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BIOTIN IN METABOLISM AND MOLECULAR BIOLOGY

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■ **Abstract** Biotin is a water-soluble vitamin required by all organisms by virtue of its essential role in carboxylation reactions. Although the metabolism and role of biotin in intermediary metabolism are well established, biotin remains one of the most poorly understood water-soluble vitamins in terms of nutritional requirements and responsiveness to physiological and pharmacological states. Significant advances in the understanding of biotin nutriture have been recently accomplished through the description of the kinetics and regulation of biotin transport and improved methods for biotin status assessment. Additionally, the potential role of biotin in the regulation of gene expression has been strengthened through description of altered gene expression during biotin deficiency and through newly described enzymatic activities of the enzyme biotinidase. Given mounting evidence of suboptimum biotin status, a more complete understanding of these aspects of biotin should lead to a greater appreciation of the ways in which biotin aids in the maintenance of health.

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INTRODUCTION

It has been approximately 12 years since the last review in this series that focused on the overall function of biotin, and in the intervening time great progress has been made in further elucidating the roles of this relatively poorly understood vitamin (30, 110). Although the metabolism of biotin and its participation in enzymatic catalysis is well established, the past decade has ushered in an improved understanding of biotin nutriture using new technologies and approaches. This includes an expanding data set on the kinetics and preliminary molecular characterization of biotin transport, new and potentially important functions of the enzyme biotinidase, new technologies for the assessment of biotin status, and an emerging appreciation for the existence of marginal biotin deficiency in both physiological and pharmacological states. Another interesting and continuing story, which has its genesis nearly 35 years ago, is also unfolding; the possible role of biotin in the control of gene expression. Although in many ways the study of biotin is now only approaching the level to which other vitamins are already understood, the sum of these studies by several excellent research groups has created an optimistic excitement that the current knowledge about this vitamin may only be the tip of the iceberg and that further study will describe a dynamic and responsive vitamin important not only in the pathology of disease states but in the maintenance of health in novel and important ways. This review begins with a brief general review to orient the reader and then focuses on the above areas of investigation.

A PERSPECTIVE ON BIOTIN METABOLISM AND FUNCTION

Biotin is a water-soluble vitamin essential for all known organisms. Whereas animals lack the ability to synthesize biotin, it is synthesized by microorganisms and plants and therefore widespread in the food supply at low concentrations relative to most water-soluble vitamins (26). The highest level of biotin is found in organ meats such as liver and kidney, but it is low in meats, most vegetables, and fruits. The bioavailability of biotin in foods can vary substantially but is in general less than 50%; in some grains, such as corn, the availability appears to be essentially 100%, whereas in other grains, such as wheat, the bioavailability may be as low as 5% (26). The relative bioavailability of biotin in different foods presumably reflects differential susceptibility of these forms to digestive breakdown, but the factors controlling biotin bioavailability are poorly understood.

For many foods the level of biotin is not known or has been indirectly estimated, and this paucity of nutrient information has led to a poor understanding of biotin intake through the national dietary surveys such as the Continuing Survey of Food Intake for Individuals (CSFII) or the National Health and Nutrition Examination Survey (NHANES). The current adequate intake estimation from the Food and

Nutrition Board suggests that adults obtain 30–100 μg/day (1). This was based upon intake of biotin in breast-fed infants extrapolated to adults based on body weight. It is recognized that this is an imprecise estimation, but little data exists to justify altering it in the absence of well-validated indices of biotin status. Very few studies have been performed that estimate biotin intake. An indirect estimation of the vitamin status of young British adults suggested that the intake of biotin is approximately 35 μg/day (18). Additional analyses of this type are warranted, because estimates of biotin intake in Swiss and Canadian diets suggest more than twice this amount (41, 43). There is currently no concern about suboptimum biotin status at the national level, but this view may in part stem from a lack of functional data about markers of biotin status and a lack of appreciation for the possible spectrum of biotin nutriture.

