11
Biotin

SUMMARY

Biotin functions as a coenzyme in bicarbonate-dependent carboxylation reactions. Values extrapolated from the data for infants and limited estimates of intake are used to set the Adequate Intake (AI) for biotin because of limited data on adult requirements. The AI for adults is 30 μg/day. There are no nationally representative estimates of the intake of biotin from food or from both food and supplements. There are not sufficient data on which to base a Tolerable Upper Intake Level (UL) for biotin.

BACKGROUND INFORMATION

After biotin's initial discovery in 1927 (Boas, 1927), it took nearly 40 years of research for it to be fully recognized as a vitamin. In mammals this colorless, water-soluble vitamin functions as a cofactor for enzymes that catalyze carboxylation retentions (Dakshinamurti, 1994).

Function

Biotin functions as a required cofactor for four carboxylases found in mammalian species, which each covalently bind the biotin moiety (Bonjour, 1991; McCormick, 1976). Of the four biotin-dependent carboxylases, three are mitochondrial (pyruvate carboxylase, methyl-

crotonyl-coenzyme A [CoA] carboxylase, and propionyl-CoA carboxylase) whereas the fourth (acetyl-CoA carboxylase) is found in both the mitochondria and the cytosol. An inactive form of acetyl-CoA carboxylase has been postulated to serve as storage for biotin in the mitochondria (Allred and Roman-Lopez, 1988; Allred et al., 1989; Shriver et al., 1993).

Acetyl-CoA carboxylase catalyzes the carboxylation of acetyl CoA to form malonyl CoA. Malonyl CoA then serves as a substrate for fatty acid elongation. The second biotin-dependent carboxylase, pyruvate carboxylase, catalyzes the carboxylation of pyruvate to form oxaloacetate, which serves as an intermediate in the tricarboxylic acid cycle. Oxaloacetate thus formed is converted to glucose in the liver, kidney, and other gluconeogenic tissues.

A third biotin-dependent carboxylase, β-methylcrotonyl-CoA carboxylase, is required for the degradation of leucine, a branch-chained amino acid. Low activity of this enzyme resulting from biotin deficiency leads to the production of 3-hydroxyisovaleric acid and 3-methylcrotonylglycine by an alternate pathway (Mock, 1996). Thus, elevated levels of these abnormal metabolites in urine reflect reduced activity of β-methylcrotonyl-CoA carboxylase, usually resulting from biotin deficiency.

A fourth biotin-dependent carboxylase, propionyl-CoA carboxylase, carboxylates propionyl-CoA to form D-methylmalonyl-CoA, which is racemized to the L-isomer, then undergoes isomerization to succinyl-CoA, and subsequently enters the tricarboxylic acid cycle. Reduction in activity of this carboxylase results in increased excretion of 3-hydroxypropionic acid and 3-methylcitric acid in urine (Mock, 1996).

In the normal breakdown of cellular proteins, these biotin-containing enzymes are degraded to biocytin (ε-*N*-biotinyl-L-lysine) or short oligopeptides containing biotin-linked lysyl residues (Mock, 1996). Biotinidase (earlier called biocytinase), a hydrolase, releases biotin from this oligopeptide for reuse (Mock, 1996).

Physiology of Absorption, Metabolism, and Excretion

Biotin exists as free biotin and in protein-bound forms in foods. The mechanism of intestinal hydrolysis of protein-bound biotin has not been well characterized, and little is known about factors that affect bioavailability. Although most dietary biotin appears to be protein bound in both meats and cereals, biotin in cereals appears to be less bioavailable (Mock, 1996). Avidin, a protein found in appreciable amounts in raw egg white, has been shown to bind

biotin in the small intestine and prevent its absorption (Mock, 1996).

Biotinidase is thought to play a critical role in the release of biotin from covalent binding to protein (Wolf et al., 1984). Doses of free (unbound) biotin in the range of the estimated typical dietary intake (50 to 150 μg/day) given to individuals who have biotinidase deficiency have been shown to prevent the symptoms seen in biotinidase deficiency, indicating that biotinidase deficiency results in a relative biotin deficiency through lack of adequate digestion of protein-bound biotin, inadequate renal reabsorption, or both.

Intestinal Absorption and Microbial Synthesis

A biotin carrier located in the intestinal brush border membrane transports biotin against a sodium ion concentration gradient and is structurally specific, temperature dependent, and electroneutral; at pharmacological concentrations, diffusion predominates (Mock, 1996).

