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Review

Biotin biochemistry and human requirements

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Human biotin turnover and requirements can be estimated on the basis of (1) concentrations of biotin and metabolites in body fluids, (2) activities of biotin-dependent carboxylases, and (3) the urinary excretion of organic acids that are formed at increased rates if carboxylase activities are reduced. Recent studies suggest that the urinary excretions of biotin and its metabolite bisnorbiotin, activities of propionyl-CoA carboxylase and β-methylcrotonyl-CoA carboxylase in lymphocytes, and urinary excretion of 3-hydroxyisovaleric acid are good indicators of marginal biotin deficiency. On the basis of studies using these indicators of biotin deficiency, an adequate intake of 30 μg (123 nmoles) of biotin per day is currently recommended for adults. The dietary biotin intake in Western populations has been estimated to be 35 to 70 μg/d (143–287 nmol/d). Recent studies suggest that humans absorb biotin nearly completely. Conditions that may increase biotin requirements in humans include pregnancy, lactation, and therapy with anticonvulsants or lipoic acid. (J. Nutr. Biochem. 10:128–138, 1999) © *Elsevier Science Inc. 1999. All rights reserved.*

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Biotin biochemistry

Biotin-dependent carboxylases

In mammals, holocarboxylase synthetase (E.C. 6.3.4.10) catalyzes the covalent binding of biotin to the ϵ-amino group of lysine in four different apocarboxylases to form the active holocarboxylases. Each of the four carboxylases catalyzes the incorporation of bicarbonate into an organic compound (*Figure 1*).[1] In the carboxylation sequence, bicarbonate and adenosine 5′-triphosphate (ATP) form carbonyl phosphate, which releases pyrophosphate; carbonyl phosphate reacts with the 1′-N of the biotinyl moiety to form 1′-N-carboxybiotinyl carboxylase. This intermediate then incorporates carboxylate into substrate in a highly specific fashion.

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For acetyl-CoA carboxylase (E.C. 6.4.1.2), a cytosolic (acetyl-CoA carboxylase α) form and a mitochondrial form (acetyl-CoA carboxylase β) have been identified; they have been reviewed in detail.[2] Both the α and β forms catalyze the binding of bicarbonate to acetyl-CoA to form malonyl-CoA; the latter is a substrate of fatty acid synthesis (*Figure 1*). Despite the fact that both the α and β forms of acetyl-CoA carboxylase catalyze the same reaction, they have different roles in intermediary metabolism due to their subcellular localization. Acetyl-CoA carboxylase α controls fatty acid synthesis in the cell cytosol by providing the substrate malonyl-CoA. In contrast, acetyl-CoA carboxylase β controls fatty acid oxidation in mitochondria. This effect of acetyl-CoA carboxylase β also is mediated by malonyl-CoA, which is an inhibitor of fatty acid transport into mitochondria. Acetyl-CoA carboxylase β also may play in important role in biotin storage.[3,4]

The three other mammalian biotin-dependent carboxylases are located exclusively in mitochondria (*Figure 1*): pyruvate carboxylase (E.C. 6.4.1.1), propionyl-CoA carboxylase (E.C. 6.4.1.3), and β-methylcrotonyl-CoA carboxylase (E.C. 6.4.1.4). Pyruvate carboxylase is a key enzyme in gluconeogenesis and also acts to provide a tricarboxylic

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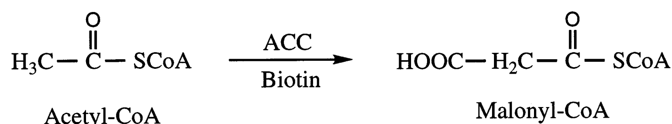
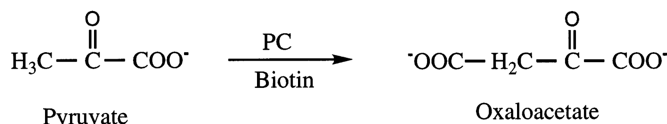
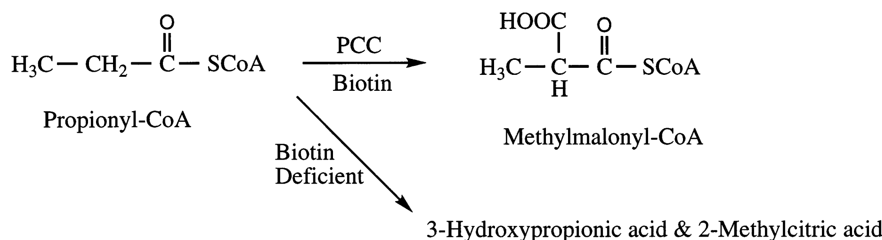
Cytosol and mitochondria