A second potential source of biotin for higher organisms is microbial synthesis by gut flora. This source was initially detected from observations that fecal biotin excretion exceeds oral biotin intake (26). While some controversy about the importance of exogenously derived biotin persists, a substantial amount of experimental evidence suggests that microbially synthesized biotin in the gut is insufficient to meet metabolic needs. Although significant microbial vitamin synthesis takes place in the proximal colon, the presence of adequate transport capacity in this region ($\sim$15–25% that of the jejunum) to absorb a significant amount of biotin remains to be established (14, 97). Additionally, carefully controlled studies of pigs suggest a relatively minor role of endogenous biotin synthesis in overall biotin status. Intracecal infusion of avidin or antibiotics, both of which should markedly reduce the amount and/or bioavailability of bacterially derived biotin in the large intestine, had no significant effect on plasma biotin concentration or urinary biotin excretion, even when microbial growth was stimulated through feeding of lactulose (50). This is in sharp contrast to the relatively large increases in fecal biotin excretion seen under these same circumstances. Together, these data suggest that although bacterial biotin synthesis may be substantial, its form or location limits its bioavailability and contribution to overall biotin status. Additional evidence exists for humans; individuals with biotinidase deficiency, despite having presumably normal diets and therefore representative gut flora, have a markedly higher biotin requirement, and only exogenous biotin supplementation is sufficient to supply it (4, 6, 7, 9, 13, 21, 40, 48).

Dietary biotin is present in two forms: free and protein bound. The protein bound form, in which the vitamin is covalently bound to polypeptides through a lysine residue occurring in a specific amino acid motif, is degraded by digestive proteases to the ε-N^1-biotinyl-lysine adduct biocytin. Although intestinal absorption of biocytin is inefficient, a second digestive enzyme known as biotinidase is thought to be responsible for the cleavage of the amino acid–vitamin bond, liberating the free vitamin (46). Absorption processes similar to that in the intestine bring biotin into the cytosolic space of other tissues (60). As with other micronutrients such as zinc, an unassociated intracellular pool of biotin has been definitively demonstrated. Although its size is substantially smaller than the pool bound to

carboxylase enzymes, in rats the size of the intracellular free pool of biotin is sensitive and directly related to dietary intake (57, 72).

The unassociated pool of biotin presumably serves as the cellular reservoir for the posttranslational process of protein biotinylation. The amount of free biotin in the cell is likely controlled by a balance between cellular uptake and release, and turnover of protein bound biotin by carboxylase degradation. Biotin destined for incorporation into apoenzymes is conjugated to polypeptides through the action of the enzyme holocarboxylase synthetase (HCS). The thermodynamic energy used to drive the addition of biotin is derived from the group transfer of the adenylate portion of adenosine triphosphate, with the formation of biotinyl-5′-adenylate and pyrophosphate. The biotinyl-5′-adenylate is a mixed-acid anhydride with high group-transfer potential. Pyrophosphate is immediately hydrolyzed by pyrophosphatase, which makes the equilibrium of the reaction physiologically favorable. Several mutations in the human HCS gene have been described, and a lack of HCS activity can result in a loss of all biotin dependent carboxylase function, termed multiple carboxylase deficiency (3, 19, 36, 39, 42, 61, 89, 101, 109). It is important to note that a deficiency in biotinidase can also result in a similar clinical presentation (7, 79, 111, 112, 121, 122). Although the exact molecular mechanisms are not well understood, pharmacological biotin supplementation of HCS deficient individuals alleviates the loss of biotin-dependent enzyme function.

Alternatively, two moieties of the vitamin can be catabolized, separately or in combination. In both microorganisms and mammals the sulfur group of the tetrathiohydrophene ring can be oxidized to *d* and *l* biotin sulfoxides and biotin sulfones, possibly through oxygen utilizing microsomal enzyme systems (15, 16, 55, 56). The valeric acid side chain can also be oxidized, probably through a β-oxidation–like pathway similar to that used for fatty acids, to generate bisnorbiotin and tetranorbiotin (128). This pathway is thought to occur through the metabolic activation of coenzyme A conjugation. The abundance of bisnorbiotin and biotin sulfoxides in both rodents and humans is substantial and may be equal that of the intact vitamin (66, 67).