Biotin is synthesized by intestinal microflora (Bonjour, 1991). Although transporter-mediated absorption of biotin is most active in the proximal small intestine of the rat, significant absorption of biotin from the proximal colon occurs, which gives credence to the concept that biotin from microbial synthesis within the colon can contribute to meeting the human requirement. From reports of increased blood concentrations after colonic instillation of biotin, it appears that biotin is absorbed from the human colon (Innis and Allardyce, 1983; Oppel, 1948; Sorrell et al., 1971). However, Kopinski and colleagues (1989a, b) have shown that biotin synthesized by enteric flora may not be present at a location or in a form that contributes importantly to absorbed biotin.

Transport

The mechanism of biotin transport to the liver and other tissues after absorption has not been well established (Mock, 1996). Biotinidase has been identified as possibly serving as a biotin-binding protein in plasma or as a transporter protein to assist biotin's entry into the cell (Chauhan and Dakshinamurti, 1988; Wolf et al., 1985). Other studies suggest that serum biotin is more than 80 percent unbound (Hu et al., 1994; Mock and Malik, 1992; Schenker et al., 1993). An acid anion carrier with relative specificity for biotin resembling the intestinal carrier appears to mediate uptake by liver cells (Bowers-Komro and McCormick, 1985). Placental uptake of

biotin and transport to the fetus have been demonstrated and appear to be specific for biotin (Hu et al., 1994; Karl and Fisher, 1992; Schenker et al., 1993); however, because the fetus does not concentrate biotin, placental transfer appears to be passive.

Metabolism and Excretion

Isolation and chemical identification of more than a dozen metabolites of biotin have established the main features of utilization in microbes and mammals (McCormick, 1976; McCormick and Wright, 1971). About half of the biotin undergoes metabolism to bisnorbiotin and biotin sulfoxide before excretion. Biotin, bisnorbiotin, and biotin sulfoxide are present in molar proportions of approximately 3:2:1 in human urine and plasma (Mock, 1996). Two additional minor metabolites, bisnorbiotin methyl ketone and biotin sulfone, were recently identified in human urine (Zempleni et al., 1997). The urinary excretion and serum concentrations of biotin and its metabolites increase roughly in the same proportion in response to either intravenous or oral administration of large doses of biotin (Mock and Heird, 1997; Zempleni et al., 1997).

Clinical Effects of Inadequate Intake

Signs of biotin deficiency in humans have been demonstrated conclusively in individuals who consume raw egg white over long periods (Baugh et al., 1968) and in total parenteral nutrition (TPN) before biotin supplementation in patients with malabsorption, including short-gut syndrome (Mock et al., 1981). The clinical findings of biotin deficiency include dermatitis, conjunctivitis, alopecia, and central nervous system abnormalities (Mock, 1996).

In adults fed raw egg white or receiving biotin-free TPN for months to years, thinning of hair, frequently with loss of hair color, was reported. Most adults with the deficiency demonstrated a red, scaly, skin rash, frequently around the eyes, nose, and mouth. Most of the adults had neurological symptoms, including depression, lethargy, hallucinations, and paresthesia of the extremities.

In infants on biotin-free TPN, symptoms of biotin deficiency begin to appear within 3 to 6 months after initiation of the TPN regimen, which is earlier than that seen in adults, probably because of the increased biotin requirement related to growth (Mock, 1996). The associated rash appears first around the mouth, eyes, and nose. The rash and the unusual distribution of facial fat observed in these infants together are called *biotin deficiency facies*. As the rash progresses,

the ears and perineal orifices are affected. The resultant rash is similar in appearance to that of cutaneous candidiasis (so termed because *Candida* can usually be cultured from the lesions) and is quite similar to that seen in zinc deficiency.

Hair loss has been noted in infants after 6 to 9 months of TPN; two infants evaluated had lost all hair, including eyelashes and eyebrows, within 3 to 6 months of the onset of hair loss (Mock, 1996). In biotin-deficient infants, hypotonia, lethargy, and developmental delay, along with a peculiar withdrawn behavior, are all characteristic of a neurological disorder resulting from a lack of biotin; it is thought that the withdrawn behavior may represent the equivalent of depression seen in adults that is due to central nervous system dysfunction.

SELECTION OF INDICATORS FOR ESTIMATING THE REQUIREMENT FOR BIOTIN

The most useful information concerning indicators of the adequacy of biotin intake arises from (1) clinical observations of patients receiving biotin-free intravenous nutrition, individuals with inborn errors of metabolism, and persons who consume large amounts of raw egg white; (2) 2 studies in which biotin deficiency was experimentally induced by feeding raw egg white; and (3) fewer than 10 studies of biotin bioavailability and pharmacokinetics.