Mitochondria





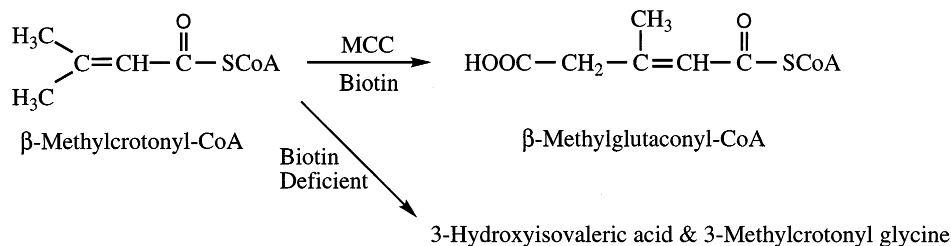


Figure 1 Biotin-dependent carboxylases in mammals. ACC–acetyl-CoA carboxylase; PC–pyruvate carboxylase; PCC–propionyl-CoA carboxylase; MCC–β-methylcrotonyl-CoA carboxylase.

acid cycle intermediate. Propionyl-CoA carboxylase catalyzes an essential step in the metabolism of isoleucine, valine, methionine, threonine, the cholesterol side chain, odd-chain fatty acids, and breakdown products of dietary carbohydrates by intestinal microorganisms. β-Methylcrotonyl-CoA carboxylase catalyzes an essential step in leucine metabolism.

Biotin deficiency causes reduced carboxylase activities; substrates are shunted to alternative pathways (*Figure 1*). For example, reduced activity of propionyl-CoA carboxylase results in increased formation of 3-hydroxypropionic acid and 2-methylcitric acid; reduced activity of β-methylcrotonyl-CoA carboxylase results in increased formation of 3-hydroxyisovaleric acid and 3-methylcrotonyl glycine. Increased urinary excretion of these organic acids have been used to diagnose isolated carboxylase deficiency, multiple carboxylase deficiency, and biotin deficiency, as discussed below.

Biotin catabolism

In a pioneering series of studies in microorganisms, McCormick and coworkers elucidated the two regions of the biotin molecule that are primarily catabolized. At the valeric acid side chain, biotin is catabolized by β-oxidation (*Figure 2*).[5–10] The repeated cleavage of two-carbon units leads to the formation of bisnorbiotin, tetranorbiotin, and related metabolites that are known to result from β-oxidation (e.g., α,β-dehydro-, β-hydroxy-, and β-keto-intermediates in successive oxidation of the five-carbon side chain). Whether

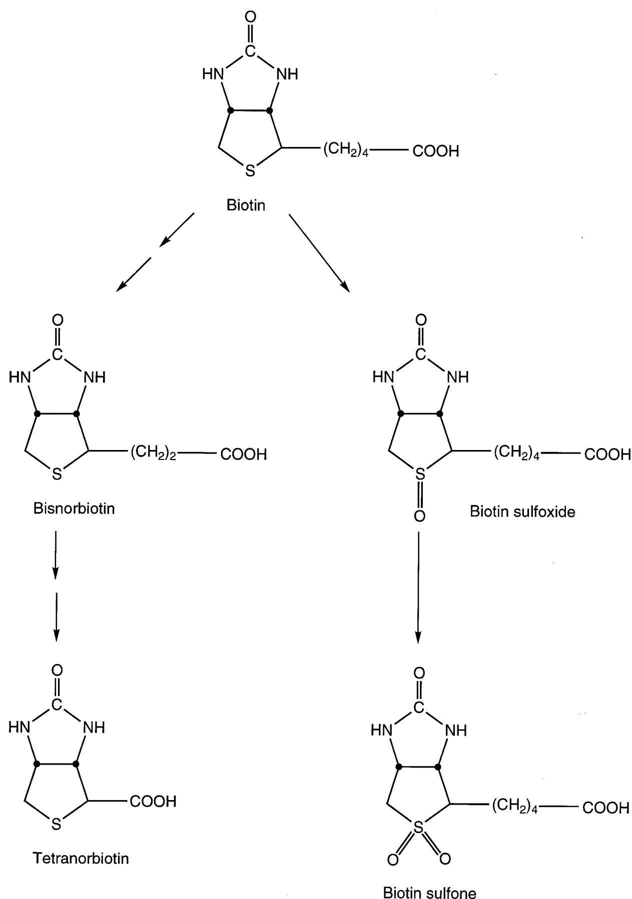


Figure 2 Metabolic pathways of biotin catabolism

the β-oxidation of biotin takes place in mitochondria or peroxisomes is uncertain.[11,12] β-Ketobiotin and β-keto-bisnorbiotin are unstable and may decarboxylate spontaneously to form bisnorbiotin methyl ketone and tetranorbiotin methyl ketone.[10,11,13] Peripheral blood mononuclear cells exhibit no detectable degradation of biotin after incubation for 168 hours.[14] After degradation of the side chain to one carbon (tetranorbiotin), microorganisms cleave and degrade the heterocyclic ring.[7]

The sulfur in the heterocyclic ring can become oxidized to biotin-*l*-sulfoxide, biotin-*d*-sulfoxide, and biotin sulfone (*Figure 2*).[6,8–10] Likely, the sulfur oxidation of biotin occurs in the smooth endoplasmic reticulum by an nicotinamide adenine dinucleotide phosphate (NADPH)-dependent process.[15] Oxidation at both sites leads to the formation of metabolites such as bisnorbiotin sulfone.[8]