Along with folic acid and S-adenosylmethionine, biotin plays a role in intermediary metabolism in the transfer of one-carbon units. In particular, biotin participates in the transfer of one-carbon units in its most oxidized form, carbon dioxide. The catalytic role of biotin in the four biotin-dependent enzymes is identical; the covalently bound vitamin acts as a mobile carrier of an activated carboxyl group obtained from dissolved bicarbonate that is incorporated into substrates. These carboxylation reactions are important in the metabolism of all macronutrients (104–106). Acetyl CoA carboxylase catalyzes the carboxylation of acetyl CoA to form malonyl CoA, representing the committed step in fatty acid synthesis. Acetyl CoA carboxylase plays a critical role in this process because the ATP driven carboxylation of biotin is conserved in the formation of malonyl CoA. The loss of the high transfer-potential carboxyl group during the condensation of malonyl CoA and acetyl CoA makes the initial step in each round of fatty acid elongation thermodynamically favorable. Pyruvate carboxylase catalyzes the carboxylation

of pyruvate to generate oxaloacetate and therefore serves as an anapleurotic reaction that replenishes citric acid cycle intermediates and catalyzes a required step in gluconeogenesis. Methylcrotonyl CoA carboxylase catalyzes a step in the degradation of the branched-chain amino acid leucine, resulting in the emergence of the deaminated carbon skeleton of leucine as the ketone body acetoacetate. Propionyl CoA carboxylase catalyzes the incorporation of carbon dioxide from bicarbonate into propionyl CoA (derived from cholesterol, odd-chain fatty acid oxidation, and select amino acids) to form methylmalonyl CoA, which enters the citric acid cycle through conversion to succinyl CoA.

ABSORPTION AND TRANSPORT OF BIOTIN

As a water soluble vitamin negatively charged at physiological pH owing to the presence of the carboxylate group, biotin requires a specific transport mechanism to cross biological membranes, where the known biological functions of biotin are manifested. In the period since approximately 1987, the definition of kinetic parameters and metabolic sensitivity of biotin uptake mechanisms has been further elucidated. The transport of biotin has been studied in several experimental systems, and for the majority of these studies common characteristics have emerged.

Several studies have described the absorption of biotin in the intestine using various models, including ligated intestinal loops, isolated enterocytes, and immortalized intestinal cell culture systems. Additionally, the analysis has taken place at several levels, from studies of intact organs to studies of purified basolateral and brush border membrane vesicles. In intact intestine, biotin transport is saturable at relatively low concentrations of biotin and nonsaturable at higher lumenal concentrations, suggesting passive diffusion across the relatively permeable intestinal surface (93). The implications for biotin-treatable disorders are that physiological intakes of biotin are subject to a carrier-mediated process, whereas pharmacological biotin supplementation experiences no such limitations. Interestingly, the absorption of biocytin, the biotinyl-lysine conjugate produced by the digestive breakdown of biotin-containing protein, is inefficient, consistent with a role for pancreatic biotinidase in the release of free vitamin from the diet. Intestinal biotin transport is clearly sodium dependent in almost all reports, as evidenced by a marked reduction in absorption when the transport medium is devoid of sodium or replaced by other monovalent cations (95). The stoichiometry of biotin and sodium transport is 1:1, consistent with a cotransport process. Structural analogs, such as dethiobiotin, are able to competitively inhibit biotin transport (95). The maximal site of biotin absorption resides in the jejunum, which is substantially greater than the ileum and markedly higher than the proximal colon (97).

The characteristics of intact intestinal transport can be extended to isolated membrane fractions derived from this tissue. Biotin transport at the brush border surface exhibits the sodium dependence observed in the intact tissue and is electroneutral (94). Although the transport of biotin is likewise sodium dependent at

the basolateral membrane surface, more sodium than biotin appears to be transported each cycle, suggesting an electrogenic process (96). Also consistent with the intact intestinal studies is the observation that brush border membrane vesicles isolated from the jejunum exhibit greater transport capacity than similar vesicles isolated from the ileum (94).

Similar characteristics have been described for the transport of biotin into immortalized cell lines that mimic intestinal cell physiology. Although the mimicry of normal tissue by cell lines is often imperfect, they offer the advantage of long-term culture that can be used to model chronic treatments on biotin uptake. In the colonic cell line CaCo-2, which can be induced to form an electrically tight monolayer displaying distinct basolateral and brush border surfaces, the uptake of biotin at the brush border surface is also sodium dependent (58, 73). Additional information gained from this model is that the flux of biotin transfer in the brush border to basolateral direction is greater than the reverse direction, consistent with a concentrative process. This same model also showed that very little biotin is metabolized during this transport process, information that would have been difficult to derive from intact tissue. Consistent with these findings are studies of the NCM460 cell line, which models the large intestinal enterocyte (92). In this model the transport of biotin is sodium dependent but also potentially regulated by a protein kinase A dependent pathway, as demonstrated by the inhibitory effect of phorbol-12 myristate acetate. A significant amount of analysis of biotin transport in human peripheral blood mononuclear cells has also been recently accomplished, providing important evidence that proliferation of these cells is linked to increased biotin uptake (129, 131, 133).

