Chapter 3

Thiamin, riboflavin, niacin, vitamin B_6, pantothenic acid and biotin

The B-complex vitamins covered here are presented in ***Table 5*** along with the biochemical and physiologic roles of the co-enzyme forms and a brief description of clinical deficiency symptoms.

Table 5

Physiologic roles and deficiency signs of B-complex vitamins

Vitamin	Physiologic roles	Deficiency
Thiamin (B_1)	Co-enzyme functions in metabolism of carbohydrates and branched-chain amino acids	Beri-beri, polyneuritis, and Wernicke-Korsakoff syndrome
Riboflavin (B_2)	Co-enzyme functions in numerous oxidation and reduction reactions	Growth, cheilosis, angular stomatitis, and dermatitis
Niacin (nicotinic acid and nicotinamide)	Co-substrate/co-enzyme for hydrogen transfer with numerous dehydrogenases	Pellagra with diarrhoea, dermatitis, and dementia
Vitamin B_6 (pyridoxine, pyridoxamine, and pyridoxal)	Co-enzyme functions in metabolism of amino acids, glycogen, and sphingoid bases	Naso-lateral seborrhoea, glossitis, and peripheral neuropathy (epileptiform convulsions in infants)
Pantothenic acid	Constituent of co-enzyme A and phosphopantetheine involved in fatty acid metabolism	Fatigue, sleep disturbances, impaired coordination, and nausea
Biotin	Co-enzyme functions in bicarbonate-dependent carboxylations	Fatigue, depression, nausea, dermatitis, and muscular pains

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 beri-beri (cardiac and dry), peripheral neuropathies, pellagra, and oral and genital lesions (related to riboflavin deficiency) – were once major public health problems in 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 of the populations.

Although these clinical manifestations of B-vitamin deficiencies have decreased, there is evidence of widespread sub-clinical deficiency of these vitamins (especially of riboflavin and pyridoxine). These sub-clinical 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 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 were selected from those published after the FAO/WHO *handbook on human nutritional requirements* was published in 1974 (*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 considering that many cases are not reported in the medical literature. Moreover, outbreaks of vitamin deficiencies in populations are usually not publicised 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 US National Academy of Sciences (*6*).

Thiamin

Background with requisite function in human metabolic processes

Deficiency

Thiamin (vitamin B_1, aneurin) deficiency results in the disease called beri-beri, which has been classically considered to exist in dry (paralytic) and wet (oedematous) forms (*7, 8*). Beri-beri 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-thiamin factors. Beri-beri is still endemic in Asia. In relatively industrialized nations, the neurologic reflections of Wernicke-Korsakoff syndrome are frequently associated with chronic alcoholism with limited food consumption (*9*). Some cases of thiamin deficiency have been observed with patients who are hypermetabolic, are on parenteral nutrition, are undergoing chronic renal dialysis, or have undergone a gastrectomy. Thiamin deficiency has also been observed in Nigerians who ate silk worms, Russian schoolchildren (in Moscow), Thai rural elderly, Cubans, Japanese elderly, Brazilian Xavante Indians, French Guyanense, Southeast Asian schoolchildren who were infected with hookworm, Malaysian detention inmates, and people with chronic alcoholism.

Toxicity

Toxicity is not a problem with thiamin because renal clearance of levels conceivably ingested is rapid.

Functions

Thiamin functions as the co-enzyme thiamin 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 thiamin, the overall decrease in carbohydrate metabolism and its inter-connection with amino acid metabolism (via α-keto acids) have severe consequences, such as a decrease in the formation of acetylcholine for neural function.

Biochemical indicators

Indicators used to estimate thiamin requirements are urinary excretion, erythrocyte transketolase activity coefficient, erythrocyte thiamin, 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 thiamin nutriture is improved with load test. Erythrocyte transketolase activity co-efficient reflects TPP levels and can indicate rare genetic defects. Erythrocyte thiamin is mainly a direct measure of TPP but also is a measure of thiamin and thiamin monophosphate by high performance liquid chromatography (HPLC) separation.

Thiamin status has been assessed by measuring urinary thiamin excretion under basal conditions or after thiamin loading, transketolase activity, and free and phosphorylated forms in blood or serum (*6, 9*). Although overlap with baseline values for urinary thiamin 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 effect when thiamin intakes were adequate 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 inter-individual and genetic factors affecting the transketolase (*16*). Baines and Davies (*17*) suggested that it is useful to determine erythrocyte TPP directly because the co-enzyme is less susceptible to factors that influence enzyme activity; however, there are also methods for determining thiamin and its phosphate esters in whole blood (*18*).

Factors affecting requirements

Because thiamin facilitates energy utilisation, its requirements have traditionally been expressed on the basis of energy intake, which can vary depending on activity levels. However, Fogeholm *et al.* (*19*) found no difference in activation coefficients for erythrocyte transketolase from a small group of skiers and from less physically active control subjects. Also, a study with thiamin-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 the decreases in indicators of thiamin status (*20*). Alcohol consumption may interfere with thiamin absorption (*9*).

Findings by age and life stage

Recommendations for infants are based on adequate food intake. Mean thiamin content of human milk is 0.21 mg/l (0.62 μmol/l) (*21*), which corresponds to 0.16 mg (0.49μmol) thiamin per 0.75 l of secreted milk per day. The blood concentration for total thiamin 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 thiamin to several indicators of thiamin status (*15*). Sauberlich *et al.* (*23*) concluded from a carefully controlled depletion-repletion study of seven healthy young men that 0.3 mg thiamin 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 thiamin intakes of 1.0 and 1.2 mg/day as minimal for women and men, respectively. Hoorn et al. (*26*) reported that 23 percent of 153 patients aged 65–93 years were deemed deficient based on a transketolase activation coefficient greater than 1.27, which was normalised after thiamin administration. Nichols and Basu (*27*) found that only 57 percent of 60 adults aged 65–74 years had TPP effects of less than 14 percent and suggested that ageing may increase thiamin requirements.

An average total energy cost of 230 MJ has been estimated for pregnancy (*28*). With an intake of 0.4 mg thiamin/4184 kJ, this amounts to 22 mg total, or 0.12 mg/day for an additional thiamin need for the second and third trimesters (180 days). Taking into account an increased growth in maternal and foetal compartments, an overall additional requirement of 0.3 mg/day is adequate (6).

Lactating women are estimated to transfer 0.2 mg thiamin in their milk each day, and an additional 0.2 mg is estimated as a need for the increased energy cost of lactation of about 2092 kJ/day.

Recommendations

The recommendations for thiamin are given in ***Table 6***.