Biotin metabolites that originate from either β-oxidation, sulfur oxidation, or both have been identified in mammals. In urine from rats, pigs, and humans, biotin metabolites accounted for 47 to 68 mole percent of total biotin plus metabolites.[11,13,16,17] In mammals, degradation of the heterocyclic ring is quantitatively minor.[11]

Metabolism of biotinyl proteins and peptides

In biotinyl proteins and biotinyl peptides, the carboxyl group of biotin is covalently attached to the ε-amino group of lysine residues. In humans, it has been assumed that biotinyl proteins and peptides arose from only two sources: protein-bound biotin in food and degradation of endogenous biotin-containing carboxylases. Recently, a third class of biotinyl proteins has been reported, as discussed below.[18,19] Hydrolysis of biotinyl proteins and peptides is thought to release free biotin, making it available for reutilization.

Food-borne biotinyl proteins. Biotin in food is largely protein bound.[20,21] Several gastrointestinal enzymes may be essential for hydrolysis of biotin-containing proteins to biotinyl peptides.[22] Biotinyl peptides are thought to be further hydrolyzed by intestinal biotinidase (E.C. 3.5.1.12) to release biotin. Intestinal biotinidase is found in pancreatic juice, secretions of the intestinal glands, bacterial flora, and the brush-border membranes.[22] Biotinidase activities are similar in mucosa from duodenum, jejunum, and ileum.[22] The primary site(s) of the hydrolysis of biotinyl peptides remains unclear. Whether intestinal absorption of biotinyl peptides can occur without prior hydrolysis remains controversial.[23–28]

Endogenous biotinyl proteins. In intermediary metabolism, biotin-containing carboxylases are degraded to biotinyl peptides. Sequential hydrolysis of biotinyl peptides leads to the formation of biocytin (ε-*N*-biotinyl-L-lysine)[29]; biotinidase hydrolyzes biocytin to biotin and lysine.[30,31] Biotinidase is not specific for biocytin but also will hydrolyze biocytin sulfoxide and biocytin sulfone.[32] The enzyme may also hydrolyze ε-*N*-lipoyl-L-lysine and derivatives thereof.[33–35] At least nine isoforms of biotinidase exist in human serum[36]; biotinidase from human serum has been sequenced and cloned.[37]

Biotin absorption and delivery to tissues

In the intestine, biotin is absorbed by a saturable, sodium-dependent transporter[24,28,38–40]; at great biotin concentrations, passive diffusion predominates.[38,39] The exit of biotin from the enterocyte (i.e., transport across the basolateral membrane) is carrier-mediated but is independent of sodium, is electrogenic, and does not accumulate biotin against a concentration gradient.[41] Some studies suggest that biotinidase serves as a biotin-binding protein in serum or perhaps even as a carrier protein for the transport of biotin into the cell.[42,43] Other studies suggest that biotin is more than 80% free with less than 10% reversibly bound and approximately 12% covalently bound to plasma protein.[44,45] Hepatocytes, cerebral capillaries, basolateral membrane vesicles of placenta, and peripheral blood mononuclear cells accumulate free biotin by a saturable transport system[14,46–51]; likely, biotin is cotransported with sodium.[14,46,48–51] Studies in peripheral blood mononuclear cells suggest that biotin uptake and efflux are mediated by the same transporter.[52]

Indicators of biotin deficiency

Clinical findings of frank biotin deficiency

Clinical findings of frank biotin deficiency have been described in patients receiving parenteral nutrition without

Table 1 Serum concentrations[63] and urinary excretions[13] of biotin and biotin metabolites*

Compound	Serum (pmol/L)	Urine (nmol/24 h)
Biotin	244 ± 61	35 ± 14
Bisnorbiotin	189 ± 135	68 ± 48
Biotin-*d,l*-sulfoxide	15 ± 33	5 ± 6
Bisnorbiotin methyl ketone	ND†	9 ± 9
Biotin sulfone	ND†	5 ± 5
Total biotin metabolites	464 ± 178‡	122 ± 66

*Means ± SD are reported (N = 15 for serum; N = 6 for urine).
†ND–not determined. Bisnorbiotin methyl ketone and biotin sulfone had not been identified at the time when this study of serum was conducted and hence these "unknowns" were not quantitated against authentic standards.
‡Includes three unidentified biotin metabolites.

biotin supplementation[53,54] and in patients with biotinidase deficiency.[55] Frank biotin deficiency also may be observed in individuals consuming large amounts of raw egg white[1,53,56]; this effect is caused by avidin, a protein in egg white. In the stomach and intestinal lumen, avidin binds biotin tightly[57] and thereby prevents absorption of biotin.[24]

Clinical findings of frank biotin deficiency induced by biotin-free intravenous feeding have included periorifacial dermatitis, conjunctivitis, alopecia, ataxia, hypotonia, ketolactic acidosis/organic aciduria, seizures, skin infection, and developmental delay in infants and children.[1,55,58] In addition, the following symptoms occurred in adults and adolescents chronically fed egg white: (1) thinning hair, often with loss of hair color; (2) skin rash described as scaly (seborrheic) and red (eczematous) that in several cases was distributed around the eyes, nose, and mouth; (3) depression, lethargy, hallucinations, and paresthesias of the extremities.[1,53,56]