INITIAL STUDIES INTO THE MOLECULAR MECHANISM OF BIOTIN TRANSPORT

Until recently, the characterization of biotin transport in various tissues has been essentially limited to kinetic characterization and substrate specificity analysis, although the regulation of these aspects has received substantial study. In contrast to many other micronutrients, however, the molecular entities behind biotin transport are poorly understood. In 1998 Prasad and colleagues isolated a cDNA from rat placenta, termed SMVT1 for sodium dependent multivitamin transporter 1, the protein product of which was capable of mediating the transport of biotin, pantothenic acid, and lipoic acid in HeLa cells (87). The underlying mechanism behind the shared substrate specificity is likely due to the valeric acid moiety present in all three compounds. The protein product predicted by the SMVT1 cDNA is 634 amino acids with a molecular mass of ~69,000 daltons. The transporter protein predicted by this sequence appears to be very similar to other nutrient transporters, as it contains 12 putative transmembrane domains. The authors suggested that this protein might be important in the transfer of biotin and pantothenic acid from the maternal supply to the fetus. The physiological relevance of SMVT1 in biotin

transport has yet to be established in vivo, and several biochemical characteristics of SMVT transport activity argue against it being important in biotin absorption. The calculated Michaelis constant for SMVT1 towards biotin transport is ~15 μM, while the concentration of free biotin in the circulation is ~0.5–2 nM. Therefore, under physiological conditions, the fractional saturation of SMVT1 with biotin would be exceedingly low and suggests that SMVT1 would not be a biologically efficient mechanism for biotin absorption. Additionally, the circulating concentration of pantothenic acid (0.886–4.59 μM) is consistent with the affinity of SMVT1 for this vitamin (5 μM) (27). Taken together, these data suggest that under physiological conditions, the transport of biotin through the SMVT1 transporter would be effectively competed by the presence of pantothenic acid. The search for the physiologically relevant molecular entities involved in biotin transport continues.

NEW ROLE FOR BIOTINIDASE?

Significant advances in the function and possible roles of the enzyme biotinidase have recently occurred. Biotinidase is an enzyme present in many prokaryotic and eukaryotic cells that exhibit hydrolase activity towards biocytin, the biotinyl-lysine conjugate (123–126). The cDNA and genomic gene structure for human biotinidase has been described, and the open reading frame predicts a protein of 543 amino acids and an overall molecular mass of approximately 57,000 daltons (24, 49). The protein has six putative glycosylation sites, which could add significantly to the mass of the mature protein. The upstream sequence of the open reading frame suggests the possible presence of a signal peptide. Overexpression of biotinidase with the putative signal peptide in a baculovirus system proved sufficient to result in a glycosylated, secreted form of the enzyme, which may explain how the protein is targeted to extracellular fluids such as serum and pancreatic secretions (74). Mutations in the biotinidase gene that result in either partial or profound biotinidase deficiency have been well described (11, 75–77, 80–85, 108). A loss of biotinidase activity results in multiple carboxylase deficiency, presumably owing to inefficient recycling and utilization of biotin (7, 112, 121).

Since the initial description of this activity and its purification from cells and serum, the proposed role of biotinidase has been one of releasing protein bound biotin in foodstuffs, and recycling biotin from biocytin resulting from carboxylase turnover in the cell. Several lines of evidence are consistent with this hypothesis. Biotinidase in serum is found in relatively high concentrations compared with circulating biotin, leading to the proposition that biotinidase may serve as a biotin binding and transport protein (22). Individuals with biotinidase deficiency have a higher biotin requirement, suggesting that the activity of biotinidase in the gut should be important. However, several recent studies have provided strong evidence that the majority (85–90%) of the biotin in serum or plasma is unassociated, although some earlier reports suggested that higher proportions of biotin are protein bound (22, 65, 68). Also arguing against a role for biotinidase as a biotin binding

protein in blood is the observation that the Km for the binding of biotin is well above normally encountered biotin concentrations. The hydrolase activity of biotinidase exhibits a pH optimum of ~5.5, substantially lower than normal cellular pH, although it may be argued that this pH optimum may be more appropriate in the proximal intestine, where biotinidase could act on protein bound biotin as it emerges from gastric processing (127).

