37:969–973.
37. Belko AZ et al. Effects of exercise on riboflavin requirements of young women. *American Journal of Clinical Nutrition*, 1983, 37:509–517.
38. Belko AZ et al. Effects of aerobic exercise and weight loss on riboflavin requirements of moderately obese, marginally deficient young women. *American Journal of Clinical Nutrition*, 1984, 40:553–561.
39. Belko AZ et al. Effects of exercise on riboflavin requirements: biological validation in weight reducing women. *American Journal of Clinical Nutrition*, 1985, 41:270–277.
40. Soares MJ et al. The effect of exercise on the riboflavin status of adult men. *British Journal of Nutrition*, 1993, 69:541–551.
41. Winters LR et al. Riboflavin requirements and exercise adaptation in older women. *American Journal of Clinical Nutrition*, 1992, 56:526–532.
42. Powers HJ et al. Bicycling performance in Gambian children: effects of supplements of riboflavin or ascorbic acid. *Human Nutrition and Clinical Nutrition*, 1987, 41:59–69.
43. Prasad AP et al. Functional impact of riboflavin supplementation in urban school children. *Nutrition Research*, 1990, 10:275–281.
44. Tremblay A et al. The effects of a riboflavin supplementation on the nutritional status and performance of elite swimmers. *Nutrition Research*, 1984, 4:201–208.
45. Weight LM, Myburgh KH, Noakes TD. Vitamin and mineral supplementation: effect on the running performance of trained athletes. *American Journal of Clinical Nutrition*, 1988, 47:192–195.
46. Zempleni J, Galloway JR, McCormick DB. Pharmacokinetics of orally and intravenously administered riboflavin in healthy humans. *American Journal of Clinical Nutrition*, 1996, 63:54–66.
47. Chia CP, Addison R, McCormick DB. Absorption, metabolism, and excre-

- tion of 8a-(amino acid)-riboflavins in the rat. *Journal of Nutrition*, 1978, 108:373–381.
48. Boisvert WA et al. Riboflavin requirement of healthy elderly humans and its relationship to macronutrient composition of the diet. *Journal of Nutrition*, 1993, 123:915–925.
 49. McCormick DB. Two interconnected B vitamins: riboflavin and pyridoxine. *Physiological Reviews*, 1989, 69:1170–1198.
 50. Roe DA et al. Factors affecting riboflavin requirements of oral contraceptive users and nonusers. *American Journal of Clinical Nutrition*, 1982, 35:495–501.
 51. Thomas MR et al. The effects of vitamin C, vitamin B₆, vitamin B₁₂, folic acid, riboflavin, and thiamin on the breast milk and maternal status of well-nourished women at 6 months postpartum. *American Journal of Clinical Nutrition*, 1980, 33:2151–2156.
 52. Roughead ZK, McCormick DB. Flavin composition of human milk. *American Journal of Clinical Nutrition*, 1990, 52:854–857.
 53. Roughead ZK, McCormick DB. A qualitative and quantitative assessment of flavins in cow's milk. *Journal of Nutrition*, 1990, 120:382–388.
 54. Bamji MS et al. Enzymatic evaluation of riboflavin status of infants. *European Journal of Clinical Nutrition*, 1991, 45:309–313.
 55. Bates CJ et al. Riboflavin status of adolescent vs. elderly Gambian subjects before and during supplementation. *American Journal of Clinical Nutrition*, 1989, 50:825–829.
 56. Kuizon MD et al. Riboflavin requirement of Filipino women. *European Journal of Clinical Nutrition*, 1992, 46:257–264.
 57. Alexander M et al. Relation of riboflavin nutriture in healthy elderly to intake of calcium and vitamin supplements: evidence against riboflavin supplementation. *American Journal of Clinical Nutrition*, 1984, 39:540–546.
 58. Bates CJ et al. Riboflavin status in Gambian pregnant and lactating women and its implications for recommended dietary allowances. *American Journal of Clinical Nutrition*, 1981, 34:928–935.
 59. Vir SC, Love AH, Thompson W. Riboflavin status during pregnancy. *American Journal of Clinical Nutrition*, 1981, 34:2699–2705.
 60. Badart-Smook A et al. Fetal growth is associated positively with maternal intake of riboflavin and negatively with maternal intake of linoleic acid. *Journal of the American Dietetic Association*, 1997, 97:867–870.
 61. McCormick DB. Niacin. In: Shils ME, Young VR, eds. *Modern nutrition in health and disease*, 6th ed. Philadelphia, PA, Lea & Febiger, 1988:370–375.