Frank biotin deficiency is rarely seen. Current research is focusing on discovery and validation of indicators of biotin status that will allow estimation of human biotin requirements and evaluation of potential deleterious effects of marginal degrees of biotin deficiency. Such indicators might include serum concentrations and urinary excretion rates of biotin and biotin metabolites, activities of the biotin-dependent carboxylases in peripheral blood mononuclear cells, and urinary excretion rates of 3-hydroxyisovaleric acid, 3-methylcrotonyl glycine, and 2-methylcitric acid. These indicators are discussed in the following sections.

Excretion of biotin and biotin metabolites in urine

In human urine, biotin accounts for only half of biotin plus biotin metabolites.[59] Bisnorbiotin, bisnorbiotin methyl ketone, biotin-*d,l*-sulfoxide, and biotin sulfone account for most of the balance.[13,59] A few metabolites have yet to be identified. *Table 1* provides data of the urinary excretion of biotin and biotin metabolites in healthy adults. The slightly smaller excretion of biotin as a percentage of biotin plus biotin metabolites compared with previous communications[59] is probably due to variation in the subject populations of the studies. Using thin-layer chromatography and derivatization with *p*-dimethylaminocinnamaldehyde, tetranorbiotin-*l*-sulfoxide was identified as another biotin metabolite in human urine.[13] However, the detectability of tetranorbiotin-*l*-sulfoxide in the high performance liquid chromatography (HPLC)/avidin assay is insufficient to allow meaningful quantitation.[60]

To validate indicators of biotin nutritional status, marginal biotin deficiency was induced experimentally in normal adults.[61,62] A beverage containing sufficient avidin to bind seven times the dietary biotin intake was ingested daily for a period of 20 days. Blood and urine specimens were collected twice weekly. The mean urinary biotin excretion for the group declined from day 0 through day 20 and was significantly different from day 0 by day 3 (*Figure 3*, top panel). Mean urinary excretion fell below the lower limit of normal by day 14. Although the urinary excretion of biotin declined from the baseline value at day 0 in every subject, biotin did not decrease to less than the lower limit of normal in two subjects.

As is depicted in the middle panel of *Figure 3*, the mean excretion of bisnorbiotin also decreased. On day 14, bisnorbiotin excretion was less than the lower limit of normal in eight of ten subjects; on day 20, bisnorbiotin excretion was less than the lower limit of normal in six. These data provide evidence that the urinary excretion rates of biotin and bisnorbiotin are early and sensitive indicators of biotin deficiency in most subjects but suggest that there is an ongoing biotin loss and obligate biotin β-oxidation in mild biotin deficiency.

As depicted in the lower panel of *Figure 3*, the mean excretion of biotin-*d,l*-sulfoxide also decreased. On day 14, biotin-*d,l*-sulfoxide excretion was less than the lower limit of normal in eight of ten subjects. However, on day 20 biotin-*d,l*-sulfoxide excretion was less than the lower limit of normal in only four subjects and was no longer significantly different from the value on day 0.

Serum concentrations of biotin and biotin metabolites

In serum, biotin accounts for only half of biotin plus biotin metabolites; bisnorbiotin, biotin-*d,l*-sulfoxide, and unidentified metabolites account for the balance.[63] *Table 1* provides data of biotin and biotin metabolites in human serum. Egg-white feeding studies suggest that serum concentrations of biotin, bisnorbiotin, and biotin-*d,l*-sulfoxide are not good indicators of marginal biotin deficiency because none decreased significantly during a period of 20 days.[61,62] Similar findings were found in patients on biotin-free total parenteral nutrition.[64] In these studies, the concentration of biotin in plasma did not decrease during the course of biotin-free parenteral nutrition although other indicators confirmed the presence of biotin deficiency.

Carboxylase activities

Activities of biotin-dependent carboxylases may be useful as indicators of biotin status in humans. On a theoretical basis, propionyl-CoA carboxylase and β-methylcrotonyl-CoA carboxylase are more promising indicators than acetyl-CoA carboxylase and pyruvate carboxylase. The latter are