The inconsistencies between the biochemical characteristics of biotinidase and the normal circulating concentration of biotin and the pH of the extracellular fluid was problematic for clearly establishing a role for biotinidase in metabolism (47). In 1995 Hymes et al. first described the covalent modification of biotinidase in the presence of biocytin and then described a novel second enzymatic activity of biotinidase; that of a biotinyl transferase that used biocytin to biotinylate histones (44, 45). This reaction could be sensitively detected in the serum of normal individuals but not in the serum of biotinidase-deficient individuals. Importantly, the biochemical parameters of the biotinyl transferase activity was biologically consistent; the affinity for the transferase activity was very close to estimates of circulating biotin concentration, and the pH optimum (~7.5) was physiogically relevant for intracellular and extracellular fluids (44, 45).

Following characterization of the biotinyltransferase activity of biotinidase, the physiological relevance of this process remained unclear. Recently, Stanley et al. analyzed the abundance of biotinylated histones in quiescent and proliferating human peripheral blood mononuclear cells. Cells at the G1, S, G2, and M phase of the cell cycle demonstrated a significantly elevated abundance of biotinylated histones compared with quiescent cells (103). The changes in biotinylated histone abundance occurred in the absence of alterations in biotinidase mRNA abundance or biotinidase activity, suggesting additional mechanisms possibly controlling this process. These important studies for the first time placed the biotinyltransferase activity of biotinidase in physiological perspective. The biotinylation of histones, as with other posttranslational modification of histones, could potentially play a role in chromatin structure and therefore influence gene expression.

NEW TECHNOLOGIES FOR THE ASSESSMENT OF BIOTIN STATUS

Analytical methods for the assessment of biotin status have been and continue to be developed. Traditional methods of quantifying biotin in biological materials include bioassay and microbiological assay, the utility and limitations of which have been previously reviewed (62). Modern methods usually rely upon the binding of biotin by either the protein avidin or streptavidin. Most assays of this type suffer from either interfering substances present in complex sample mixtures, or are confounded by the presence of biotin metabolites that do not behave similarly to intact biotin in the binding assay (62). Refinement of the measurement has been accomplished through prior separation and purification of biotin and its metabolites by high-performance liquid chromatography followed by the competitive binding

assay (62). Several pools of biotin have been analyzed for their ability to serve as an accurate and sensitive indicator of biotin status. In humans, circulating biotin, measured in either serum or plasma, has not proved to be a reliable measure of status because depression of serum biotin is not consistently observed in biotin deficiency (63, 72). Urinary biotin excretion, extended to the excretion of its metabolites, has proved more sensitive and accurate (63). Recently, further advances in the assessment of biotin status have been accomplished through the quantification of 3-hydroxyisovaleric acid (3-HIA), a metabolic intermediate that accumulates and is excreted owing to loss of methylcrotonyl CoA carboxylase activity during biotin deficiency (130). Importantly, alterations in urinary 3-HIA has proved more sensitive than urinary biotin; alterations in the urinary excretion of 3-HIA can be detected as early as 15 days after the consumption of a biotin-deficient diet (72).

It is important to note that alterations in biotin and its metabolites, or organic acids that accumulate owing to insufficient biotin-dependent enzyme function, are detected prior to the manifestation of outward signs of biotin deficiency. These new methods of assessment open the possibility that marginal biotin status, if hidden by the absence of outward signs of deficiency, may be more prevalent than previously appreciated. Conditions such as those above may not require extended periods of time in order to affect biotin status: recent evidence suggests the clearance of biotin from the circulation is quite rapid (62). Also potentially complicating the determination of biotin status is the impact of physiological and pharmacological status. For example, glucocorticoid administration has recently been shown to have significant effects upon urinary biotin excretion in rats; the elevated urinary biotin excretion observed under these conditions might potentially mislead biotin status estimates (88).

