

Janos Zempleni,\* Subhashinee S.K. Wijeratne and Yousef I. Hassan

Department of Nutrition and Health Sciences, University of Nebraska-Lincoln, Lincoln, NE

## Abstract.

Biotin is a water-soluble vitamin and serves as a coenzyme for five carboxylases in humans. Biotin is also covalently attached to distinct lysine residues in histones, affecting chromatin structure and mediating gene regulation. This review describes mammalian biotin metabolism, biotin analysis, markers of biotin status, and biological functions of

biotin. Proteins such as holocarboxylase synthetase, biotinidase, and the biotin transporters SMVT and MCT<sub>1</sub> play crucial roles in biotin homeostasis, and these roles are reviewed here. Possible effects of inadequate biotin intake, drug interactions, and inborn errors of metabolism are discussed, including putative effects on birth defects.

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E-mail: jzempeni2@unl.edu

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## 1. History

Boas was the first to demonstrate the requirement for the water-soluble vitamin biotin in mammals [1]. Subsequently, biotin was isolated [2], its chemical structure was identified [3], and its chemical synthesis was accomplished [4].

## 2. Biosynthesis and catabolism of biotin

Mammals cannot synthesize biotin but depend on dietary intake from microbial and plant sources. The route of microbial biosynthesis of biotin was largely elaborated by Eisenberg and coworkers in studies of *Escherichia coli*. In this pathway, dethiobiotin is formed from the oleate metabolite pimelyl-CoA and carbamyl phosphate [5]. Sulfur is incorporated into dethiobiotin in a synthase-dependent step, generating biotin [6].

Early studies of biotin catabolism were primarily conducted by using microbes as model organisms but largely apply to mammals as well [7–9]. McCormick and Wright identified two pathways of biotin catabolism (Fig. 1). In one pathway, biotin is catabolized by  $\beta$ -oxidation of the valeric acid side chain [7]. This pathway leads to the formation of bisnorbiotin, tetranorbiotin, and intermediates known to

result from  $\beta$ -oxidation of fatty acids (*i.e.*  $\alpha,\beta$ -dehydro-,  $\beta$ -hydroxy, and  $\beta$ -keto-intermediates). Spontaneous decarboxylation of  $\beta$ -ketobiotin and  $\beta$ -ketobisnorbiotin yields bisnorbiotin methyl ketone and tetranorbiotin methyl ketone [7,9]. After degradation of biotin to tetranorbiotin, microorganisms cleave and degrade the heterocyclic ring [7]; degradation of the heterocyclic ring is quantitatively minor in mammals [8]. In a second pathway of biotin catabolism, the sulfur in the heterocyclic ring is oxidized to produce biotin-*l*-sulfoxide, biotin-*d*-sulfoxide, and biotin sulfone [7,9]. Sulfur oxidation in the biotin molecule occurs in the smooth endoplasmic reticulum in a reaction that depends on nicotinamide adenine dinucleotide phosphate [10]. Biotin is also catabolized by a combination of both  $\beta$ -oxidation and sulfur oxidation, producing compounds such as bisnorbiotin sulfone [7,9].

## 3. Biological functions of biotin

Biotin has long been recognized for its role as a covalently bound coenzyme for carboxylases [11]. More recently, evidence emerged that biotin also plays unique roles in cell signaling, epigenetic regulation of genes, and chromatin structure [12].

### 3.1. Biotin-dependent carboxylases

In mammals, biotin serves as a covalently bound coenzyme for acetyl-CoA carboxylases 1 and 2 (E.C. 6.4.1.2), pyruvate carboxylase (E.C. 6.4.1.1), propionyl-CoA carboxylase (E.C. 6.4.1.3), and 3-methylcrotonyl-CoA carboxylase (E.C. 6.4.1.4) [11,13]. The attachment of biotin to the  $\epsilon$ -amino group of a specific lysine residue in apocarboxylases is catalyzed by holocarboxylase synthetase (HCS) (E.C. 6.3.4.10); biotinylation of carboxylases requires ATP and proceeds in the following two steps [14]:

Abbreviations: BTM, biotinidase; HCS, holocarboxylase synthetase; K, lysine.

\*Address for correspondence: Janos Zempleni, Department of Nutrition and Health Sciences, University of Nebraska-Lincoln, 316 Ruth Levertson Hall, Lincoln, NE 68583-0806, USA. Tel.: +402 472 3270; Fax: +402 472 1587; E-mail: jzempeni2@unl.edu.