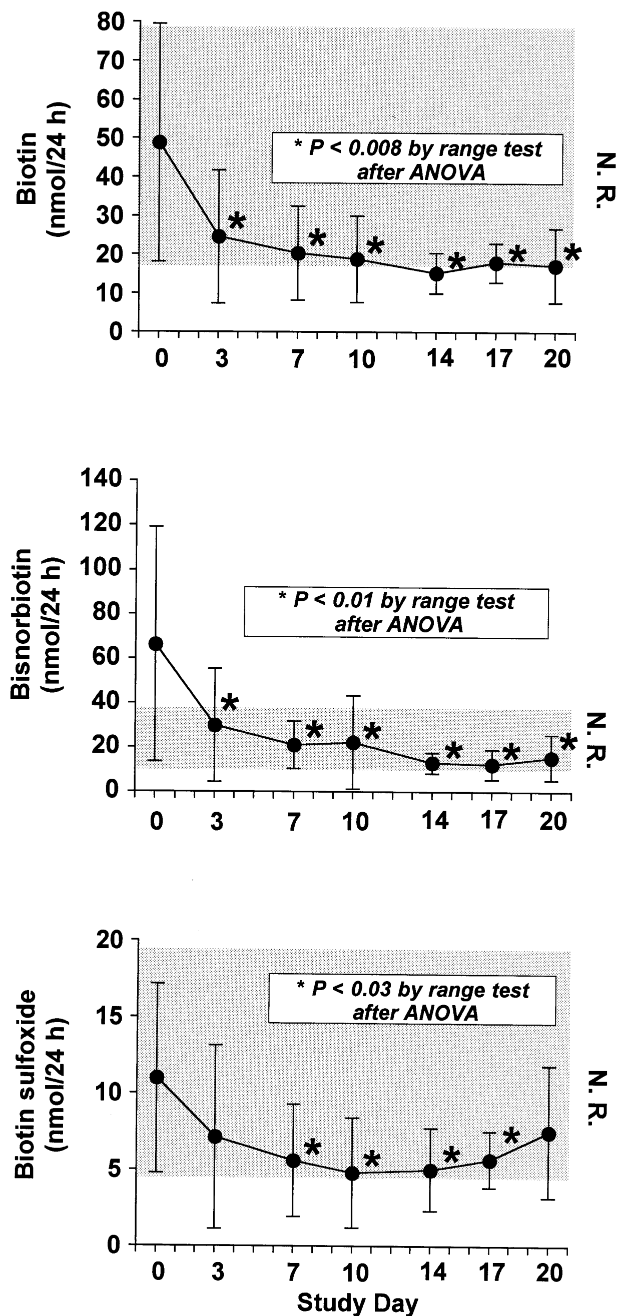


Figure 3 Mean (±SD) urinary excretion of biotin, bisnorbiotin, and biotin-*d,l*-sulfoxide in ten subjects over 20 days of egg-white feeding. *Significantly different from day 0 [repeated-measures analysis of variance (ANOVA)]: *P*-values are the least significant of the individual *P*-values derived from posthoc testing. N.R.–normal range. (Reprinted with permission from reference.[61])

under tight hormonal and allosteric control because they are key enzymes in central, essential metabolic pathways (e.g., fatty acid synthesis, gluconeogenesis).[65–68] Teleologically, it is less likely that the activities of acetyl-CoA carboxylase and pyruvate carboxylase will decrease in response to marginal biotin deficiency. In contrast, propionyl-CoA carboxylase and β-methylcrotonyl-CoA carboxylase catalyze fewer central steps in metabolism; notwithstanding, homozygous inborn deficiency (e.g., greater than 90%) of either enzyme can cause death in infancy.[55,69]

Lymphocytes are easily accessible in human blood; using quantities of blood available from adults, lymphocyte carboxylase activities can be measured. The use of an activation index of carboxylases in lymphocytes has been proposed to assess biotin status.[64] The carboxylase activation index is the ratio of carboxylase activity in cells incubated with biotin to activity in cells without biotin. High values for the activation index suggest that a substantial fraction of the carboxylase is in the apo form and is suggestive of biotin deficiency.

In biotin-deficient rats and biotin-deficient pregnant mice, we have shown that the activities of propionyl-CoA carboxylase and β-methylcrotonyl-CoA carboxylase in liver and lymphocytes are reduced compared with normal controls (D.M. Mock, unpublished observations). In patients on biotin-free total parenteral nutrition for 24 to 40 days, propionyl-CoA carboxylase activity in lymphocytes decreased to less than 50% compared with before parenteral nutrition; the mean activation index was greater than 2.[64] The activation index for control biotin-sufficient lymphocytes was 1. These data provide evidence that carboxylase activities in lymphocytes are sensitive indicators of biotin deficiency.

Excretion of organic acids in urine

Reduced activity of β-methylcrotonyl-CoA carboxylase causes a metabolic block in leucine catabolism. As a consequence, β-methylcrotonyl-CoA is shunted to alternative pathways, leading to the formation of 3-hydroxyisovaleric acid and 3-methylcrotonyl glycine. The normal urinary excretion of 3-hydroxyisovaleric acid in healthy adults is 112 ± 38 μmol/24 hr (minimum to maximum: 77–195 μmol/24 hr).[61] Biotin-deficiency studies in humans provide evidence that the urinary excretion of 3-hydroxyisovaleric acid is an early and sensitive indicator of biotin deficiency.[61,62] In these studies, marginal biotin deficiency was induced by 20 days of egg-white feeding. 3-Hydroxyisovaleric acid excretion increased steadily during the 20 days of avidin feeding; the increase was significant by day 3 of the study (*Figure 4*). By day 14, 3-hydroxyisovaleric acid excretion was greater than the upper limit of normal for all ten subjects and remained abnormal on day 20 for nine of the subjects.