Biotin and 3-Hydroxyisovalerate Excretion

The indicators of biotin status that have been validated to the greatest extent are an abnormally decreased urinary excretion of biotin and an abnormally increased urinary excretion of 3-hydroxyisovaleric acid (NI Mock et al., 1997). The urinary excretion of biotin decreased dramatically with time in normal subjects on a raw egg white diet, reaching markedly abnormal values in 9 of 10 subjects by day 20. Bisnorbiotin excretion declined in parallel, providing evidence for regulated catabolism of biotin. By day 14 of egg white feeding, 3-hydroxyisovalerate acid excretion was abnormally increased (greater than 195 μmol/day) in all 10 subjects, providing evidence that biotin depletion decreased the activity of β-methylcrotonyl-coenzyme A (CoA) and altered leucine metabolism relatively early in biotin deficiency (NI Mock et al., 1997). Normal values are 77 to 195 μmol/day (112 ± 38 [standard deviation]); at day 10 the values for the deficient people were 272 ± 92 μmol/day. Abnormally decreased excretion of biotin, abnormally increased excretion

of 3-hydroxyisovalerate acid, or both have also been reported in several overt cases of biotin deficiency (Carlson et al., 1995; Gillis et al., 1982; Kien et al., 1981; Lagier et al., 1987; Mock et al., 1981, 1985). Gender differences are not apparent in these two indicators.

Plasma Biotin

A low plasma biotin concentration is not a sensitive indicator of inadequate biotin intake. Abnormally decreased plasma biotin was absent in half the subjects fed raw egg white (NI Mock et al., 1997) and in some overt case reports of biotin deficiency (Carlson et al., 1995; Khalidi et al., 1984; Kien et al., 1981; Matsusue et al., 1985; Mock et al., 1981, 1985).

Odd-Chain Fatty Acid Composition of Plasma Lipids

Odd-chain fatty acid composition in plasma lipids may reflect biotin status (Kramer et al., 1984; Liu et al., 1994; Mock et al., 1988a, b), but the sensitivity and clinical utility of this measurement remains to be determined. The accumulation of odd-chain fatty acids is thought to result from propionyl-CoA carboxylase deficiency.

Methodology

All published studies on biotin nutriture (Zempleni et al., 1997) have used one of three basic types of assays to estimate biotin: bioassays (most studies), avidin-binding assays, or fluorescent derivative assays. Recent modifications of bioassays generally have adequate sensitivity to measure biotin in blood, urine, and foods. For example, the bioassay based on *Kloeckera brevis* has both excellent sensitivity and metabolite discrimination (Guilarte, 1985). However, the bacterial bioassays (and perhaps the eukaryotic bioassays as well) suffer interference from unrelated substances and variable growth response to biotin metabolites. The acid hydrolysis or protein digestion required to release bound biotin may also release other compounds that support bacterial growth.

There are major discrepancies among the various bioassays and avidin-binding assays concerning the true concentration of biotin in human plasma. Reported mean values range from approximately 0.5 nmol/L to more than 10 nmol/L. The avidin-based radioimmunoassay with high-performance liquid chromatography is among the best current assays (Mock, 1996).

FACTORS AFFECTING THE BIOTIN REQUIREMENT

Several factors have been identified that affect the biotin requirement: the ingestion of large quantities of raw eggwhite, which contains a substance (avidin) that binds biotin; biotinidase deficiency (a genetic defect); the use of anticonvulsants that induce biotin catabolism in some individuals; and pregnancy. In the latter two conditions, the ratio of biotin metabolites to biotin in urine is increased (Mock and Dyken, 1997; Mock and Stadler, 1997; DM Mock et al., 1997b). Inherited biotinidase deficiency is particularly relevant to understanding biotin deficiency because the clinical manifestations appear to result largely from a secondary biotin deficiency in the presence of normal dietary intakes.

FINDINGS BY LIFE STAGE AND GENDER GROUP

Infants Ages 0 through 12 Months

Method Used to Set the Adequate Intake

An Adequate Intake (AI) is used as the goal for intake by infants. The AI reflects the observed mean biotin intake of infants fed principally with human milk.