Table 6

Recommended nutrient intakes for thiamin

Group	Recommended nutrient intake mg/day
Infants and children	
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
Adolescents, 10–18 years	
Females	1.1
Males	1.2
Adults	
Females, 19+ years	1.1
Males, 19+ years	1.2
Pregnancy	1.4
Lactation	1.5

Riboflavin

Background with requisite function in human metabolic processes

Deficiency

Riboflavin (vitamin B_2) 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 bone marrow (*8, 29*). Because the deficiency almost invariably occurs combined 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 hypo-riboflavinosis 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 causes of deficiency are inborn errors in which the genetic defect is in the formation of a flavoprotein (e.g., acyl-co-enzyme A [co-A] dehydrogenases). Also at risk are those receiving phototherapy for neonatal jaundice and perhaps those with inadequate thyroid hormone. Some cases of riboflavin deficiency were also observed in Russian schoolchildren (Moscow) and Southeast Asian schoolchildren (infected with hookworm).

Toxicity

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

Functions

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

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 vitamin and 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 mainly 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 returns tests (amounts measured after a specific amount of riboflavin is given orally); erythrocyte glutathione reductase; 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 biologic response because certain catabolites can inhibit cellular uptake (*33*). Under conditions of adequate riboflavin uptake (≈1.5 mg/day) by adults, an estimated 120 μg (320 nmol) total riboflavin or 80 μg/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, but some limits 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 hundreds of 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 are considered adequate.

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 "normalised" with 20 percent additional riboflavin. However, riboflavin supplementation did not lead to an increase in work performance when such subjects were not clinically deficient (*42-45*).

Bio-availability of riboflavin in foods, mostly as digestible flavoco-enzymes, is excellent at nearly 95 percent (*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 percent of food flavin is found as 8α-FAD covalently attached to certain flavoprotein enzymes. Although some portions of the 8α-(amino acid)-riboflavins are released by proteolysis of these flavoproteins, they do not have vitamin activity (*47*). A lower fat-to-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 B_6, which requires FMN for conversion of the phosphates of pyridoxine and pyridoxamine to the co-enzyme 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/418 kJ was given in a 2-week acclimation period (*50*).

Findings by age and life stage

As reviewed by Thomas et al. (*51*), early estimates of riboflavin content in human milk showed changes during the post-partum 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 0.35mg (931 nmol) /l (*6*) or 0.26 mg (691nmol) /0.75 l of milk per day. For low-income Indian women with erythrocyte glutathione reductase activity ratios averaging 1.80 and a milk riboflavin content of 0.22 mg/l, 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 sub-clinical 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 1.6–3.4 µg (4.2 to 9.0 nmol) /mg creatinine and a decrease in AC to 1.25. Boisvert *et al.* (*48*) obtained normalisation 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 the seven non-pregnant women. Maternal riboflavin intake was positively associated with foetal 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 foetal compartments. For lactating women, an estimated 0.3 mg riboflavin is transferred in milk daily and, because utilisation for milk production is assumed to be 70 percent efficient, the value is adjusted upward to 0.4 mg/day.

Recommendations

The recommendations for riboflavin are given in ***Table 7***.

Table 7

Recommended nutrient intakes for riboflavin

Group	Recommended nutrient intake mg/day
Infants and children	
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
Adolescents, 10–18 years	
Females	1.0
Males	1.3
Adults	
Females, 19+ years	1.1
Males, 19+ years	1.3
Pregnancy	1.4
Lactation	1.6

Niacin

Background with requisite function in human metabolic processes

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 India, China, and Africa. Its cause has been mainly attributed to a deficiency of niacin; however, its biochemical inter-relationship to riboflavin and vitamin B_6, 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) (*61*). Pellagra also occurs in people with chronic alcoholism (*61*). Cases of niacin deficiency have been found in people suffering from Crohn's 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 US Food and Nutrition Board (*6*) was adopted by this consultation.

Functions

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, which does not have the pharmacologic action of the acid that is administered at high doses to lower blood lipids. It is the amide form that exists within the redox-active co-enzymes, 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 mobilisation. 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 functions 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.

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 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*). Similarly to the situation for erythrocyte pyridine nucleotide (nicotinamide co-enzymes), NAD concentration decreased 70 percent whereas NADP remained unchanged in adult males fed diets with only 6 or 10 mg NEs/day (*67*). Erythrocyte

NAD provided a marker at least as sensitive as urinary metabolites of niacin in this study (*67*) and in a niacin depletion study of elderly subjects (*68*).

Factors affecting requirements

The biosynthesis of niacin derivatives on the pathway to nicotinamide co-enzymes stems from tryptophan, an essential amino acid found in protein, and as such this source of NEs increases niacin intake. There are several dietary, drug, and disease factors that reduce the conversion of tryptophan to niacin (*61*) (e.g. the use of oral contraceptives [*69*]). Although a 60-to-1 conversion factor represents the average for human utilisation of tryptophan as NEs, there are substantial individual differences (*70, 71*). There is also an interdependence of enzymes within the tryptophan-to-niacin pathway where vitamin B_6 (as pyridoxal phosphate) and riboflavin (as FAD) are functional. Further, riboflavin (as FMN) is required for the oxidase that forms co-enzymic PLP from the alcohol and amine forms of phosphorylated vitamin B_6 (*49*).

Findings by age and life stage

Niacin content of human milk is approximately 1.5 mg (12.3 μmol) /l and the tryptophan content is 210 mg (1.0mmol) /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 kjoule (1,000 kcal) × 230,000 kjoule (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 percent increase. Also about 2 mg NEs/day is required for growth in maternal and foetal 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 added need attributable to lactation.

Recommendations

The recommendations for niacin are given in ***Table 8***.

Table 8

Recommended nutrient intakes for niacin

Group	Recommended nutrient intake, NEs/day[a]
Infants and children	
0–6 months	2[b]
7–12 months	4
1–3 years	6
4–6 years	8
7–9 years	12
Adolescents, 10–18 years	16
Adults	
Females, 19+ years	14
Males, 19+ years	16
Pregnancy	18
Lactation	17

[a] NEs, niacin equivalents. [b] Preformed.

Vitamin B_6

Background with requisite function in human metabolic processes

Deficiency

A deficiency of vitamin B_6 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 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 urinary xanthurenate and a decreased glutamate conversion to the anti-neurotransmitter γ-aminobutyrate. Hypovitaminosis B_6 may often occur with riboflavin deficiency, because riboflavin is needed for the formation of the co-enzyme PLP. Infants are especially susceptible to insufficient intakes, which can lead to epileptiform convulsions. Skin changes include dermatitis with cheilosis and glossitis. There is usually a decrease in circulating lymphocytes and possibly a normocytic, microcytic, or sideroblastic anaemia (*9*). The sensitivity of such systems as sulphur amino acid metabolism to vitamin B_6 availability is reflected in homo-cysteinemia. A decrease in the metabolism of glutamate in the brain, which is found in vitamin B_6 insufficiency, reflects a nervous system dysfunction. As is the case with other micronutrient deficiencies, vitamin B_6 deficiency results in an impairment of the immune system. A current concern is for the rather pandemic occurrence of somewhat low vitamin B_6 intakes in many people who eat poorly (e.g., people with eating disorders). Vitamin B_6 deficiency has also been observed in Russian schoolchildren (Moscow), Southeast Asian schoolchildren (infected with hookworm), elderly Europeans (Dutch), and in some individuals with hyperhomo-cysteinemia or on chronic hemodialysis. Several medical conditions can also affect vitamin B_6 metabolism and lead to deficiency symptoms.