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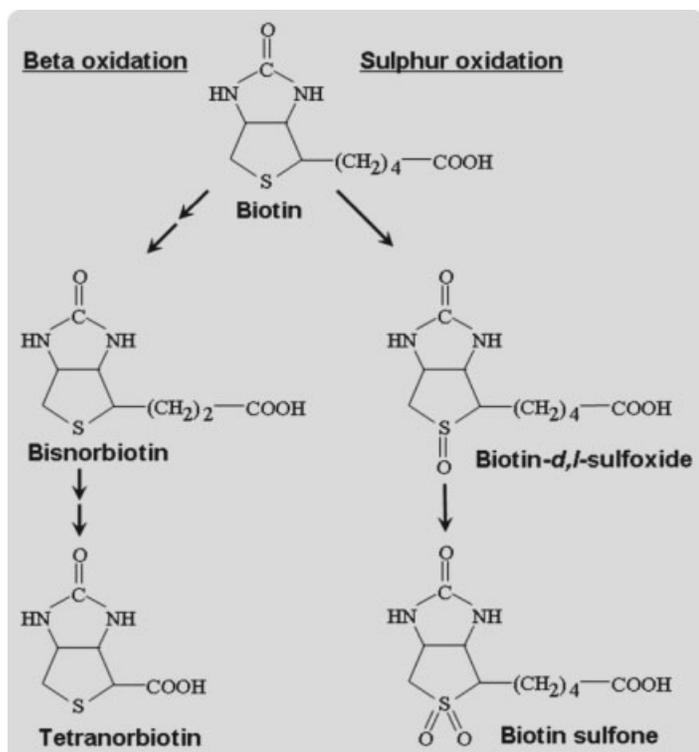


Fig. 1. Pathways of biotin catabolism.

- (1)  $\text{ATP} + \text{biotin} + \text{HCS} \rightarrow \text{Biotin-AMP-HCS} + \text{pyrophosphate}$
- (2)  $\text{Biotin-AMP-HCS} + \text{apocarboxylase} \rightarrow \text{holocarboxylase} + \text{AMP} + \text{HCS}$

(Net)  $\text{ATP} + \text{biotin} + \text{apocarboxylase} \rightarrow \text{holocarboxylase} + \text{AMP} + \text{pyrophosphate}$

Biotin-dependent carboxylases mediate the covalent binding of bicarbonate to organic acids by the following carboxylation sequence [15]. First, bicarbonate and ATP form carboxy phosphate, releasing ADP. Second, carboxy phosphate reacts with the 1'-N of the biotinyl moiety in holocarboxylases ("biotinyl carboxylase") to form 1'-N-carboxybiotinyl carboxylase and to release inorganic phosphate, P<sub>i</sub>. Third, 1'-N-carboxybiotinyl carboxylase incorporates carboxylate into an acceptor, *i.e.* a specific organic acid for each of the carboxylases.

Two isoforms of acetyl-CoA carboxylase have been identified: the cytoplasmic acetyl-CoA carboxylase 1 and the mitochondrial acetyl-CoA carboxylase 2 [13]. Both acetyl-CoA carboxylase 1 and 2 catalyze the binding of bicarbonate to acetyl-CoA, generating malonyl-CoA (Fig. 2). Acetyl-CoA carboxylase 1 is a key enzyme in fatty acid synthesis in the cytoplasm. In contrast, acetyl-CoA carboxylase 2 participates in the regulation of fatty acid oxidation in mitochondria. This effect of acetyl-CoA carboxylase 2 is mediated by malonyl-CoA, which is an inhibitor of fatty acid transport into mitochondria.

Pyruvate carboxylase localizes to mitochondria and is a key enzyme in gluconeogenesis (Fig. 2). The mitochondrial

propionyl-CoA carboxylase and 3-methylcrotonyl-CoA carboxylase comprise biotin-containing alpha subunits and biotin-free beta subunits. Propionyl-CoA carboxylase catalyzes an essential step in the metabolism of isoleucine, valine, methionine, threonine, the cholesterol side chain, and odd-chain fatty acids.  $\beta$ -Methylcrotonyl-CoA carboxylase catalyzes an essential step in leucine metabolism. Additional carboxylases have been identified in some microbes but are not further discussed here [11].