Ages 0 through 6 Months. In early and transitional human milk, the concentration of biotin metabolites is nearly twice the concentration of biotin in samples (DM Mock et al., 1997a). With postpartum maturation of milk production, the biotin concentration increases but the metabolites still account for approximately one-third of total biotin at 5 weeks postpartum. In mature human milk (greater than 21 days postpartum) the concentration of biotin varies substantially (Mock et al., 1992); it exceeds the concentration in serum by one to two orders of magnitude. This suggests that there is an active biotin transport system into milk. According to Hirano and coworkers (1992), estimates of the biotin content of milk are 3.8 ± 1.2 (standard deviation) μg/L as free biotin determined microbiologically and 5.2 ± 2.1 μg/L after acid hydrolysis—slightly higher than earlier estimates of 4.5 μg/L (Salmenpera et al., 1985) and 7 μg/L for total biotin from bioassays (Paul and Southgate, 1978). With greatest weight given to the recent results (Hirano et al., 1992), but with the value within the range of the two other studies (Paul and Southgate, 1978; Salmenpera et al., 1985), the biotin content of human milk was estimated to be 6 μg/L. The adequate intake for biotin for

infants ages 0 through 6 months is based on the reported mean volume of milk consumed by this age group (0.78 L/day; see Chapter 2) and the estimate of the biotin concentration in human milk of 6 μg/L (0.78 L × 6 μg/L = 5 μg).

Ages 7 through 12 Months. If the reference body weight ratio method described in Chapter 2 to extrapolate up from the AI for biotin for infants ages 0 through 6 months is used, the AI for biotin for the older infants is 6 μg/day after rounding.

Biotin AI Summary, Ages 0 through 12 Months

AI for Infants		
0–6 months	**5 μg/day of biotin**	**≈0.7 μg/kg**
7–12 months	**6 μg/day of biotin**	**≈0.7 μg/kg**

Children and Adolescents Ages 1 through 18 Years

Method Used to Set the AI

Evidence concerning the biotin requirement is minimal and does not justify the setting of an Estimated Average Requirement (EAR). No definitive studies demonstrate evidence of biotin deficiency in normal individuals in any age group resulting from inadequate intakes. In the absence of additional information, including data on needs of adults, AIs for children and adolescents have been extrapolated from values for infants by using the formula

$$AI_{child} = (AI_{young\ infant})\ (weight_{child}/weight_{infant})^{0.75}.$$

Biotin AI Summary, Ages 1 through 18 Years

AI for Children	
1–3 years	**8 μg/day of biotin**
4–8 years	**12 μg/day of biotin**
AI for Boys	
9–13 years	**20 μg/day of biotin**
14–18 years	**25 μg/day of biotin**
AI for Girls	
9–13 years	**20 μg/day of biotin**
14–18 years	**25 μg/day of biotin**

Adults Ages 19 Years and Older

Method Used to Set the AI

In the absence of data on biotin deficiencies in normal individuals, a reasonable inference would be that the average current dietary intake of biotin should meet the dietary requirement. With this approach, the AI for biotin might be set at either 40 or 60 μg/day depending on the data set used (see "Dietary Intake"). Extrapolation from the AI for infants exclusively fed human milk would be expected to overestimate the requirement for adults because adults require biotin only for maintenance. The result of such an extrapolation using the formula

$$AI_{adult} = (AI_{young\ infant})\ (weight_{adult}/weight_{infant})^{0.75}$$

is 30 μg/day of biotin. Based on this very limited evidence, the AI for adults is set at 30 μg/day of biotin. This value should be adequate for maintaining normal excretion of 3-hydroxyisovaleric acid in adults (NI Mock et al., 1997). Data are not sufficient to set separate values for men and women or for the elderly.

Biotin AI Summary, Ages 19 Years and Older

AI for Men	
19–30 years	**30 μg/day of biotin**
31–50 years	**30 μg/day of biotin**
51–70 years	**30 μg/day of biotin**
> 70 years	**30 μg/day of biotin**
AI for Women	
19–30 years	**30 μg/day of biotin**
31–50 years	**30 μg/day of biotin**
51–70 years	**30 μg/day of biotin**
> 70 years	**30 μg/day of biotin**

Pregnancy

Evidence Considered in Setting the AI

Two recent studies (Mock and Stadler, 1997; DM Mock et al., 1997b) have raised questions, previously expressed (NRC, 1989), about the adequacy of biotin status during pregnancy. Some studies

have detected low plasma concentrations of biotin (Bhagavan, 1969; Dostalova, 1984); others have not (Mock and Stadler, 1997). DM Mock and colleagues (1997b) detected increased 3-hydroxyisovaleric acid in more than half of healthy pregnant women by the third trimester, and urinary excretion of biotin was decreased in about 50 percent of the women studied. It is not known whether these changes in values are normal for pregnant women or indicate low biotin intake relative to need. However, these data are not sufficient to justify an increase in the AI to meet the needs of pregnancy except for pregnant adolescents.