Toxicity

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

Functions

There are three natural vitamers (different forms of the vitamin) of vitamin B_6, namely pyridoxine, pyridoxamine, and pyridoxal. These must be phosphorylated and the 5'-phosphates of the first two oxidized to the functional PLP, which serves as a carbonyl-reactive co-enzyme to diverse enzymes involved in the metabolism of amino acids. Such enzymes include aminotransferases, decarboxylases, and dehydratases, δ-aminolevulinate synthase in heme biosynthesis, phosphorylase in glycogen breakdown, and sphingoid base biosynthesis, etc. (*10, 11*).

Biochemical indicators

Indicators used to estimate vitamin B_6 requirements are PLP, urinary excretion, erythrocyte aminotransferases activity coefficients, tryptophan catabolites, erythrocyte and whole blood PLP, and plasma homo-cysteine. PLP is the major vitamin B_6 form in tissue and reflects liver PLP; it changes fairly slowly in response to vitamin intake. The excretion rate of vitamin and particularly 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 used especially after a tryptophan load test.

Vitamin B_6 status is most appropriately evaluated by using a combination of indicators, including those considered direct (e.g., vitamer concentration in cells or fluids) and 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 B_6 given to 11 young women led to abnormal electroencephalograph patterns in 2 of the women and a plasma PLP of approximately 9 nmol. Hence, a level of about 10 nmol is considered sub-optimal. A cut-off for plasma PLP of 20 nmol 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 (*76*). Urinary 4-pyridoxic acid level changes promptly with changes in vitamin B_6 intake (*73*) and is therefore of questionable value in assessing status. However, a value higher than 3 μmol/day , achieved with an intake of ~1 mg/d, 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 B_6 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 B_6 status. In a review of such literature, Leklem (*77*) suggested that a 24-hour urinary excretion less than 65 μmol xanthurenate after a 2-g oral dose of tryptophan indicates normal vitamin B_6 status.

Factors affecting requirements

A recent review by Gregory (*78*) shows that bio-availability of vitamin B_6 in a mixed diet is about 75 percent (*79*), with approximately 8 percent of this total contributed by pyridoxine β-D-glucoside, which is about half as effectively utilised (*78*) as free B vitamers or their phosphates. The amine and aldehyde forms of vitamin B_6 may be about 10 percent 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 B_6 status (*81*). Studies reported decreases in indicators of vitamin B_6 status in women receiving oral contraceptives (*82, 83*), but this probably reflects hormonal stimulation of tryptophan catabolism rather than any deficiency of vitamin B_6 per se. Subjects with pre-eclampsia or eclampsia have lowered plasma PLP levels than do healthy pregnant women (*84, 85*).

Findings by age and life stage

The average intake for infants, based on human-milk content, is 0.13 mg/l (*86*) or 0.1 mg/0.75 l/day. With an average maternal dietary intake of vitamin B_6 of 1.4 mg/day, human milk was found to contain 0.12 mg/l, and plasma PLP of nursing infants averaged 54 nmol (*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 B_6 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.

In a review of earlier studies of men with various protein intakes, Linkswiler (*89*) concluded that normalisation of a tryptophan load test required 1–1.5 mg vitamin B_6. Miller *et al.* (*90*) found that 1.6 mg vitamin B_6 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 1–1.2 mg vitamin B_6 could be estimated.

Limited studies of the elderly indicate that requirements may be somewhat higher, at least to maintain plasma PLP above a 20-nmolar cut-off level (*95, 96*).

For pregnancy it was confirmed that indicators of vitamin B_6 status decrease, especially in the third trimester (*85, 97, 98*). It is not clear, however, whether this is a normal physiologic phenomenon. For a maternal body store of 169 mg and foetal plus placental accumulation of 25 mg vitamin B_6, 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 about 75 percent of bio-availability, an additional average requirement in pregnancy of 0.25 mg can be assumed. Because most of this need is in the latter stages of pregnancy and vitamin B_6 is not stored to any significant extent, an extra 0.5 mg/day of vitamin B_6 may be justified to err on the side of safety.

For lactation, it may be prudent to add 0.6 mg vitamin B_6 to the base requirement for women because low maternal intakes could lead to a compromised vitamin B_6 status in the infant (*99*).

Recommendations

The recommendations for vitamin B_6 are given in ***Table 9***.

Table 9

Recommended nutrient intakes for vitamin B_6

Group	Recommended nutrient intake mg/day
Infants and children	
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
Adolescents, 10–18 years	
Females	1.2
Males	1.3
Adults	
Females, 19–50 years	1.3
Males, 19–50 years	1.3
Females, >50 years	1.5
Males, >50 years	1.7
Pregnancy	1.9
Lactation	2.0

Pantothenate

Background with requisite function in human metabolic processes

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-methyl-pantothenate) 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, rapid heart rate on exertion, epigastric distress with anorexia and constipation, 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.

Functions

Pantothenic acid is a component of CoA, a cofactor that carries acyl groups for many enzymatic processes, and of phosphopantetheine within acyl carrier protein, a component of the fatty acid synthase complex (*10, 11*). Pantothenate is most especially involved in fatty acid metabolism but has a wide-ranging function as a prosthetic group that adds specificity to binding with appropriate enzymes.

Biochemical indicators

Indicators used to estimate pantothenate requirements are urinary excretion and blood levels. Excretion rate reflects intake. Whole blood, which contains vitamin and pantothenate-containing metabolites, has a general correlation with intake; erythrocyte levels 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 3–0.8 mg/day (13.7–3.6 μmol/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 (12μmol)/d, but it was strongly dependent on diet (*79*). Pantothenate intake estimated for adolescents was significantly correlated with pantothenate in urine (*103*). Whole-blood pantothenate fell from 1.95–1.41 μg/ml (8.8–6.4 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– 12.1μmol/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.

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 percent of the pantothenate present in natural foods may be bio-available (*79*).

Findings by age and life stage

Infant requirements are based on an estimation of pantothenic acid content of human milk, which according to reported values is approximately 2 mg/l (*20, 105*). For a reported average human-milk intake of 0.75 l/day (*106-108*) these values suggest that 1.6 mg/day is an adequate intake by the younger (0–6 months) infants. Taking into consideration growth and body size, 1.9 mg/day may be extrapolated for the older (6–12 months) infant (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 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*).

For lactation blood pantothenate concentrations were found significantly lower at 3 months post-partum (*112*). With a loss of 1.7 mg/day (7.8 μmol/d) from a lactating woman and lower maternal blood concentrations found with intakes of about 5–6 mg/d, a recommended intake may be 7 mg/d.

Recommendations

The recommendations for pantothenate are given in ***Table 10***.

Table 10

Recommended nutrient intakes for pantothenate

Group	Recommended nutrient intake, mg/day
Infants and children	
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
Adolescents, 10–18 years	5.0
Adults	
Females, 19+	5.0
Males, 19+	5.0
Pregnancy	6.0
Lactation	7.0

Biotin

Background with requisite function in human metabolic processes

Deficiency

Biotin deficiency in humans has been clearly documented with prolonged consumption of raw egg whites, which contain biotin-binding avidin. Biotin deficiency was also 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 were noted in infants with intractable diaper 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 limited intestinal absorption of biotin.