Proteolytic degradation of holocarboxylases leads to the formation of biotinyl peptides. These peptides are further degraded by biotinidase (BTD) (E.C. 3.5.1.12) to release biotin which is recycled in holocarboxylase synthesis [16].

### 3.2. Epigenetics

**3.2.1. Chromatin and gene regulation.** Chromatin comprises of DNA and DNA-binding proteins, *i.e.* histones and nonhistone proteins (Fig. 3). Histones play a crucial role in the folding of DNA in chromatin [17]. Five major classes of histones have been identified in mammals: linker histone H1, and core histones H2A, H2B, H3, and H4. Histones consist of a globular domain and a more flexible N-terminal tail. DNA and histones form repetitive nucleoprotein units, the nucleosomes [17]. Each nucleosome ("nucleosomal core particle") consists of 147 base pairs of DNA wrapped around an octamer of core histones (one H3-H3-H4-H4 tetramer and two H2A-H2B dimers).

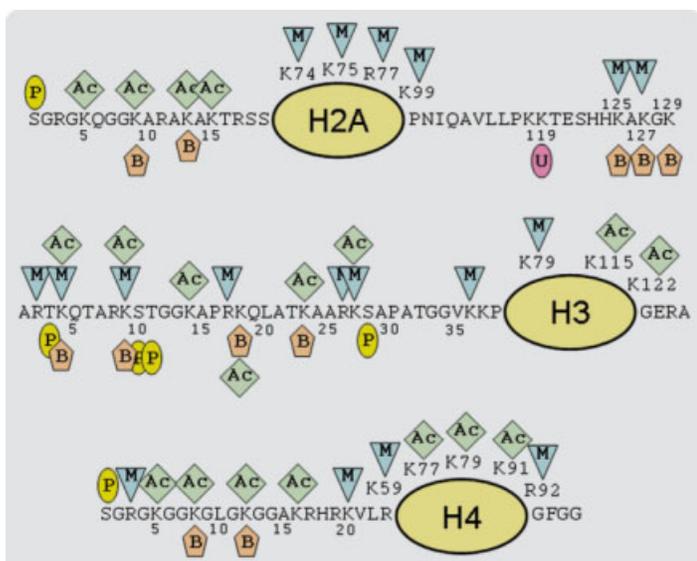
The N-terminal tails of core histones protrude from the nucleosomal surface; covalent modifications of these tails affect the structure of chromatin and form the basis for gene regulation [18,19]. Amino acid residues in histone tails are modified by covalent acetylation, methylation, phosphorylation, ubiquitination, and poly(ADP-ribosylation) [17–19]. The various modifications of histones have distinct functions. For example, trimethylation of K4 in histone H3 is associated with transcriptional activation of surrounding DNA, whereas dimethylation of K9 is associated with transcriptional silencing [18,19]. Covalent modifications of histones are reversible [19].

**3.2.2. Histone biotinylation.** Evidence has been provided that biotin is attached to histones (DNA-binding proteins) via an amide bond [20,21]. The following 11 biotinylation sites have been identified in human histones: lysine (K)-9, K13, K125, K127, and K129 in histone H2A [22]; K4, K9, K18, and perhaps K23 in histone H3 [23,24]; and K8 and K12 in histone H4 [25] (Fig. 4).

Functions of histone biotinylation in chromatin biology are emerging. For example, biotinylation of K12 in histone H4 plays roles in gene repression, DNA repair, heterochromatin structures, and repression of transposons, thereby promoting genomic stability [26–30]. Importantly, biotinylation of histones depends on dietary biotin supply [31,32].

Originally, it was believed that biotinylation of histones is catalyzed by BTD. Hymes et al. have proposed a reaction mechanism by which cleavage of biocytin (biotin- $\epsilon$ -lysine) by BTD leads to the formation of a biotinyl-thioester intermediate at or near the active site of BTD [20,33]. In a next step,





**Fig. 4. Modification sites in histones H2A, H3, and H4.** Ac, acetate; B, biotin; M, methyl; P, phosphate; U, ubiquitin. [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

### 3.3. Gene expression

About 40 years ago, evidence emerged suggesting that biotin may affect gene expression [41]; these pioneering studies provided evidence that expression of rat liver glucokinase (E.C. 2.7.1.2) depends on biotin. Since then, more than 2,000 biotin-dependent genes have been identified in human lymphoid and liver cells [12]. These genes are not randomly distributed in the human genome but can be assigned to gene clusters, based on signaling pathways, chromosomal location, cellular localization of gene products, biological function, and molecular function [12]. Evidence has been provided that bisnorbiotin also affects gene expression, suggesting that biotin catabolites might have biotin-like activities in humans [12]. Effects of biotin on gene expression are mediated by a variety of cell signals, including biotinyl-AMP, cGMP, NF- $\kappa$ B, Sp1, and Sp3, and receptor tyrosine kinases [12,42].