Reduced activity of propionyl-CoA carboxylase causes a metabolic block in propionic acid metabolism. As a consequence of reduced propionyl-CoA carboxylase activities, propionic acid is shunted to alternative metabolic pathways. In these pathways, 3-hydroxypropionic acid and 2-methylcitric acid are formed. However, recent studies in biotin-deficient individuals, early in the course of development of deficiency, showed that urinary excretion of 3-hydroxypropionic acid was not significantly different from normal controls.[70] Hence, these studies suggest that urinary excretion of 3-hydroxypropionic acid is not a good indicator of marginal biotin deficiency. Theoretically, the accumulated propionic acid may be consumed as substrate for synthesis of odd-chain fatty acids. Accumulation of odd-chain fatty acids in hepatic tissue, cardiac tissue, and serum phospho-

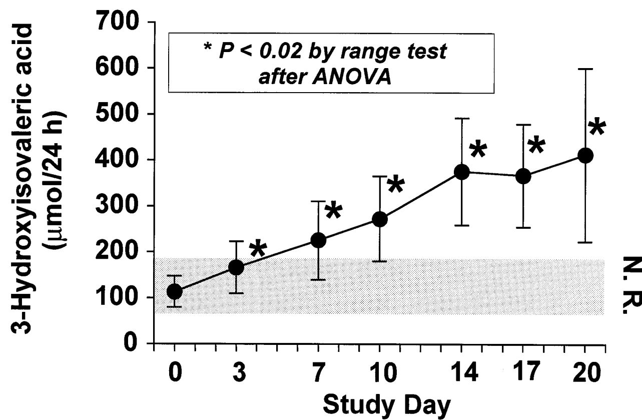


Figure 4 Mean (±SD) urinary excretion of 3-hydroxyisovaleric acid in ten subjects over 20 days of egg-white feeding. *Significantly different from day 0 [repeated measures analysis of variance (ANOVA)]: *P*-values are the least significant of the individual *P*-values derived from posthoc testing. N.R.–normal range. (Reprinted with permission from reference [61].)

lipids has been detected in biotin-deficient rats and chicks[71–73] and in biotin-deficient patients on parenteral nutrition.[74]

In severe biotin deficiency, activities of acetyl-CoA carboxylase and pyruvate carboxylase may be reduced. Reduced activity of acetyl-CoA carboxylase causes abnormal synthesis of long-chain fatty acids, including polyunsaturated fatty acids.[71,73,75–77] This may result in abnormal metabolism of prostaglandin and related substances.[78,79]

Biotinidase deficiency and biotin deficiency in brain may cause low pyruvate carboxylase activity, which leads to lactic acid accumulation.[80,81] This may mediate hypotonia, seizures, ataxia, and delayed development observed in both biotin deficiency and biotinidase deficiency.

Effects of biotin deficiency on the immune system

Biotin deficiency has adverse effects on cellular and humoral immune functions. For example, children with hereditary abnormalities of biotin metabolism developed *Candida* dermatitis; these children had absent delayed hypersensitivity skin test responses, immunoglobulin A deficiency, and subnormal percentages of T lymphocytes in peripheral blood.[82] Hence, abnormal values of variables of the immune system may indicate biotin deficiency. In biotin-deficient rats, synthesis of antibodies was reduced.[83,84] Biotin deficiency in mice decreased the activities of biotin-dependent carboxylases in spleen lymphocytes, the number of spleen cells, and the percentage of B lymphocytes in spleen; in contrast, the percentage of T lymphocytes was increased.[85] In biotin-deficient guinea pigs, the number of circulating neutrophils was increased whereas the number of B and T lymphocytes was decreased.[86] Incubation of cells from mouse spleen in biotin-deficient media reduced cytotoxic T-lymphocyte generation.[87] However, it seems likely that changes in these variables of immune function are not specific for biotin deficiency.[88–91]

Biotin requirements and current recommendations for dietary intake

The estimated average requirement (EAR) of a nutrient is defined as the lowest continuing intake level that will maintain a defined level of nutriture in a person.[92] The EAR is the amount of nutrient that is estimated to meet the nutrient requirement of *one half* of healthy individuals in a life stage and gender group; life stage considers age and pregnancy or lactation. EARs serve as a base to calculate recommended dietary allowances (RDAs); the RDA of a given nutrient equals its EAR plus two standard deviations of the EAR.[92] Unlike EARs, RDAs are the amounts of intake of essential nutrients that are adequate to meet the known nutrient needs of *practically all* healthy persons.[93] Thus, RDAs are goals for nutrient intakes of individuals. For certain nutrients including biotin, scientific knowledge is insufficient to provide EARs and RDAs. In these cases, adequate intakes (AIs) are provided.[92] AIs are based on observed or experimentally determined estimates of nutrient intakes by a group of healthy people. Like RDAs, AIs are goals for the nutrient intake of individuals.