Biotin AI Summary, Pregnancy

AI for Pregnancy	
14–18 years	**30 μg/day of biotin**
19–30 years	**30 μg/day of biotin**
31–50 years	**30 μg/day of biotin**

Lactation

Method Used to Set the AI

To cover the amount of biotin secreted in milk, the AI is increased by 5 μg/day for lactating adolescents and women. No distinction is made for the stage of lactation or age.

Biotin AI Summary, Lactation

AI for Lactation	
14–18 years	**35 μg/day of biotin**
19–30 years	**35 μg/day of biotin**
31–50 years	**35 μg/day of biotin**

Special Considerations

Persons receiving hemodialysis or peritoneal dialysis may have an increased requirement for biotin (Livaniou et al., 1987; Yatzidis et al., 1984) as would persons with genetic biotinidase deficiency (Mock, 1996).

INTAKE OF BIOTIN

Food Sources

Biotin contents have been determined for relatively few foods and are not ordinarily included in food composition tables. Although biotin is widely distributed in natural foodstuffs, its concentration varies substantially. For example, liver contains biotin at about 100 μg/100 g whereas fruits and most meats contain only about 1 μg/100 g.

Dietary Intake

The U.S. Department of Agriculture Continuing Survey of Food Intakes by Individuals, the Third National Health and Nutrition Examination Survey (NHANES III), and the Boston Nutritional Status Survey do not report biotin intake. Murphy and Calloway (1986), using food intake data from the NHANES II, estimated the mean biotin intake of young women aged 18 to 24 years to be 39.9 ± 26.9 (standard deviation) μg/day. This result is considerably lower than the estimated dietary intake of biotin in a composite Canadian diet (62 μg/day) and an actual analysis of the diet (60 μg/day) (Hoppner et al., 1978). Calculated average intakes of biotin for the British population of adults and children were similar to the U.S. estimate—33 and 35 μg/day (Bull and Buss, 1982; Lewis and Buss, 1988).

Intake from Supplements

According to the 1986 National Health Interview Survey, approximately 17 percent of U.S. adults take a supplement containing biotin (Moss et al., 1989). Specific data on intake from supplements are not available.

TOLERABLE UPPER INTAKE LEVELS

Hazard Identification

No reported adverse effects of biotin in humans or animals were found. Toxicity has not been reported in patients treated with daily doses up to 200 mg orally and up to 20 mg intravenously to treat biotin-responsive inborn errors of metabolism and acquired biotin deficiency (Mock, 1996).

Several studies reported that acute doses of biotin (10 mg/100 g body weight) in pregnant rats (at the pre- and postimplantation stages) caused inhibition of fetal and placental growth and resorption of fetuses and placentae (Paul and Duttagupta, 1975, 1976). The dose used was equivalent to a human dose of 7 g for a 70-kg person, which is considerably greater than the recommended intake. These results are not considered useful for deriving a Tolerable Upper Intake Level (UL) for human intakes because of the high doses used, mode and vehicle of administration used (subcutaneous injection administration of 0.1 mol/L of NaOH, which would itself be toxic), and lack of an adequate control group.

Dose-Response Assessment

The data on adverse effects from high biotin intake are not sufficient for a quantitative risk assessment, and a UL cannot be derived. Several studies involving high biotin intakes reported no adverse effects. Koutsikos et al. (1996) found no adverse effects after intravenous administration of 50 mg of biotin to hemodialysis patients. Roth et al. (1982) administered 10 mg/day of biotin during the ninth month of pregnancy and found no adverse effects in the mother or infant. Ramaekers et al. (1993) reported no adverse effects in a 15-year-old boy given 10 mg/day of biotin to treat multiple carboxylase deficiency resulting from an inborn error of metabolism. Colamaria et al. (1989) similarly found no adverse effects in an infant treated with 10 mg/day of biotin to reverse a syndrome consisting of lethargy, sparse scalp hair, autistic-like behavior, myoclonus, and drug-resistant seizures. Taken together, these studies indicate a possible range for intake levels in any future studies of toxic effects of biotin.

Intake Assessment and Risk Characterization

Neither an intake assessment nor a risk characterization is currently possible because national surveys do not provide data on the dietary intake of biotin.

RESEARCH RECOMMENDATIONS FOR BIOTIN

There is a serious lack of data useful for setting Estimated Average Requirements (EARs) for biotin. The understanding of the nutrition of biotin is rudimentary compared with that of some other B vitamins. Although the limited information seems to indicate that

there is little cause for concern about the adequacy of biotin intake for healthy people, information on the human requirements, intake, bioavailability, toxicity, and metabolic effects of this compound is needed.

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