Functions

Biotin functions as a co-enzyme within several carboxylases after the carboxyl function of the vitamin 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 both in mitochondria and the cytosol. In all these cases biotin serves as carrier for the transfer of active bicarbonate into a substrate to generate a carboxyl product.

Biochemical indicators

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

The present indicators for biotin status are its urinary excretion, as assessed with an avidin-based radioimmunoassay with HPLC, and 3-hydroxyisovalerate excretion (*115*). The isolation and chemical identification of more than a dozen metabolites of biotin established the main features of its use in microbes and mammals (*116, 117*). Quantification of the major biotin metabolites was done by Zempleni *et al*. (*118*). Both biotin and bisnorbiotin excretions 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 μmol/24 hours. Decreased excretion of biotin, abnormally increased excretion of 3-hydroxyisovalerate, or both have been reported for 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.

Findings by age and life stage

The biotin content of human milk is estimated to be approximately 6 μg (24nmol)/l based on several studies (*125-127*) that report values ranging from near 4–7 μg (16.4–28.9 nmol) /l. Hence, the estimated intake of biotin for an infant consuming 0.75 l is 5 μg/day during the first half year and for older infants is perhaps 6 μg/day.

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

Adequate intake for child or adult = (adequate intake young infant) (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 lactation the intake may need to be increased by an additional 5 μg/day to cover the losses due to human-milk secretion.

Recommendations

The recommendations for biotin are given in ***Table 11***.

Table 11

Recommended nutrient intakes for biotin

Group	Recommended nutrient intake μg/day
Infants and children	
0–6 months	5
7–12 months	6
1–3 years	8
4–6 years	12
7–9 years	20
Adolescents, 10–18 years	25
Adults	
Females, 19+	30
Males, 19+	30
Pregnancy	30
Lactation	35

General considerations for B-complex vitamins

Notes on suggested recommendations

The recommendations for infants are based largely on the composition and quantity of human milk consumed and are formally considered to be adequate intakes. Younger (0–6 months) infants are considered to derive adequate intake from milk alone; recommendations for older (7–12 months) infants are adjusted by metabolic scaling such that a factor – (weight of 7-12 mo/weight of 0-5 mo) $^{0.75}$ – is multiplied by the recommendation for the younger infant (*6*). Recommendations have been given to use the higher (6–11 months) level for the first year of life.

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 has been used where a factor – (weight child/weight adult) $^{0.75}$ × (1 + growth factor) – is multiplied by the adult recommendation (*6*).

For most of the B-complex vitamins covered here, data are not sufficient to justify altering recommendations for the elderly.

For pregnancy and lactation, increased maternal needs related to increases in energy and replacement of secretion losses were considered.

Dietary sources of B-complex vitamins

A listing of some better and usual food sources for the vitamins considered is given in ***Table 12.***

Table 12

Dietary sources of water-soluble vitamins

Vitamin	Good-to-moderate dietary sources[a]
Thiamin (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]Foods are listed according to the concentrations of vitamin which they contain.

Research suggestions

In view of the issues raised in this section on B-complex vitamins, the following suggestions are noted:

- Actual requirements are least certain for children, adolescents, pregnant and lactating women, and the elderly, and as such they 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.
- 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 bio-availability 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; care necessary for their storage and preparation; and achievable means for adjusting intake with age, sex, and health status.

REFERENCES

1. **United Nations Sub-Committee on Nutrition.** 1988. *Report on the Nutrition Situation of Refugees and Displaced Populations*. No. 25; p.5., Geneva, Switzerland.
2. **Sadun, J. Martone, R. Muci-Mendoza, L. Reyes, J. C. Silva & B. Caballero**. 1994 Epidemic optic neuropathy in Cuba: eye findings. *Arch. Ophthalmol.,* 112: 691-699.
3. **O. Ordunez-Garcia, J. F. Nieto, A. D. Espinosa-Brito & B. Caballero**. 1996. Cuban epidemic neuropathy, 1991-1994: history repeats itself a century after the "amblyopia of the blockade". *Am. J. Public Health,* 86 (5): 738-743.
4. **R. Hedges, K. Tucker, M. Hirano & B. Caballero**. 1997 Epidemic optic and peripheral neuropathy in Cuba: the unique geopolitical public health problem. *Surveys Ophthalmol.,* 41: 341-353.
5. **FAO/WHO.** 1974. Handbook on Human Nutritional Requirements, eds. Passmore R, Nicol BM, Rao M. Narayana, Beaton GH, de Mayer EM. FAO Nutritional Studies No.28; WHO Monograph Series No. 61, Rome, Italy.
6. **Food and Nutrition Board, Institute of Medicine/National Academy of Sciences-National Research Council.** 1998. *Dietary Reference Intake: Folate, Other B Vitamins, and Choline*. Washington, D.C., National Academy Press.
7. **McCormick, D.B**. Thiamin. 1988. In: *Modern Nutrition in Health and Disease, 6th edition.* Shils, M.E., Young V.R., eds. Philadelphia: Lea & Febiger, p. 355-61.
8. **McCormick, D.B**. 1997. Vitamin, Structure and Function of. In: *Encyclopedia of Molecular Biology and Molecular Medicine, Vol. 6.* Meyers, R.A., ed. Weinheim: VCH, p. 244-52.
9. **McCormick, D.B & Greene, H.L**. 1994. Vitamins. In: *Tietz Textbook of Clin Chem., 2nd edition.* Burtis, V.A., Ashwood, E.R., eds. Philadelphia: W.B. Saunders, p. 1275-1316.
10. **McCormick, D.B.** 1996. Co-enzymes, Biochemistry of. In: *Encyclopedia of Molecular Biology and Molecular Medicine, Vol. 1.* Meyers, R.A., ed. Weinheim: VCH, p. 396-406.
11. **McCormick, D.B.** 1997. Co-enzymes, Biochemistry. In: *Encyclopedia of Human Biology 2nd edition.* Dulbecco, R., ed.-in-chief. San Diego: Academic Press, p. 847-64.
12. **Bayliss, R.M., Brookes, R., McCulloch, J., Kuyl, J.M. & Metz, J.** 1984. Urinary thiamine excretion after oral physiological doses of the vitamin. *Int. J. Vit. Nutr. Res.,* 54: 161-4.
13. **Gans, D.A. & Harper, A.E.** 1991. Thiamin status of incarcerated and nonincarcerated adolescent males: dietary intake and thiamin pyrophosphate response. *Am. J. Clin. Nutr.,* 1991; 53: 1471-5.
14. **Schrijver**, J. Biochemical markers for micronutrient status and their interpretation. *In: Modern Lifestyles, Lower Energy Intake and Micronutrient Status.* Pietrzik, K., ed. Heidelberg: Springer-Verlag, p. 55-85.
15. **Bailey, A.L., Finglas, P.M., Wright, A.J. & Southon, S.** 1994. Thiamin intake, erythrocyte transketolase (EC 2.2.1.1) activity and total erythrocyte thiamin in adolescents. *Br. J. Nutr.,* 72: 111-25.
16. **Singleton, C.K., Pekovich, S.R., McCool, B.A. & Martin, P.R**. 1995. The thiamine-dependent hysteretic behavior of Human transketolase: implications for thiamine deficiency. *J. Nutr.,* 125: 189-194.