Evidence has been provided that biotin also affects gene expression at the post-transcriptional level. For example, the expression of asialoglycoprotein receptor in HepG2 hepatocarcinoma cells and propionyl-CoA carboxylase in rat hepatocytes depends on biotin; these effects are not caused by alterations in the abundance of mRNA coding for these proteins [12].

## 4. Methods of biotin analysis

### 4.1. Microbial growth assays

Microorganisms such as *Lactobacillus plantarum*, *Lactobacillus casei*, *Ochromonas danica*, *Escherichia coli* C162, *Saccharomyces cerevisiae*, and *Kloeckera brevis* depend on biotin for growth and are commonly used in microbial growth assays [43]. These test organisms show variable growth

responses to biotin and biotin precursors, catabolites, and analogs. For some microbes (e.g. *Ochromonas danica*, *Lactobacillus plantarum*), acid or enzymatic hydrolysis of samples is required to release biotin from protein. In contrast, for other organisms (e.g. *Kloeckera brevis*) the detectable biotin decreases with enzymatic hydrolysis.

### 4.2. Avidin-binding assays

The proteins avidin and streptavidin are widely used in biotin analysis because they bind biotin with exceptional strength and specificity; the dissociation constant of the avidin-biotin complex is  $1.3 \times 10^{-15}$  M [44]. Avidin-binding assays generally measure the ability of biotin to compete with [ $^3$ H]biotin or [ $^{14}$ C]biotin for binding to avidin; to prevent binding of enzyme-conjugated avidin to biotinylated protein adhered to plastic; or to prevent the binding of biotinylated enzyme to avidin [43]. Streptavidin has greater specificity for biotin than avidin and, therefore, is typically the protein of choice in (strept)avidin-binding assays [44].

Avidin-binding assays are subject to the following potential pitfalls. First, avidin binds biotin catabolites less tightly compared with biotin [43]. Hence, avidin-binding assays may underestimate the true concentration of biotin plus catabolites if calibrated by using biotin. Second, compounds structurally similar to biotin (e.g. lipoic acid, urea, hexanoic acid) may bind to avidin and cause artificially large readings for “apparent biotin.” Taken together, avidin-binding compounds in biological samples need to be resolved by chromatography prior to analysis of individual chromatographic fractions against authentic standards. Appropriate analytical procedures have been reviewed elsewhere [43].

### 4.3. 4'-Hydroxyazobenzene-2-carboxylic acid dye assay

This is a simple assay to quantify biotin at concentrations that exceed those typically found in biological samples. In the absence of biotin, 4'-hydroxyazobenzene-2-carboxylic acid forms noncovalent complexes with avidin at its biotin-binding sites to produce a characteristic absorption band at 500 nm [43]. The addition of biotin to this complex results in displacement of 4'-hydroxyazobenzene-2-carboxylic acid from the binding sites. As 4'-hydroxyazobenzene-2-carboxylic acid is displaced, the absorbance of the complex decreases proportionally.

## 5. Digestion, absorption, storage, and excretion

### 5.1. Digestion

A large fraction of dietary biotin is covalently linked to lysine residues in proteins [16,45]. Gastrointestinal proteases and peptidases digest biotin-containing proteins to release biocytin (biotinyl- $\epsilon$ -lysine) and biotin-containing peptides [16]. BTD releases free biotin from biocytin and biotinylated peptides; BTD activity can be found in pancreatic fluids and other intestinal secretions, intestinal flora, and brush-border

membranes [16,46,47]. The primary site(s) for hydrolysis of biotinylated peptides is unknown. Small quantities of biotinylated peptides might be absorbed without prior hydrolysis [48].