For biotin, the Food and Nutrition Board of the National Research Council released an AI of 30 μg/d (123 nmol/d) for adults and pregnant women.[92] During lactation, the AI of biotin is 35 μg/d (143 nmol/d); for infants (0–5 months), the AI is 5 μg/d (20 nmol/d). These recommendations are based mainly on two studies, which found that (1) a daily dose of 60 μg (246 nmoles) biotin has maintained adults on parenteral nutrition symptom-free for 6 months[94] and (2) diets supplying 28 to 42 μg (115–172 nmoles) of biotin per day did not cause any indication of inadequate biotin status.[93]

Factors that affect biotin requirements

Pregnancy

Some of the early studies of biotin status in pregnancy detected decreased maternal plasma concentrations of biotin[95,96]; others did not.[97] Biotin status in pregnancy is potentially important because biotin deficiency in pregnant mice and hamsters is very teratogenic.[98–100] The predominant malformations were skeletal malformations such as micrognathia, cleft palate, and micromelia.

In two studies, we assessed biotin nutritional status during normal human gestation.[101,102] In a cross-sectional study of biotin status in pregnancy we detected deficiency in both early and late pregnancy, based on increased urinary excretion of 3-hydroxyisovaleric acid.[101] However, the urinary excretion of biotin, bisnorbiotin, and biotin-*d,l*-sulfoxide increased during late pregnancy. In a second longitudinal study, the urinary excretion of 3-hydroxyisovaleric acid increased significantly in both early and late pregnancy.[102] Moreover, the urinary excretion of biotin and bisnorbiotin and the plasma concentration of biotin decreased during late pregnancy. These data provide evidence that biotin status decreased during pregnancy. Thus, the data from the cross-sectional and the longitudinal studies partially conflict. Mock and Stadler[101] proposed that the increased urinary excretion of biotin and metabolites in the

cross-sectional study might be caused by an impairment of renal reclamation of biotin, bisnorbiotin, and biotin-*d,l*-sulfoxide in late pregnancy. Increased urinary excretion of biotin metabolites also may be caused by accelerated biotransformation of biotin during pregnancy.[62] Increased excretion of bisnorbiotin is induced in male rats by treatment with either dexamethasone or dehydroepiandrosterone,[12] providing evidence that pregnancy causes accelerated biotin biotransformation.

Lactation

The content of biotin and biotin metabolites have been studied in human milk. Biotin in the skim fraction of milk accounts for greater than 95% of total biotin; biotin in the cell pellet and the fat layer accounts for less than 0.5% and less than 4%, respectively.[103] Human milk shows a considerable variation in biotin excretion over 24 hours and with time postpartum.[104] Biotin, bisnorbiotin, and biotin-*d,l*-sulfoxide have been measured in human milk using the HPLC/avidin-binding assay.[105] At 8 days postpartum, biotin was approximately 8 nmol/L and accounted for 44% of biotin plus measured metabolites; bisnorbiotin and biotin-*d,l*-sulfoxide accounted for 48% and 8%, respectively. Although the percentage of biotin increased postpartum, bisnorbiotin and biotin-*d,l*-sulfoxide remained important portions of the total. By 6 weeks postpartum, the biotin concentration had increased to approximately 30 nmol/L and accounted for approximately 70% of biotin plus metabolites; bisnorbiotin and biotin-*d,l*-sulfoxide accounted for approximately 20% and less than 10%, respectively. Because bisnorbiotin and biotin-*d,l*-sulfoxide do not have vitamin activity, assays that are not chemically specific for individual metabolites (e.g., total avidin-binding assays) will likely overestimate the biotin content of human milk.

Anticonvulsants

Biotin requirements may be increased during anticonvulsant therapy. The anticonvulsants primidone and carbamazepine inhibit biotin uptake into brush-border membrane vesicles from human intestine.[106,107] Long-term therapy with anticonvulsants increases the urinary excretion of biotin catabolites and of 3-hydroxyisovaleric acid.[108,109] Phenobarbital, phenytoin, and carbamazepine displace biotin from biotinidase, conceivably affecting plasma transport, renal handling, or cellular uptake of biotin.[43] During anticonvulsant therapy, the plasma concentrations of biotin may be decreased.[110,111]

Potential interaction between lipoic acid and biotin

Large doses of lipoic acid have been administered successfully to treat heavy metal intoxication,[112,113] reduce signs of diabetic neuropathy in patients or test animals,[114,115] and enhance glucose disposal in patients with noninsulin-dependent diabetes mellitus.[116] Because lipoic acid and biotin have structural similarities, the potential exists for competition for intestinal or cellular uptake. Indeed, we have shown that chronic administration of pharmacologic doses of lipoic acid decreases the activities of pyruvate carboxylase and β-methylcrotonyl-CoA carboxylase in rat liver to 64 to 72% of controls.[117] However, the pathologic implications of such a decrease are not clear. Individuals who are heterozygous for pyruvate carboxylase deficiency, β-methylcrotonyl-CoA carboxylase deficiency, and propionyl-CoA carboxylase deficiency have carboxylase activities approximately 50% of normal and yet are characteristically asymptomatic.[69,118]