17. **Baines, M. & Davies, G**. 1988. The evaluation of erythrocyte thiamin diphosphate as an indicator of thiamin status in man, and its comparison with erythrocyte transketolase activity measurements. *Annals Clin. Biochem.,* 1988; 25 (Part 6): 698-705.
18. **Gerrits, J., Eidhof, H., Brunnekreeft, J.W. & Hessels, J.** Determination of thiamin and thiamin phosphates in whole blood by reversed-phase liquid chromatography with precolumn derivatization. *In: Methods in Enzymology. Vitamins and Co-enzymes.* McCormick, D.B., Suttie, J.W., Wagner, C., eds. San Diego: Academic Press, 279: 74-82.
19. **Fogelholm, M., Rehunen, S., Gref, C.G., Laakso, J.I., Lehto, J., Ruskonen, I. & Himberg, J.J. 1992.** Dietary intake and thiamin, iron, and zinc status in elite Nordic skiers during different training periods. *Int. J. Sport Nutr.,* 2: 351-65.
20. **van der Beek, E.J., van Dokkum, W., Wedel, M., Schrijver & van den Berg,** H. 1994. Thiamin, riboflavin and vitamin B6: impact of restricted intake on physical performance in man. *J. Am. Coll. Nutr.,* 13: 629-40.
21. **Committee on Nutrition.** 1985. Composition of Human milk: normative data. In: Pediatric Nutrition Handbook, 2nd Edition. Elk Grove Village, IL: *Am. Acad. Pediatr.,* p.363-368.
22. **Wyatt, D.T., Nelson, D. & Hillman, R.E.** 1991. Age-dependent changes in thiamin concentrations in whole blood and cerebrospinal fluid in infants and children. *Am. J. Clin. Nutr.,* 53: 530-6.
23. **Sauberlich, H.E., Herman, Y.F., Stevens, C.O. & Herman, R.H.** 1979. Thiamin requirement of the adult Human. *Am. J. Clin. Nutr.,* 32: 2237-48.
24. **Wood, B., Gijsbers, A., Goode, A., Davis, S., Mulholland, J. & Breen, K.** 1980. A study of partial thiamin restriction in Human volunteers. *Am. J. Clin. Nutr.,* 33: 848-61.
25. **Anderson, S. H., Charles, T.J. & Nicol, A.D.** 1985. Thiamine deficiency at a district general hospital: report of five cases. *Q. J. Med.,* 55: 15-32.
26. **Hoorn, R.K., Flikweert, J.P. & Westerink, D**. 1975. Vitamin B_1, B_2 and B_6 deficiencies in geriatric patients, measured by co-enzyme stimulation of enzyme activities. *Clinica Chimica Acta*, 61: 151-62.
27. **Nichols, H.K. & Basu, T.K.** 1994. Thiamin status of the elderly: dietary intake and thiamin pyrophosphate response. *J. Am. Coll. Nutr.,* 13: 57-61.
28. **Food and Nutrition Board, Institute of Medicine/National Academy of Sciences-National Research Council.** 1990. *Nutrition During Pregnancy.* Part I Weight Gain. Part II Nutrient Supplements. Washington, D.C, National Academy Press.
29. **McCormick, D.B**. 1994. Riboflavin. In: Modern Nutrition in Health and Disease, 8th edition. Shils, M.E., Olson, J.A., Shike, M., eds. Philadelphia: Lea & Febiger, p. 366-75.
30. **Smith, M.D.** 1980. Rapid method for determination of riboflavin in urine by high-performance liquid chromatography. *J. Chromatogr.*, 182: 285-91.
31. **Chastain, J.L. & McCormick, D.B.** 1987. Flavin catabolites: identification and quantitation in Human urine. *Am. J. Clin. Nutr.,* 46: 830-4.
32. **Roughead, Z.K. & McCormick, D.B.** 1991. Urinary riboflavin and its metabolites: effects of riboflavin supplementation in healthy residents of rural Georgia (USA). *Eur. J. Clin. Nutr.,* 45: 299-307.
33. **Aw, T.-Y., Jones, D.P. & McCormick, D.B.** 1983. Uptake of riboflavin by isolated rat liver cells. *J. Nutr.,* 113: 1249-54.
34. **Nichoalds, G.E.** 1981. Riboflavin. Symposium in Laboratory Medicine. In: Labbac RF, ed. *Symposium on Laboratory Assessment of Nutritional Status*. Clinics in Laboratory Medicine. Philadelphia: W.B. Saunders, 1: 685-98.

35. **Sadowski, J.A.** 1992. Riboflavin. In: *Nutrition in the Elderly.* The Boston Nutritional Status Survey. Hartz, S.C., Russell, R.M., Rosenberg, I.H. eds. London: Smith-Gordon, p. 119-25.

36. **Ramsay, V.P., Neumann, C., Clark, V. & Swenseid, M.E.** 1983. Vitamin cofactor saturation indices for riboflavin, thiamine, and pyridoxine in placental tissue of Kenyan women. *Am. J. Clin. Nutr.,* 37: 969-73.

37. **Belko, A.Z., Obarzanek, E., Kalkwarf, H.J., Rotter, M.A., Bogusz, S., Miller, D., Haas, J.D. & Roe, D.A.** 1983. Effects of exercise on riboflavin requirements of young women. *Am. J. Clin. Nutr.,* 37: 509-17.

38. **Belko, A.Z., Obarzanek, E., Roach, R., Rotten, M., Urban, G., Weinberg, S. & Roe, D.A.** 1984. Effects of aerobic exercise and weight loss on riboflavin requirements of moderately obese, marginally deficient young women. *Am. J. Clin. Nutr.,* 40: 553-61.

39. **Belko, A.Z., Meredith, M.P., Kalkwarf, H.J., Obarzanek, E., Weinberg, S., Roach, R., McKeon, G. & Roe, D.A.** 1985. Effects of exercise on riboflavin requirements: biological validation in weight reducing women. *Am. J. Clin. Nutr.,* 41: 270-7.

40. **Soares, M.J., Satyanarayana, K., Bamji, M.S., Jacob, C.M., Ramana, Y.V. & Rao, S.S.** 1993. The effect of exercise on the riboflavin status of adult men. *Br. J. Nutr.,* 69: 541-51.

41. **Winters, L.R., Yoon, J.S., Kalkwarf, H.J., Davies, J.C., Berkowitz, M.G., Haas, J. & Roe, D.A.** 1992. Riboflavin requirements and exercise adaptation in older women. *Am. J. Clin. Nutr.,* 56: 526-32.