## 5.2. Absorption

Biotin transport across the apical membrane in the brush-border membrane occurs through a sodium-dependent, electroneutral mechanism, whereas the transport across the basolateral membrane is sodium-independent and electrogenic [49–52]. The apparent Michaelis–Menten constant of the biotin transporter in mouse jejunum is 22  $\mu\text{mol/L}$  [53]. The intestinal, sodium-dependent uptake of biotin is mediated by the sodium-dependent multivitamin transporter SMVT, which also has affinity for pantothenic acid and lipoic acid [54–57]. Passive diffusion across cell membranes may contribute to biotin uptake if extracellular biotin concentrations exceed 25  $\mu\text{mol/L}$ ; carrier-mediated uptake predominates at biotin concentrations of less than 5  $\mu\text{mol/L}$  [47]. Intestinal biotin transporter activity depends on dietary biotin supply [58], and is regulated by protein kinase C in concert with  $\text{Ca}^{2+}$ /calmodulin-mediated pathways [52] and transcription factors KLF-4 and AP-2 [59,60]. A mechanism has been proposed by which biotinylation of histones at the SMVT promoter locus regulates expression of the SMVT gene and, hence, biotin transport rates [31]. Doses of biotin that exceed the normal dietary intake 600 times are 100% bioavailable [61].

## 5.3. Uptake, storage, and distribution in liver and peripheral tissues

SMVT not only mediates intestinal absorption of biotin, but also is crucial for biotin uptake into liver and peripheral tissues and for renal reabsorption [52,57,62,63]. Evidence has been provided that monocarboxylate transporter 1 might account for biotin uptake in some cell lineages such as lymphoid cells and keratinocytes [64,65].

A relatively large fraction of intravenously administered biotin accumulates in rat liver, consistent with a role for this organ in biotin storage [66]. Depletion and repletion experiments of biotin-dependent carboxylases in rat liver provided evidence that mitochondrial acetyl-CoA carboxylase 2 may serve as a reservoir for biotin [67]. Note that the central nervous system (CNS) retains most of its biotin during phases of depletion at the expense of other tissues such as liver [68]. In rat CNS, biotin is enriched in specific regions such as the cerebellar motor system and brainstem auditory nuclei that correlates to the associated neurological symptoms associated with biotin deficiency [69].

## 5.4. Cellular compartments

Biotin is distributed unequally across cellular compartments [66]. The vast majority of biotin in rat liver localizes to mitochondria and cytoplasm, whereas only a small fraction localizes to microsomes [66]. The relative enrichment of biotin in mitochondria and cytoplasm is consistent with the role of biotin as a coenzyme for carboxylases in these compartments.

**Table 1**

**Serum concentrations [71] and urinary excretions [9] of biotin and catabolites**

Compound	Serum (pmol/L)	Urine (nmol/24 h)
Biotin	244 $\pm$ 61	35 $\pm$ 14
Bisnorbiotin	189 $\pm$ 135	68 $\pm$ 48
Biotin- <i>d,l</i> -sulfoxide	15 $\pm$ 33	5 $\pm$ 6
Bisnorbiotin methyl ketone	ND <sup>a</sup>	9 $\pm$ 9
Biotin sulfone	ND <sup>a</sup>	5 $\pm$ 5
Total biotinyl compounds	464 $\pm$ 178 <sup>b</sup>	122 $\pm$ 66

Means  $\pm$  SD are reported ( $n = 15$  for serum;  $n = 6$  for urine).

<sup>a</sup> 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, quantification of these “unknowns” was based on using biotin as a standard.

<sup>b</sup> Including three unidentified biotin catabolites.

A quantitatively small but qualitatively important fraction of biotin localizes to the cell nucleus, *i.e.* about 0.7% of total biotin in human lymphoid cells can be recovered from the nuclear fraction [21]. The relative abundance of nuclear biotin increases to about 1% of total biotin in response to proliferation, consistent with a role for histone in cell proliferation [21,70].

## 5.5. Urinary and biliary excretion

Healthy adults excrete  $\sim 100$  nmol of biotin and catabolites per day into urine [9] (Table 1). Biotin accounts for approximately half of the total; the catabolites bisnorbiotin, biotin-*d,l*-sulfoxides, bisnorbiotin methyl ketone, biotin sulfone, and tetranorbiotin-*l*-sulfoxide account for most of the balance [9]. If physiological or pharmacological doses of biotin are administered parenterally to humans, rats, or pigs, 43–75% of the dose is excreted into urine [8,61]. Renal epithelia reclaim biotin that is filtered in the glomeruli in an SMVT-mediated process [72]. The biliary excretion of biotin and catabolites is quantitatively minor. Less than 2% of an intravenous dose of [<sup>14</sup>C]biotin was recovered in rat bile but more than 60% of the dose was excreted in urine [73].