Other conditions that may affect biotin status

Biotin deficiency has been reported in several other circumstances. Low plasma concentrations of biotin have been observed in patients on chronic hemodialysis.[119] Nine hemodialysis patients who developed encephalopathy or peripheral neuropathy responded to biotin therapy.[120] In contrast, other researchers observed increased concentrations of plasma and red cell biotin in hemodialysis patients compared with controls.[121] Reduced blood concentrations of biotin, hepatic content of biotin, or urinary excretion of biotin have been reported in alcoholics, patients with gastric disease, patients with inflammatory bowel disease, and children with severe protein-energy malnutrition.[122–125]

Dietary intake of biotin

The content of free (i.e., water extractable) biotin and protein-bound (i.e., released by acidic or enzymatic hydrolysis) biotin varies among foods. The majority of biotin in meats and cereals appears to be protein bound.[20,21,126] Most measurements of biotin content in food have used bioassays. Despite potential analytical limitations due to interfering endogenous substances, protein binding, and lack of chemical specificity for biotin versus metabolites, there is reasonably good agreement among the published reports, and some worthwhile generalizations can be made.[127–131] Biotin is widely distributed in natural foodstuffs, but the absolute content of even the richest sources is low when compared with the content of most other water-soluble vitamins. Foods relatively rich in biotin include egg yolk, liver, and some vegetables. The dietary biotin intake in Western populations has been estimated to be 35 to 70 μg/d (143–287 nmol/d).[127,132–134]

Full-term infants on parenteral nutrition (20 μg biotin/d) had normal plasma levels; preterm infants on parenteral nutrition (13 μg biotin/d) had increased plasma levels.[135] Our studies of biotin concentration in human breast milk suggest that an infant who ingests 800 mL of mature breast milk per day ingests approximately 6 μg (24 nmoles) of biotin.[105] It remains unclear whether the contribution of biotin synthesis by gut microorganisms is important to the total biotin absorbed.[136–139] However, an infant who recovered from protein intolerance is documented to have developed biotin deficiency while consuming a biotin-free elemental formula.[140]

Bioavailability of biotin

In pioneering studies of biotin bioavailability, pharmaceutical preparations of biotin were given orally to healthy adults.[141,142] These studies estimated biotin bioavailability at 24 to 58%, based on the urinary recovery of the vitamin.

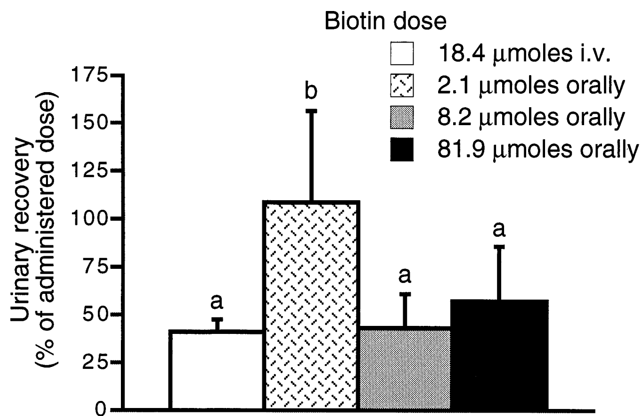


Figure 5 Urinary recovery of biotin plus metabolites within 24 hours of biotin administration ($N = 6$ adults; values are means ±SD). Columns not sharing the same superscript are significantly different ($P < 0.05$).

However, in these studies, the urinary excretion of biotin was analyzed using microorganisms that grow on biotin but not on most biotin metabolites,[141] or by avidin-binding assays[142] that do not take into account the smaller binding affinities of biotin metabolites for avidin compared with that of biotin.[60,143] As a result, these assays likely underestimated the true concentration of biotin plus metabolites excreted in urine.[59,144] For accurate estimations of biotin in biological fluids, we contend that it is necessary to separate biotin and biotin metabolites chromatographically before quantitation by avidin-binding assays; the HPLC/avidin-binding assay fulfills this analytical necessity.[145]

Recently, we quantitated the bioavailability of biotin in healthy adults.[146] Various doses of biotin were given either orally (2.1, 8.2, or 81.9 μmoles) or intravenously (18.4 μmoles). Before and after each administration, 24-hour urine samples were collected; the urinary excretion of biotin and biotin metabolites was measured using a chemically specific HPLC/avidin-binding assay. We compared the urinary recovery of the oral doses to the intravenous dose as a standard for complete availability. Urinary recovery of biotin plus metabolites was similar (approximately 50%) following the two largest oral doses and the one intravenous dose, suggesting 100% bioavailability of the two largest oral doses (*Figure 5*). The apparent recovery of the smallest oral dose was approximately twice that of other doses for unknown reasons. Overall, these findings suggest that free biotin is absorbed nearly completely even when pharmacologic doses of biotin are administered. However, uncertainties remain regarding the bioavailability of free and bound biotin in foodstuffs. In the published bioavailability studies, biotin was ingested either as a pure substance in tablet form or water solution.[141,142,146] Effect of inclusion in a meal and completeness of release from protein have yet to be examined. Thus, estimates of estimated safe and adequate daily dietary intakes, AIs, or RDAs are less precise than those for most other vitamins.

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