 62. Carpenter KJ, Lewin WJ. A re-examination of the composition of diets associated with pellagra. *Journal of Nutrition*, 1985, 115:543–552.
 63. Berger NA. Poly (ADP-ribose) in the cellular response to DNA damage. *Radiation Research*, 1985, 101:4–15.
 64. Shibata K, Matsuo H. Effect of supplementing low protein diets with the limiting amino acids on the excretion of N'-methylnicotinamide and its pyridones in rat. *Journal of Nutrition*, 1989, 119:896–901.
 65. Jacob RA et al. Biochemical markers for assessment of niacin status in young men: urinary and blood levels of niacin metabolites. *Journal of Nutrition*, 1989, 119:591–598.
 66. Dillon JC et al. The urinary metabolites of niacin during the course of pellagra. *Annals of Nutrition and Metabolism*, 1992, 36:181–185.
 67. Fu CS et al. Biochemical markers for assessment of niacin status in young

- men: levels of erythrocyte niacin coenzymes and plasma tryptophan. *Journal of Nutrition*, 1989, 119:1949–1955.
68. Ribaya-Mercado JD et al. Effect of niacin status on gastrointestinal function and serum lipids [abstract]. *FASEB Journal*, 1997, 11:179.
 69. Rose DP, Braidman IP. Excretion of tryptophan metabolites as affected by pregnancy, contraceptive steroids, and steroid hormones. *American Journal of Clinical Nutrition*, 1971, 24:673–683.
 70. Patterson JI et al. Excretion of tryptophan- niacin metabolites by young men: effects of tryptophan, leucine, and vitamin B6 intakes. *American Journal of Clinical Nutrition*, 1980, 33:2157–2167.
 71. Horwitt MK, Harper AE, Henderson LM. Niacin-tryptophan relationships for evaluating niacin equivalents. *American Journal of Clinical Nutrition*, 1981, 34:423–427.
 72. McCormick DB. Vitamin B₆. In: Shils ME, Young VR, eds. *Modern nutrition in health and disease*, 6th ed. Philadelphia, PA, Lea & Febiger, 1988:376–382.
 73. Liu A et al. Relationship between body store of vitamin B6 and plasma pyridoxal-P clearance: metabolic balance studies in humans. *Journal of Laboratory and Clinical Medicine*, 1985, 106:491–497.
 74. Kretsch MJ, Sauberlich HE, Newbrun E. Electroencephalographic changes and periodontal status during short-term vitamin B-6 depletion of young, nonpregnant women. *American Journal of Clinical Nutrition*, 1991, 53:1266–1274.
 75. Bailey AL, Wright AJA, Southon S. Pyridoxal-5-phosphate determination in human plasma by high performance liquid chromatography: how appropriate are cut-off values for vitamin B6 deficiency? *European Journal of Clinical Nutrition*, 1999, 53:448–455.
 76. Hamfelt A, Tuvemo T. Pyridoxal phosphate and folic acid concentration in blood and erythrocyte aspartate aminotransferase activity during pregnancy. *Clinica Chemica Acta*, 1972, 41:287–298.
 77. Leklem JE. Vitamin B-6: a status report. *Journal of Nutrition*, 1990, 120(Suppl. 11):S1503–S1507.
 78. Gregory JF III. Bioavailability of vitamin B-6. *European Journal of Clinical Nutrition*, 1997, 51(Suppl. 1):S43–S48.
 79. Tarr JB, Tamura T, Stokstad EL. Availability of vitamin B₆ and pantothenate in an average American diet in man. *American Journal of Clinical Nutrition*, 1981, 34:1328–1337.
 80. Wozenski JR, Leklem JE, Miller LT. The metabolism of small doses of vitamin B-6 in men. *Journal of Nutrition*, 1980, 110:275–285.
 81. Pannemans DLE, van den Berg H, Westerterp KR. The influence of protein intake on vitamin B-6 metabolism differs in young and elderly humans. *Journal of Nutrition*, 1994, 124:1207–1214.
 82. Shane B, Contractor SF. Assessment of vitamin B₆ status. Studies on pregnant women and oral contraceptive users. *American Journal of Clinical Nutrition*, 1975, 28:739–747.
 83. Rose DP. Oral contraceptives and vitamin B6. In: *Human vitamin B₆ requirements. Proceedings of a workshop: Letterman Army Institute of Research, Presidio of San Francisco, California, June 11–12 1976*. Washington, DC, National Academy Press, 1978:193–201.