## 6. Biotin status

### 6.1. Direct measures

The urinary excretion of biotin and biotin catabolites decreases rapidly and substantially in biotin-deficient individuals, suggesting that the urinary excretion is an early and sensitive indicator of biotin deficiency [74]. A decreased urinary excretion of biotin, together with an increase in the ratios of bisnorbiotin and biotin-*d,l*-sulfoxide to biotin, reflect increased biotin catabolism as observed in women smokers [75]. In contrast, serum concentrations of biotin, bisnorbiotin,

and biotin-*d,l*-sulfoxide do not decrease in biotin-deficient individuals [74] and in patients on biotin-free total parenteral nutrition [76] during reasonable periods of observation. Hence, serum concentrations are not good indicators of marginal biotin deficiency.

## 6.2. Indirect measures

Activities of biotin-dependent carboxylases in lymphocytes are reliable markers for assessing the biotin status in humans [77,78]. Some investigators used a modified approach and calculated a “carboxylase activation index,” representing the ratio of carboxylase activities in cells incubated with excess supplemental biotin to cells without supplemental biotin [76]. High values for the activation index suggest that a substantial fraction of a given carboxylase was present as apo-enzyme, indicating biotin deficiency.

Reduced carboxylase activities in biotin deficiency affects intermediary metabolism (Fig. 2). Reduced activity of  $\beta$ -methylcrotonyl-CoA carboxylase impairs leucine catabolism. As a consequence,  $\beta$ -methylcrotonyl-CoA is shunted to alternative pathways, leading to an increased formation of 3-hydroxyisovaleric acid and 3-methylcrotonyl glycine. Biotin deficiency studies in humans suggest that the urinary excretion of 3-hydroxyisovaleric acid is an early and sensitive indicator of biotin status [75,79].

Reduced activity of propionyl-CoA carboxylase causes a metabolic block in propionic acid metabolism. Consequently, 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 suggest that the urinary excretion of 3-hydroxypropionic acid and 2-methylcitric acid is not a good indicator of marginal biotin deficiency [80]. Theoretically, propionic acid might be consumed in the synthesis of odd-chain fatty acids [81]. In severe biotin deficiency, the activity of pyruvate carboxylase may be reduced, leading to the accumulation of lactate [32].

## 7. Biotin deficiency

### 7.1. Clinical findings of frank biotin deficiency

Signs of frank biotin deficiency have been described in patients with BTB deficiency [35], in severely malnourished children in developing countries [82], and in individuals consuming large amounts of raw egg white which contains the protein avidin [83]. Binding of biotin to avidin in the gastrointestinal tract prevents absorption of biotin. Clinical findings of frank biotin deficiency include periorificial dermatitis, conjunctivitis, alopecia, ataxia, hypotonia, ketolactic acidosis/organic aciduria, seizures, skin infection, and developmental delay in infants and children [35,45].

### 7.2. Immune system, cell proliferation, and stress resistance

Biotin deficiency has adverse effects on cellular and humoral immune functions. For example, children with hereditary

abnormalities of biotin metabolism developed candida dermatitis and presented with absent delayed-hypersensitivity skin-tests responses, IgA deficiency, and subnormal percentages of T lymphocytes in peripheral blood [84]. In rodents, biotin deficiency decreases antibody synthesis [85], decreases the number of spleen cells and the percentage of B lymphocytes in spleen [86], and impairs thymocyte maturation [87]. Decreased rates of cell proliferation may cause some of the effects of biotin on immune function [88–90].

Biotin deficiency is linked also to cell stress, enhancing the nuclear translocation of the transcription factor NF- $\kappa$ B in human lymphoid cells [91]. NF- $\kappa$ B mediates activation of anti-apoptotic genes; this is associated with enhanced survival of biotin-deficient cells in response to cell death signals compared with biotin-sufficient controls [91]. Stress-resistant *Drosophila* can be selected by feeding biotin-deficient diets for multiple generations [32].