CONDITIONS OF SUBOPTIMUM BIOTIN STATUS

Despite the prevailing assumption that biotin deficiency is rare, there is mounting evidence indicating the existence of several physiological and pharmacological states in which biotin status is compromised. Inborn errors of biotin metabolism, which impinge on every aspect of biotin metabolism and function, result in secondary or functional biotin deficiency (5, 7, 8, 12, 20, 35, 38). Deficiency specifically in biotinidase results in a secondary biotin deficiency, because the vitamin is inefficiently absorbed in the gut. Biotinidase deficiency is now recognized as a condition of sufficient incidence that it is screened neonatally worldwide (37). Biotin deficiency has been associated with protein-energy malnutrition, presumably owing to the lack of intake of protein-bound biotin; the biotin deficiency may exacerbate the inability of these individuals to derive energy from fuel (114–116). In an important related condition, individuals admitted to a hospital setting for inflammatory bowel disease also exhibited protein energy malnutrition. Half of these patients were marginally deficient in biotin, along with deficiencies in other vitamins. Following total enteral nutrition therapy, the biotin status of these individuals was unchanged, suggesting that current nutritional therapy for inflammatory

bowel disease does not meet metabolic needs for biotin, as well as for other select vitamins (2).

Three studies have presented data indicating that a high proportion of individuals treated with the antiepileptic medications phenytoin, primidone, phenobarbital, or carbamazepine exhibit reduced plasma levels of biotin and increased urinary excretion of organic acids associated with loss of biotin-dependent enzyme function (52–54). More recently, improved methods of status assessment were used to confirm these results and extend them to include the observation that individuals treated with anticonvulsants demonstrate an accelerated catabolism of biotin, consistent with a decrease in the active form of the vitamin (64, 69). A second mechanism of depressed biotin status in these individuals is mediated through competition of the drug for biotin transport in the intestine (98). The functional implications of this depressed biotin status are not inconsequential; the accumulation of lactate in the brain that may stem from insufficient pyruvate carboxylase activity is potentially neurotoxic in part owing to the poor passage of lactic acid across the blood brain barrier (78, 86). The importance of induced biotin deficiency during the treatment of epilepsy is further illustrated by the observation that children presenting with symptomatic biotinidase deficiency often exhibit associated epileptic seizures that do not respond well to anticonvulsant treatment (100). Regional distribution of biotin in the brain has been described, and therefore the loss of this biotin may underlie part of the lesion in biotin function during anticonvulsant administration (28).

Although early cross-sectional studies suggested no alteration in biotin status during pregnancy, more recent longitudinal evidence has now firmly established that a significant proportion of women from mid to late pregnancy enter into negative biotin balance (70, 71). This is supported by both the observation of increased excretion of 3-hydroxyisovaleric acid and decreased urinary biotin excretion longitudinally during pregnancy. Marginal biotin deficiency during pregnancy may represent a potential concern owing to the related observation that in rodent models maternal biotin deficiency is teratogenic (118–120, 132). Alcoholism may be yet another condition in which biotin status is impaired, evidenced by the substantial inhibition of intestinal biotin transport by chronic ethanol feeding in rats (99). Interestingly, the one study of the effect of aging on biotin metabolism in rodents suggests that circulating biotin concentrations and intestinal biotin transport appear to be elevated in aged rats, although additional investigation is required to place these findings into relevance (90).

BIOTIN: A POTENTIAL ROLE IN GENE EXPRESSION?

As with many nutrients, a growing body of evidence suggests that biotin may be involved in the regulation of gene expression. This potential involvement could be either direct, as in the case of zinc, in which transcription factors responsive to zinc per se interact with promoter regions contained in select genes to elevate or

suppress the expression of that gene, or indirect, as when some process or product of a mechanism that involves the nutrient is the active factor in regulating the expression of genes (29). With respect to a potential role for biotin, evidence as early as 1965 demonstrated that the administration of biotin to biotin-deficient rats increased incorporation of ^{14}C-labeled amino acids into specific polypeptides (34). Although the resolution of this technique was relatively low, it proved for the first time that at least in a condition of poor biotin nutriture, recovery of circulating and tissue levels of biotin was associated with transcriptional and/or translational processes. In an extension of this work, it was later demonstrated that in fasted, but not biotin-deficient, rats the administration of biotin resulted in a marked increase in the activity of glucokinase, the high-Km, low-affinity hexokinase expressed primarily in liver and pancreas (31). When the mechanism behind the elevated activity was investigated, it was found that biotin caused an upregulation in the amount of glucokinase messenger RNA that kinetically preceded the increase in enzymatic activity (23).