42. **Powers, H.J., Bates, C.J., Eccles, M., Brown, H. & George, E.** 1987. Bicycling performance in Gambian children: effects of supplements of riboflavin or ascorbic acid. Human. *J. Clin. Nutr.*, 41: 59-69.

43. **Prasad, A.P., Bamji, M.S., Lakshmi, A.V. & Satyanarayana, K.** 1990. Functional impact of riboflavin supplementation in urban school children. *Nutr Res.,* 10: 275-81.

44. **Tremblay, A., Boilard, M., Bratton, M.F., Bessette, H. & Roberge, A.B.** 1984. The effects of a riboflavin supplementation on the nutritional status and performance of elite swimmers. *Nutr Res.,* 4: 201-8.

45. **Weight, L.M., Myburgh, K.H. & Noakes, T.D.** 1988. Vitamin and mineral supplementation: effect on the running performance of trained athletes. *Am. J. Clin. Nutr.,* 47: 192-5.

46. **Zempleni, J., Galloway, J.R. & McCormick, D.B.** 1996. Pharmacokinetics of orally and intravenously administered riboflavin in healthy Humans. *Am. J. Clin. Nutr.,* 63: 54-66.

47. **Chia, C.P., Addison, R. & McCormick, D.B.** 1978. Absorption, metabolism, and excretion of 8a-(amino acid)-riboflavins in the rat. *J. Nutr.,* 108: 373-81.

48. **Boisvert, W.A., Mendoza, I., Castañeda, C., DePortocarrero, L., Solomons, N.W., Gershoff, S.N. & Russell, R.M.** 193. Riboflavin requirement of healthy elderly Humans and its relationship to macronutrient composition of the diet. *J. Nutr.,* 123: 915-25.

49. **McCormick, D.B.** 1989. Two interconnected B vitamins: riboflavin and pyridoxine. *Physiol. Revs.,* 69: 1170-98.

50. **Roe, D.A., Bogusz, S., Sheu, J. & McCormick, D.B.** 1982. Factors affecting riboflavin requirements of oral contraceptive users and nonusers. *Am. J. Clin. Nutr.,* 35: 495-501.

51. **Thomas, M.R., Sneed, S.M., Wei, C., Nail, P.A., Wilson, M. & Sprinkle, E.E., III.** 1980. 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. *Am. J. Clin. Nutr.,* 33: 2151-6.

52. **Roughead, Z.K. & McCormick, D.B.** 1990. Flavin composition of Human milk. *Am. J. Clin. Nutr.,* 52: 854-7.

53. **Roughead, Z.K. & McCormick, D.B.** 1990. A qualitative and quantitative assessment of flavins in cow's milk. *J. Nutr.,* 120: 382-8.

54. **Bamji, M.S., Chowdhury, N., Ramalakshmi, B.A. & Jacob, C.M.** 1991. Enzymatic evaluation of riboflavin status of infants. *Eur. J. Clin. Nutr.,* 45: 309-13.

55. **Bates, C.J., Powers, H.J., Downes, R., Brubacher, D., Sutcliffe, V. & Thurnhill, A.** 1989. Riboflavin status of adolescent vs. elderly Gambian subjects before and during supplementation. *Am. J. Clin. Nutr.,* 50: 825-9.

56. **Kuizon, M.D., Natera, M.G., Alberto, S.P., Perlas, L.A., Desnacido, J.A., Avena, E.M., Tajaon, R.T. & Macapinlac, M.P.** 1992. Riboflavin requirement of Filipino women. *Eur. J. Clin. Nutr.,* 46: 257-64.

57. **Alexander, M., Emanuel, G., Golin, T., Pinto, J.T. & Rivlin, R.S.** 1984. Relation of riboflavin nutriture in healthy elderly to intake of calcium and vitamin supplements: evidence against riboflavin supplementation. *Am. J. Clin. Nutr.,* 39: 540-6.

58. **Bates, C.J., Prentice, A.M., Paul, A.A., Sutcliffe, B.A., Watkinson, M. & Whitehead, R.G.** 1981. Riboflavin status in Gambian pregnant and lactating women and its implications for recommended Dietary Allowances. *Am. J. Clin. Nutr.,* 34: 928-35.

59. **Vir, S.C., Love, A.H. & Thompson, W.** 1981. Riboflavin status during pregnancy. *Am. J. Clin. Nutr.,* 34: 2699-705.

60. **Badart-Smook, A., van Houwelingen, A.C., Al, M.D., Kester, A.D. & Hornstra, G. 1997.** Foetal growth is associated positively with maternal intake of riboflavin and negatively with maternal intake of linoleic acid. *J. Am. Diet. Assoc.*, 97: 867-70.

61. **McCormick, D.B.** 1988. Niacin. *In: Modern Nutrition in Health and Disease, 6th edition*. Shils, M.E., Young, V.R., eds. Philadelphia: Lea & Febiger, 370-5.

62. **Carpenter, K.J. & Lewin, W.J.** 1985. A reexamination of the composition of diets associated with pellagra. *J. Nutr.,* 115: 543-52.

63. **Berger, N.A.** 1985. Poly(ADP-ribose) in the cellular response to DNA damage. *Radiat. Res.,* 101: 4-15.

64. **Shibata, K. & Matsuo, H.** 1989. Effect of supplementing low protein diets with the limiting amino acids on the excretion of N^1-methylnicotinamide and its pyridones in rat. *J. Nutr.,* 119: 896-901.

65. **Jacob, R.A., Swendseid, M.E., McKee, R.W., Fu, C.S. & Clemens, R.A.** 1989. Biochemical markers for assessment of niacin status in young men: urinary and blood levels of niacin metabolites. *J. Nutr.,* 119: 591-8.

66. **Dillon, J.C., Malfait, P., Demaux, G. & Foldi-Hope, C.** 1992. The urinary metabolites of niacin during the course of pellagra. *Ann. Nutr. Metab.,* 36: 181-5.

67. **Fu, C.S., Swendseid, M.E., Jacob, R.A. & McKee, R.W.** 1989. Biochemical markers for assessment of niacin status in young men: levels of erythrocyte niacin co-enzymes and plasma tryptophan. *J. Nutr.,* 119: 1949-55.

68. **Ribaya-Mercado. J.D., Russell, R.M., Rasmussen, H.M., Crim, M.C., Perrone-Petty, G. & Gershoff, S.N.** 1997. Effect of niacin status on gastrointestinal function and serum lipids. *FASEB J.*, 11: A179 abstract.

69. **Rose, D.P. & Braidman, I.P.** 1971. Excretion of tryptophan metabolites as affected by pregnancy, contraceptive steroids, and steroid hormones. *Am. J. Clin. Nutr.,* 24: 673-83.