 84. Brophy MH, Siiteri PK. Pyridoxal phosphate and hypertensive disorders of pregnancy. *American Journal of Obstetrics and Gynecology*, 1975, 121:1075–1079.

85. Shane B, Contractor SF. Vitamin B₆ status and metabolism in pregnancy. In: Tryfiates GP, ed. *Vitamin B6 metabolism and role in growth*. Westport, CT, Food & Nutrition Press, 1980:137–171.
86. West KD, Kirksey A. Influence of vitamin B6 intake on the content of the vitamin in human milk. *American Journal of Clinical Nutrition*, 1976, 29:961–969.
87. Andon MB et al. Dietary intake of total and glycosylated vitamin B-6 and the vitamin B-6 nutritional status of unsupplemented lactating women and their infants. *American Journal of Clinical Nutrition*, 1989, 50:1050–1058.
88. Heiskanen K et al. Vitamin B-6 status during childhood: tracking from 2 months to 11 years of age. *Journal of Nutrition*, 1995, 125:2985–2992.
89. Linkswiler HM. Vitamin B6 requirements of men. In: *Human vitamin B₆ requirements. Proceedings of a workshop: Letterman Army Institute of Research, Presidio of San Francisco, California, June 11–12 1976*. Washington, DC, National Academy Press, 1978:279–290.
90. Miller LT, Leklem JE, Shultz TD. The effect of dietary protein on the metabolism of vitamin B-6 in humans. *Journal of Nutrition*, 1985, 115:1663–1672.
91. Brown RR et al. Urinary 4-pyridoxic acid, plasma pyridoxal phosphate, and erythrocyte aminotransferase levels in oral contraceptive users receiving controlled intakes of vitamin B6. *American Journal of Clinical Nutrition*, 1975, 28:10–19.
92. Kretsch MJ et al. Vitamin B-6 requirement and status assessment: young women fed a depletion diet followed by a plant- or animal-protein diet with graded amounts of vitamin B-6. *American Journal of Clinical Nutrition*, 1995, 61:1091–1101.
93. Hansen CM, Leklem JE, Miller LT. Vitamin B-6 status of women with a constant intake of vitamin B-6 changes with three levels of dietary protein. *Journal of Nutrition*, 1996, 126:1891–1901.
94. Hansen CM, Leklem JE, Miller LT. Changes in vitamin B-6 status indicators of women fed a constant protein diet with varying levels of vitamin B-6. *American Journal of Clinical Nutrition*, 1997, 66:1379–1387.
95. Ribaya-Mercado JD et al. Vitamin B-6 requirements of elderly men and women. *Journal of Nutrition*, 1991, 121:1062–1074.
96. Selhub J et al. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. *Journal of the American Medical Association*, 1993, 270:2693–2698.
97. Cleary RE, Lumeng L, Li T-K. Maternal and fetal plasma levels of pyridoxal phosphate at term: adequacy of vitamin B6 supplementation during pregnancy. *American Journal of Obstetrics and Gynecology*, 1975, 121:25–28.
98. Lumeng L et al. Adequacy of vitamin B6 supplementation during pregnancy: a prospective study. *American Journal of Clinical Nutrition*, 1976, 29:1376–1383.
99. Borschel MW. Vitamin B6 in infancy: requirements and current feeding practices. In: Raiten DJ, ed. *Vitamin B-6 metabolism in pregnancy, lactation and infancy*. Boca Raton, FL, CRC Press, 1995:109–124.
100. McCormick DB. Pantothenic acid. In: Shils ME, Young VR, eds. *Modern nutrition in health and disease*, 6th ed. Philadelphia, PA, Lea & Febiger, 1988:383–387.
101. Plesofsky-Vig N. Pantothenic acid and coenzyme A. In: Shils ME, Olson JA, Shike M, eds. *Modern nutrition in health and disease*, 8th ed. Philadelphia, PA, Lea & Febiger, 1994:395–401.

102. Fry PC, Fox HM, Tao HG. Metabolic response to a pantothenic acid deficient diet in humans. *Journal of Nutritional Science and Vitaminology*, 1976, 22:339–346.
103. Eissenstat BR, Wyse BW, Hansen RG. Pantothenic acid status of adolescents. *American Journal of Clinical Nutrition*, 1986, 44:931–937.
104. Wittwer CT et al. Enzymes for liberation of pantothenic acid in blood: use of plasma pantothenase. *American Journal of Clinical Nutrition*, 1989, 50:1072–1078.