The regulation of a non-biotin-dependent enzyme has important implications for the regulation of carbohydrate flux in the opposing pathways of glycolysis and gluconeogenesis. The effect of biotin on the expression of phosphoenolpyruvate carboxykinase (PEPCK) has been analyzed in diabetic rats. Treatment of rats with streptozotocin resulted in the destruction of the pancreatic β-cells, which are critically important in the sensing and control of blood glucose. In these states the activity and expression of hepatic PEPCK is elevated. The administration of biotin, in a manner similar to insulin, resulted in a marked suppression of PEPCK expression with rapid kinetics; significant ($\sim$sixfold) downregulation of PEPCK expression was observed within 15 min of the injection of biotin (33). At a higher level of resolution of this phenomenon, the transcription rate of PEPCK in diabetic and biotin-treated diabetic rats was analyzed using nuclear run-on assay, which directly measures the synthesis of specific messenger RNA in the nucleus. The administration of biotin elicited a significant reduction in PEPCK transcription after 1 h. Interestingly, this suppression is similar to the reduction in PEPCK expression elicited by the administration of insulin. Although these regulatory phenomena centered around the effect of biotin on altered states of carbohydrate metabolism, another metabolic derangement observed in biotin deficiency is hyperammonemia. The effect of biotin deficiency on the activity of enzymes involved in amino acid metabolism, however, had not been assessed during altered biotin status until the studies of Maeda and colleagues. In this report, the activities of enzymes involved in the urea cycle were analyzed in biotin-adequate and biotin-deficient animals. In biotin-deficient rats, the activity of arginosuccinate synthetase, arginosuccinate lyase, and arginase was unchanged, but the activity of the critical enzyme ornithine transcarbamoylase was significantly reduced (59). This study provides a possible molecular mechanism for the hyperammonemia commonly observed during biotin deficiency.

Another interesting example of possible biotin-regulated gene expression comes from a cell culture model system. The asialoglycoprotein receptor on the surface

of hepatocytes is involved in the internalization of circulating glycoproteins that display a terminal sialic acid on the oligosaccharide chain. In cultured hepatocytes the absence of biotin in the culture medium prevents not the expression of the receptor, but rather its proper cell surface targeting (25). Although the messenger RNA for the receptor appeared not to be altered by the lack of medium biotin, additional analysis of the transcription of the receptor would be helpful for a full understanding of the role of biotin in this process.

It should be noted that in all these studies the potential regulatory role of biotin is on metabolic pathways that are perturbed a priori; there have not been reports in which the administration of biotin to biotin-adequate, energy-normalized animals results in detectable alterations in gene expression. This may suggest that in metabolic perturbations, especially in carbohydrate and amino acid metabolism, some factor is either deficient or in excess, for which high doses of biotin substitutes.

PERSPECTIVE AND FUTURE DIRECTIONS OF BIOTIN RESEARCH

The study of biotin nutriture is proceeding on several fronts. Clearly, the manifestation of frank biotin deficiency is recognized as occurring relatively rarely, although in those circumstances rapid treatment is required because of the potentially irreversible consequences that can arise from prolonged biotin deficiency (124). Given the mounting evidence of suboptimal biotin status, however, a need for experimental models that mimic this marginal state, and its potential effects, are needed. Traditionally, many studies that analyzed the effects of biotin deficiency utilized animal models that exhibited severe and frank biotin deficiency (10, 17, 32, 51, 59, 91, 102, 107, 113, 117). More recently, however, the effect of subclinical biotin deficiency in rats has begun to be analyzed (57, 72). In these reports a marginal biotin deficiency can be induced and clear biochemical deficiency at the cellular level can be detected prior to the outward signs of frank deficiency. These studies suggest that outward signs of biotin deficiency may not be the most reliable method for determining the need for more widespread biotin status measurements. A greater appreciation for the occurrence of marginal biotin deficiency in the general population is needed, and the work currently addressing techniques and approaches to the determination of biotin status should yield the tools necessary to accomplish this goal. Additional work is required also in the elucidation of genes involved in biotin transport. As in many other nutrient transporters, it can be expected that several closely related genes might encode biotin transporters that are distinguished by their tissue specificity, activity, and possible regulation. Several of the conditions under which biotin status is impaired, such as in the case of anticonvulsant treatment, could be in part explained in terms of transport competition and function. The new functional data on the activity of biotinidase as a biotinyltransferase that modifies histones, and therefore potentially impact gene regulation in a more broad sense, also require further study.

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