70. **Patterson, J.I., Brown, R.R., Linkswiler, H. & Harper, A.E.** 1980. Excretion of tryptophan-niacin metabolites by young men: effects of tryptophan, leucine, and vitamin B6 intakes. *Am. J. Clin. Nutr.,* 33: 2157-67.
71. **Horwitt, M.K., Harper, A.E. & Henderson, L.M.** 1981. Niacin-tryptophan relationships for evaluating for evaluating niacin equivalents. *Am. J. Clin. Nutr.,* 34: 423-7.
72. **McCormick, D.B.** 1988. Vitamin B_6. In: *Modern Nutrition in Health and Disease, 6th edition.* Shils, M.E., Young, V.R., eds. Philadelphia: Lea & Febiger, p. 376-82.
73. **Liu, A., Lumeng, L., Aronoff, G.R. & Li, T.-K.** 1985. Relationship between body store of vitamin B_6 and plasma pyridoxal-P clearance: metabolic balance studies in Humans. *J. Lab. Clin. Med.,* 106: 491-7.
74. **Kretsch, M.J., Sauberlich, H.E. & Newbrun, E.** 1991. Electroencephalographic changes and periodontal status during short-term vitamin B_6 depletion of young, nonpregnant women. *Am. J. Clin. Nutr.,* 53: 1266-74.
75. **Bailey, A.L., Wright, A.J.A. & Southon, S.** 1999. Pyridoxal-5-phosphate determination in Human plasma by high performance liquid chromatography: How appropriate are cut-off values for vitamin B_6 deficiency? *Eur. J. Clin. Nutr.,* 53(6): 448-55.
76. **Hamfelt, A. & Tuvemo, T.** 1972. Pyridoxal phosphate and folic acid concentration in blood and erythrocyte aspartate aminotransferase activity during pregnancy. *Clinica Chimica Acta*, 41: 287-98.
77. **Lekem, J.E.** 1990. Vitamin B_6: a status report. *J. Nutr.,* 120(11): 1503-7.
78. **Gregory, J.F. III.** 1997. Bio-availability of vitamin B_6. *Eur. J. Clin. Nutr.,* 51(1): S43-8.
79. **Tarr, J.B., Tamura, T. & Stokstad, E.L**. 1981. Availability of vitamin B_6 and pantothenate in an average American diet in man. *Am. J. Clin. Nutr.,* 34: 1328-37.
80. **Wozenski, J.R., Leklen, J.E. & Miller, L.T.** 1980. The metabolism of small doses of vitamin B_6 in men. *J. Nutr.,* 110: 275-85.
81. **Pannemans, D.L.E., van den Berg, H. & Westerterp, K.R.** 1994. The influence of protein intake on vitamin B_6 metabolism differs in young and elderly Humans. *J. Nutr.,* 124: 1207-14.
82. **Shane, B. & Contractor, S.F.** 1975. Assessment of vitamin B_6 status. Studies on pregnant women and oral contraceptive users. *Am. J. Clin. Nutr.,* 28: 739-47.
83. **Rose, D.P.** 1978. Oral contraceptives and vitamin B_6. *In: Human Vitamin B_6 Requirements* (Proceedings of a Workshop). P. 193-201. Washington, D.C., National Academy Press.
84. **Brophy, M.H. & Siiteri, P.K.** 1975. Pyridoxal phosphate and hypertensive disorders of pregnancy. *Am. J. Obstet. Gynecol.*, 121: 1075-9.
85. **Shane, B. & Contractor, S.F.** 1980.Vitamin B_6 Status and metabolism in pregnancy. *In: Vitamin B6 metabolism and role in growth.* Tryfiates, G.P., ed. Westport, Connecticut: Food & Nutrition Press, p. 137-71.
86. **West, K.D. & Kirksey, A.** 1976. Influence of vitamin B6 intake on the content of the vitamin in Human milk. *Am. J. Clin. Nutr.,* 29: 961-9.
87. **Andon, M.B., Reynolds, R.D., Moser-Veillon, P.B. & Howard, M.P.** 1989. Dietary intake of total and glycosylated vitamin B_6 and the vitamin B_6 nutritional status of unsupplemented lactating women and their infants. *Am. J. Clin. Nutr.,* 50: 1050-8.
88. **Heiskanen, K., Kallio, M., Salmenperä, L., Siimes, M.A., Roukonen, I. & Perkeentupa, J.** 1995. Vitamin B_6 status during childhood: tracking from 2 months to 11 years of age. *J. Nutr.,* 125: 2985-92.

89. **Linkswiler, H.M.** 1978. Vitamin B6 requirements of men. In: *Human Vitamin B6 Requirements* (Proceedings of a workshop). p. 279-90. Washington, D.C., National Academy Press.
90. **Miller, L.T., Leklem, J.E. & Shultz, T.D.** 1985. The effect of dietary protein on the metabolism of vitamin B_6 in Humans. *J. Nutr.,* 115: 1663-72.
91. **Brown R.R., Rose, D.P., Leklem, J. E., Linkswiler, H. & Anand, R.** 1975. Urinary 4-pyridoxic acid, plasma pyridoxal phosphate, and erythrocyte aminotransferase levels in oral contraceptive users receiving controlled intakes of vitamin B6. *Am. J. Clin. Nutr.,* 28: 10-19.
92. **Kretsch, M.J., Sauberlich, H.E., Skala, J.H. & Johnson, H.L.** 1995. 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. *Am. J. Clin. Nutr.,* 61: 1091-101.
93. **Hansen, C.M., Leklem, J.E. & Miller, L.T.** 1996. Vitamin B_6 status of women with a constant intake of vitamin B_6 changes with three levels of dietary protein. *J. Nutr.,* 126: 1891-901.
94. **Hansen, C.M., Leklem, J.E. & Miller, L.T.** 1997. Changes in vitamin B_6 status indicators of women fed a constant protein diet with varying levels of vitamin B-6. *Am. J. Clin. Nutr.,* 66: 1379-87.
95. **Ribaya-Mercado, J.D., Russell, R.M., Sahyoun, N., Morrow, F.D. & Gershoff, S.N.** 1991. Vitamin B_6 requirements of elderly men and women. *J. Nutr.,* 121: 1062-74.
96. **Selhub, J., Jacques, P.F., Wilson, P.W.F., Rush, D. & Rosenherg, I.H.** 1993 .Vitamin status and intake as primary determinants of homo-cysteinemia in an elderly population. *JAMA*, 270: 2693-8.
97. **Cleary, R.E., Lumeng, L. & Li, T.-K.** 1975. Maternal and foetal plasma levels of pyridoxal phosphate at term: adequacy of vitamin B6 supplementation during pregnancy. *Am. J. Obstet. Gynecol.*, 121: 25-8.
98. **Lumeng, L., Cleary, R.E., Wagner, R., Yu, P.-L. & Li, T.-K.** 1976. Adequacy of vitamin B6 supplementation during pregnancy: a prospective study. *Am. J. Clin. Nutr.,* 29: 1376-83.
99. **Borschel, M.W.** 1995. Vitamin B6 in infancy: requirements and current feeding practices. *In: Vitamin B_6 Metabolism in Pregnancy, Lactation and Infancy.* Raiten, D.J., ed. Boca Raton, Florida: CRC Press, Inc., p. 109-24.
100. **McCormick, D.B.** 1988. Pantothenic Acid. In: Modern Nutrition in Health and Disease, 6th edition. p.383-7. Shils, M.E., Young, V.R., eds. Philadelphia: Lea & Febiger.
101. **Plesofsky-Vig, N.** 1994. Pantothenic acid and co-enzyme A. In: Modern Nutrition in Health and Disease, 8th edition. Shils, M.E., Olson, J.A., Shike, M., eds. Philadelphia, Lea & Febiger, p. 395-401.
102. **Fry, P.C., Fox, H.M. & Tao, H.G.** 1976. Metabolic response to a pantothenic acid deficient diet in Humans. *J. Nat. Sci.Vit.*, 22: 339-46.
103. **Eissenstat, B.R., Wyse, B.W. & Hansen, R.G.** 1986. Pantothenic acid status of adolescents. *Am. J. Clin. Nutr.,* 44: 931-7.
104. **Wittwer, C.T., Schweitzer, C., Pearson, J., Song, W.O., Windham, C.T., Wyse, B.W. & Hansen, R.G.** 1989. Enzymes for liberation of pantothenic acid in blood: use of plasma pantetheinase. *Am. J. Clin. Nutr.,* 50: 1072-8.
105. **Picciano, M.F.** 1995. Vitamins in milk. A. Water-soluble vitamins in Human milk. In: *Handbook of Milk Composition.* Jensen, R.G., ed. San Diego: Academic Press.