105. Picciano MF. Vitamins in milk. A. Water-soluble vitamins in human milk. In: Jensen RG, ed. *Handbook of milk composition*. San Diego, CA, Academic Press, 1995.
106. Butte NF et al. Human milk intake and growth in exclusively breast-fed infants. *Journal of Pediatrics*, 1984, 104:187–195.
107. Allen JC et al. Studies in human lactation: milk composition and daily secretion rates of macronutrients in the first year of lactation. *American Journal of Clinical Nutrition*, 1991, 54:69–80.
108. Heinig MJ et al. Energy and protein intakes of breast-fed and formula-fed infants during the first year of life and their association with growth velocity: the DARLING Study. *American Journal of Clinical Nutrition*, 1993, 58:152–161.
109. Kathman JV, Kies C. Pantothenic acid status of free living adolescent and young adults. *Nutrition Research*, 1984, 4:245–250.
110. Srinivasan V et al. Pantothenic acid nutritional status in the elderly—institutionalized and noninstitutionalized. *American Journal of Clinical Nutrition*, 1981, 34:1736–1742.
111. Bul NL, Buss DH. Biotin, pantothenic acid and vitamin E in the British household food supply. *Human Nutrition: Applied Nutrition*, 1982, 36A:125–129.
112. Song WO, Wyse BW, Hansen RG. Pantothenic acid status of pregnant and lactating women. *Journal of the American Dietetic Association*, 1985, 85:192–198.
113. McCormick DB. Biotin. In: Shils ME, Young VR, eds. *Modern nutrition in health and disease*, 6th ed. Philadelphia, PA, Lea & Febiger, 1988:436–439.
114. Mock DM. Biotin. In: Ziegler EE, Filer LJ Jr, eds. *Present knowledge in nutrition*, 7th ed. Washington, DC, International Life Sciences Institute, The Nutrition Foundation, 1996:220–235.
115. Mock NI et al. Increased urinary excretion of 3-hydroxyisovaleric acid and decreased urinary excretion of biotin are sensitive early indicators of decreased status in experimental biotin deficiency. *American Journal of Clinical Nutrition*, 1997, 65:951–958.
116. McCormick DB, Wright LD. The metabolism of biotin and analogues. In: Florkin M, Stotz EH, eds. *Comprehensive biochemistry*, Vol. 21. Amsterdam, Elsevier, 1971:81–110.
117. McCormick DB. Biotin. In: Hegsted M, ed. *Present knowledge in nutrition*, 4th ed. Washington, DC, The Nutrition Foundation, 1976:217–225.
118. Zempleni J, McCormick DB, Mock DM. Identification of biotin sulfone, bisnorbiotin methylketone, and tetranorbiotin-l-sulfoxide in human urine. *American Journal of Clinical Nutrition*, 1997, 65:508–511.
119. Mock DM et al. Biotin deficiency: an unusual complication of parenteral alimentation. *New England Journal of Medicine*, 1981, 304:820–823.

120. Kien CL et al. Biotin-responsive in vivo carboxylase deficiency in two siblings with secretory diarrhea receiving total parenteral nutrition. *Journal of Pediatrics*, 1981, 99:546–550.
121. Gillis J et al. Biotin deficiency in a child on long-term TPN. *Journal of Parenteral and Enteral Nutrition*, 1982, 6:308–310.
122. Mock DM et al. Biotin deficiency complicating parenteral alimentation: diagnosis, metabolic repercussions, and treatment. *Journal of Pediatrics*, 1985, 106:762–769.
123. Lagier P et al. Zinc and biotin deficiency during prolonged parenteral nutrition in infants. *Presse Médicale*, 1987, 16:1795–1797.
124. Carlson GL et al. Biotin deficiency complicating long-term parenteral nutrition in an adult patient. *Clinical Nutrition*, 1995, 14:186–190.
125. Holland B et al. *McCance & Widdowson's the composition of foods*. 5th revised and extended edition. London, Her Majesty's Stationery Office, 1991.
126. Salmenpera L et al. Biotin concentrations in maternal plasma and milk during prolonged lactation. *International Journal of Vitamin and Nutrition Research*, 1985, 55:281–285.
127. Hirano M et al. Longitudinal variations of biotin content in human milk. *International Journal for Vitamin and Nutrition Research*, 1992, 62:281–282.
128. Mock DM et al. Biotin status assessed longitudinally in pregnant women. *Journal of Nutrition*, 1997, 127:710–716.