### 7.3. Lipid metabolism

Consistent with roles of biotin-dependent acetyl-CoA carboxylases 1 and 2, and propionyl-CoA carboxylase in lipid metabolism, biotin deficiency causes alterations of the fatty acid profile in liver, skin, and serum of several animal species [92]. Biotin deficiency is associated with increased abundance of odd-chain fatty acids, suggesting that odd-chain fatty acid accumulation may be a marker for reduced propionyl-CoA carboxylase activity in biotin deficiency. Biotin deficiency does not affect the fatty acid composition in brain tissue to the same extent as in liver [92].

Biotin deficiency also causes abnormalities in fatty acid composition in humans. In patients who developed biotin deficiency during parenteral alimentation, the percentage of odd-chain fatty acids (15:0, 17:0) in serum increased for each of the four major lipid classes, *i.e.* cholesterol esters, phospholipids, triglycerides, and free fatty acids [92]. However, the relative changes in these four classes of lipids have not always been consistent among studies [92].

### 7.4. Teratogenic effects of biotin deficiency

Mock and coworkers suggested that about half of the pregnant women in the U.S. are marginally biotin deficient despite a normal dietary biotin intake [93–95]. If there was a link between marginal biotin deficiency and fetal malformations in humans, the findings by Mock and coworkers would have important implications for health policies and intake recommendations. As of today, this link is somewhat uncertain. While animal studies have clearly demonstrated that biotin deficiency is teratogenic, the severity of deficiency in these animal studies typically exceeded what was observed in pregnant women. Notwithstanding these limitations, the teratogenic effects of biotin deficiency in animal models warrant a brief summary [92,96,97]. In some strains of mice, biotin deficiency during pregnancy causes substantial increases in fetal malformations and mortality [92,97]. The most common fetal malformations in biotin-deficient rats include cleft palate, micrognathia, and micromelia. Evidence

has been provided that biotin catabolism to bisnorbiotin is increased in pregnant women compared with nonpregnant controls [93,98]. Mock and coworkers reported that the activity of propionyl-CoA carboxylase is reduced by ~90% in the fetus at term in response to feeding an egg-white that causes only a 50% reduction in maternal hepatic propionyl-CoA carboxylase activity [99].

### 7.5. Biotin homeostasis in the CNS

Disturbances in biotin homeostasis in the CNS cause encephalopathies [12]. Factors leading to biotin imbalances in CNS include deficiencies of BTM, HCS, and perhaps biotin transporters [12] as described below. Afflicted patients typically respond to the administration of large doses of biotin with maintaining normal neurological function [12].

Moderate dietary biotin deficiency is typically not associated with neurological symptoms. This is consistent with the hypothesis that under conditions of moderate biotin deficiency the CNS maintains normal concentrations of biotin at the expense of other tissues. Indeed, evidence has been provided that biotin deficiency causes a >90% decrease of biotinylated carboxylases in rat liver, whereas brain carboxylases remain unchanged [68]. The tissue-specific response to biotin supply is probably due to tissue-specific changes in expression.

## 8. Inborn errors of biotin metabolism

### 8.1. BTM deficiency

Low activities of the enzyme BTM cause a failure to recycle biotin from degraded carboxylases, *i.e.* to release biotin from biocytin. Substantial amounts of biocytin are excreted into urine [100], eventually leading to biotin deficiency. Thus, clinical and biochemical features in children with BTM deficiency are similar to those described earlier for biotin deficiency [35].

Typically, symptoms of BTM deficiency appear at age 1 week to >1 year [35]. Wolf distinguishes between patients with profound BTM deficiency (less than 10% of normal serum BTM activity) and patients with partial deficiency (10–30% of normal BTM activity) [101]. Procedures for prenatal diagnosis of BTM activity in cultured amniotic fluid cells and for neonatal screening using blood samples have been proposed [35]. BTM activity is measured by quantitating the release of *p*-aminobenzoic acid from *N*-biotinyl-*p*-aminobenzoate [102]. The mean ( $\pm$ SD) normal activity of BTM is  $5.8 \pm 0.9$  nmol *p*-aminobenzoate liberated per minute per milliliter of serum [102].

The combined incidence of profound and partial deficiency is 1 in 60,089 live births; an estimated 1 in 123 individuals is heterozygous for the disorder [101]. Mutations of the *BTM* gene have been well characterized at the molecular level [103–105]. Children with profound BTM deficiency are treated with 5–20 mg of biotin per day [35]. If identified early, symptomatic patients improve rapidly after biotin therapy is initiated; therapy must be continued throughout life [35].