106. **Butte, N.F., Garza, G., Smith, E.O. & Nichols, B.L.** 1984. Human milk intake and growth in exclusively breast-fed infants. *J. Pediatr.*, 104: 187-95.

107. **Allen, J.C., Keller, R.P., Archer, P. & Neville, M.C.** 1991. Studies in Human lactation: milk composition and daily secretion rates of macronutrients in the first year of lactation. *Am. J. Clin. Nutr.,* 54: 69-80.

108. **Heinig, M.J., Nommsen, L.A., Peerson, J.M., Lonnerdal, B. & Dewey, K.G.** 1993. Energy and protein intakes of breast-fed and formula-fed infants during the first year of life an their association with growth velocity: the DARLING Study. *Am. J. Clin. Nutr.,* 58: 152-61.

109. **Kathman, J.V. & Kies, C.** 1984. Pantothenic acid status of free living adolescent and young adults. *Nutr. Res.,* 4: 245-50.

110. **Srinivasan, V., Christensen, N., Wyse, B.W. & Hansen, R.G.** Pantothenic acid nutritional status in the elderly-institutionalized and noninstitutionalized. *Am. J. Clin. Nutr.,* 34: 1736-42.

111. **Bul, N.L., Buss & D.H. Biotin.** 1982. Pantothenic acid and vitamin E in the British household food supply. *Hum. Nutr: Appl. Nutr.*, 36A: 125-9.

112. **Song, W.O., Wyse, B.W. & Hansen, R.G.** 1985. Pantothenic acid status of pregnant and lactating women. *J. Am. Diet. Assoc.*, 85: 192-8.

113. **McCormick, D.B.** 1988. *Biotin. In: Modern Nutrition in Health and Disease, 6th edition*. Shils, M.E., Young, V.R., eds. Philadelphia: Lea & Febiger, p. 436-9.

114. **Mock, D.M.** 1996. *Biotin.* In: *Present Knowledge in Nutrition, 7th edition.* Ziegler, E.E., Filer, L.J., Jr., eds. p. 220-35. Washington, D.C.: International Life Sciences Institute-Nutrition Foundation.

115. **Mock, N.I., Malik, M.I., Stumbo, P.J., Bishop, W.P. & Mock, D.M.** 1997. Increased urinary excretion of 3-hydroxyisovaleric acid and decreased urinary excretion of biotin are sensitive early indicators of decreased status in experimental biotin deficiency. *Am. J. Clin. Nutr.,* 65: 951-8.

116. **McCormick, D.B. & Wright, L.D**. 1971. The metabolism of biotin and analogues. In: Comprehensive Biochemistry, Vol. 21. Florkin, M., Stotz, E.H., eds. p. 81-110. Amsterdam: Elsevier.

117. **McCormick, D.B.** 1976. Biotin. In: Present Knowledge in Nutrition, 4th edition. Hegsted, M., ed. Washington, D.C.: *The Nutrition Foundation*, p. 217-25.

118. **Zempleni, J., McCormick, D.B. & Mock, D.M.** 1997. Identification of biotin sulfone, bisnorbiotin methylketone, and tetranorbiotin-l-sulfoxide in Human urine. *Am. J. Clin. Nutr.,* 65: 508-11.

119. **Mock, D.M., deLorimer, A.A., Liebman, W.M., Sweetman, L. & Baker, H.** 1981. Biotin deficiency: an unusual complication of parenteral alimentation. *N. Engl. J. Med.,* 304: 820-3.

120. **Kien, C.L., Kohler, E., Goodman, S.I., Berlow, S., Hong, R., Horowitz, S.P. & Baker, H.** 1981. Biotin-responsive in vivo carboxylase deficiency in two siblings with secretory diarrhea receiving total parenteral nutrition. *J. Pediatr.,* 99: 546-50.

121. **Gillis, J., Murphy, F.R., Boxall, L.B. & Pencharz, P.B.** 1982. Biotin deficiency in a child on long-term TPN. *J. Parenteral Enternal Nutr.*, 6: 308-10.

122. **Mock, D.M., Baswell, D.L., Baker, H., Holman, R.T. & Sweetman, L.** 1985. Biotin deficiency complicating parenteral alimentation: diagnosis, metabolic repercussions, and treatment. *J. Pediatr.,* 106: 762-9.

123. **Lagier, P., Bimar, P., Seriat-Gautier, S., Dejode, J.M., Brun, T. & Bimar, J.** 1987. *Zinc and biotin deficiency during prolonged parenteral nutrition in infant.* Press Medicine, 16: 1795-7.

124. **Carlson, G.L., Williams, N., Barber, D., Shaffer, J.L., Wales, S., Isherwood, D., Shenkin, A. & Irving, M.H.** 1995. Biotin deficiency complicating long-term parenteral nutrition in an adult patient. *Clin. Nutr.,* 14: 186-90.

125. **Paul, A.A., Southgate, D.A.T. McCance & Widdowson's.** 1978. *The Composition of Foods.* London: H.M. Stationery Office.

126. **Salmenpera, L., Perheentupa, J., Pipsa, J.P. & Siimes, M.A.** 1985. Biotin concentrations in maternal plasma and milk during prolonged lactation. *Int. J. Vit. Nutr. Res.,* 55: 281-5.

127. **Hirano, M., Honma, K., Daimatsu, T., Hayakawa, K., Oizumi, J., Zaima, K. & Kanke, Y.** 1992. Longitudinal variations of biotin content in Human milk. *Int. J. Vit. Nutr. Res.,* 62: 281-2.

128. **Mock, D.M., Stadler, D.D., Stratton, S.L. & Mock, N.I.** 1997. Biotin status assessed longitudinally in pregnant women. *J. Nutr.,* 127: 710-6.