### 8.2. Deficiencies of carboxylases and HCS

Afflicted individuals present with either isolated deficiencies of individual carboxylases or multiple deficiencies of biotin-dependent carboxylases because of defective HCS [106]. Patients with multiple carboxylase deficiency characteristically exhibit low activities of all five biotin-dependent carboxylases. Mutations of the *HCS* gene have been well characterized at the molecular level [107]. It has been estimated that the incidence of HCS deficiency is less than 1 in 1,000 live births in Japan [107]. Afflicted individuals typically respond well to administration of pharmacological doses of biotin, in particular if the mutation of the gene resides in the biotin-binding region of the protein [107]. Only a few cases of isolated carboxylase deficiencies have been reported [105,108–111].

### 8.3. Biotin transporter deficiency

Recently, a case of inborn biotin transporter deficiency has been identified [12]. The afflicted patient exhibited the typical signs of biotin deficiency despite normal biotin intake; transport rates of biotin in lymphoid tissues were substantially smaller compared with healthy controls. Clinical features are similar to those described for multiple carboxylase deficiency and BTM deficiency.

## 9. Biotin–drug interactions

### 9.1. 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 [45]. Long-term therapy with anticonvulsants increases both biotin catabolism and urinary excretion of 3-hydroxyisovaleric acid [45]. Phenobarbital, phenytoin, and carbamazepine displace biotin from BTM, conceivably affecting plasma transport, renal handling, or cellular uptake of biotin [45].

### 9.2. Lipoic acid

Lipoic acid competes with biotin for binding to SMVT [45], potentially decreasing the cellular uptake of biotin. Indeed, chronic administration of pharmacological doses of lipoic acid decreased the activities of pyruvate carboxylase and  $\beta$ -methylcrotonyl-CoA carboxylase in rat liver to 64–72% of controls [45].

## 10. Requirements and recommended intakes

### 10.1. Adequate intakes

The Food and Nutrition Board of the National Research Council has released recommendations for adequate intake of biotin, ranging from 5  $\mu$ g/day in newborn infants to 35  $\mu$ g/day in lactating women (21–143 nmol/day) [112]. These recommendations are based on estimated biotin intakes (not

to be confused with “requirements” and “Recommended Dietary Allowances”) in a group of healthy people. Adequate intakes are set when requirements of a certain nutrient are unknown; they may serve as goals for the nutrient intake of individuals. Biotin supplements may contribute substantially to biotin intake. For example, 15–20% of individuals in the U.S. report consuming biotin-containing dietary supplements [112].

## 10.2. Factors that affect biotin requirements

Pregnancy may be associated with an increased demand for biotin. Recent studies provide evidence for marginal biotin deficiency in human gestation as judged by increased urinary excretion of 3-hydroxyisovaleric acid [98]. Pregnancy and smoking accelerate biotin catabolism in women [75,93].

Lactation may generate an increased demand for biotin. At 8 days postpartum, biotin in human milk was ~8 nmol/L and accounted for 44% of total avidin-binding substances; bisnorbiotin and biotin-*d,l*-sulfoxides accounted for 48% and 8%, respectively [113]. By 6 weeks postpartum, the biotin concentration had increased to ~30 nmol/L and accounted for about 70% of total avidin-binding substances; bisnorbiotin and biotin-*d,l*-sulfoxides accounted for about 20% and less than 10%, respectively.

## 11. Intake and food sources

The majority of biotin in meats and cereals appears to be protein bound [45]. Most studies of biotin content in foods depended on using bioassays. Biotin is widely distributed in natural foodstuffs. Foods relatively rich in biotin include egg yolk, liver, and some vegetables. The dietary biotin intake in Western populations is about 35–70 µg/day (143–287 nmol/day) [45]. Infants consuming 800 mL of mature breast milk per day ingest ~6 µg (24 nmol) of biotin [113]. It remains unclear whether biotin synthesis by gut microorganisms contributes importantly to the total biotin absorbed [45].

## 12. Excess and toxicity

Empirically, ingestion of pharmacological doses of biotin is considered safe. For example, lifelong treatment of BTM deficiency patients with biotin doses that exceed the normal dietary intake by 300 times does not produce frank signs of toxicity [35]. Likewise, no signs of biotin overdose were reported after acute oral and intravenous administration of doses that exceeded the dietary biotin intake by up to 600 times [61]. Biotin supplementation affects the expression of numerous genes. It is unknown whether any of these alterations are undesirable.

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