Biotinidase Deficiency: New Directions and Practical Concerns

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Opinion statement

Biotinidase deficiency is a readily treatable inherited disorder. Discovery of the enzyme deficiency as the cause for late-onset multiple carboxylase deficiency initially seemed to answer almost all of the questions about the disorder. However, as is the case for most inborn errors of metabolism, finding the enzyme that causes the disorder, cloning the gene, and determining the spectrum of clinical features of the disease only opens a Pandora's box. As researchers have found, there are still many important and interesting questions about this disorder that must be addressed and answered. However, when compared with other inherited metabolic diseases, biotinidase deficiency is still one of the most readily treatable. If a child must have an inborn error of metabolism, let it be biotinidase deficiency and let it be identified by newborn screening.

Introduction

Biotin is a water-soluble B-complex vitamin and the coenzyme for four carboxylases in humans involved in glucogenesis, fatty acid synthesis, and the catabolism of several branch-chain amino acids (Fig. 1) [1•,2,3]. Biotin is covalently attached to the various apocarboxylases by biotin holocarboxylase synthetase. The carboxyl group of biotin is linked through an amide bond to an epsilon (ϵ)-amino group of specific lysine residues of the apoenzymes. Ultimately, the carboxylases are degraded proteolytically to biocytin (biotinyl-ϵ-lysine) or biotinyl peptides. These products then become the substrates for biotinidase (EC 3.5.1.12), which cleaves the amide bond and recycles free biotin and lysine or lysyl peptides [4,5]. Biotinidase also has a role in making biotin available by processing protein-bound biotin [6].

Profound biotinidase deficiency (less than 10% of mean normal activity in serum) is an autosomal-recessive inherited disorder characterized by neurologic and cutaneous symptoms [7–9]. A booklet about biotinidase deficiency for professionals and families of children with the disorder is available on an Internet web site [10•]. If untreated, most enzyme-deficient children exhibit seizures, hypotonia, skin rash, and alopecia during the second to fifth month of life. Many children have ataxia, developmental delay, conjunctivitis, hearing loss, and visual problems, including optic atrophy. Most, but not all, symptomatic affected children have metabolic ketoacidosis or organic aciduria. Some affected individuals have severe metabolic compromise that results in coma or death. Patients with biotinidase deficiency may only manifest one or two of these features, whereas others exhibit the full spectrum of findings. This variability in expression can even occur in members of the same family. Once hearing loss, visual abnormalities, and moderate to severe developmental delay occur, they are not usually reversible by treatment with biotin. If treatment is initiated before the development of symptoms, these findings can be prevented. A group of children with profound biotinidase deficiency may not develop symptoms until later in childhood or during adolescence [11]. They usually exhibit motor limb weakness, spastic paresis, and eye problems, such as loss of visual acuity and scotomata, rather than the

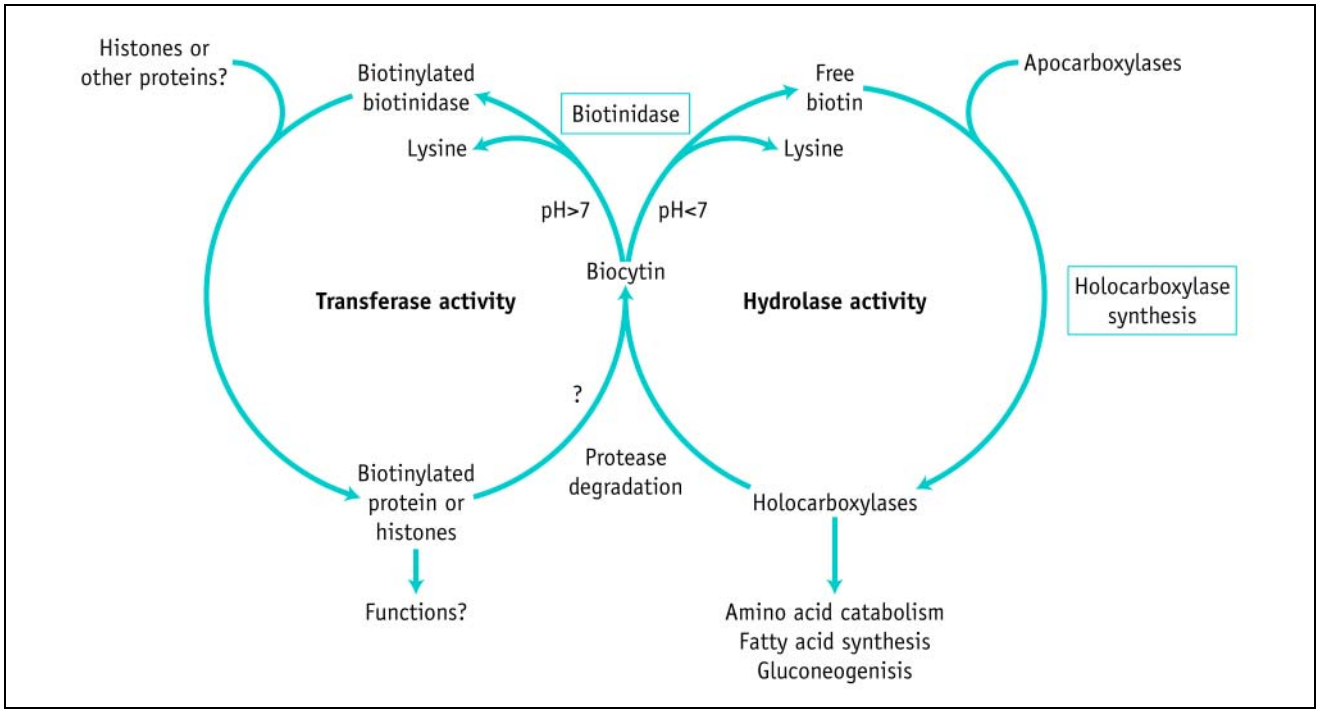

Figure 1. The biotin bi-cycle. The cycles have biotinyl-hydrolase and biotinyl-transferase activities. The two enzymes involved are in boxes.

more characteristic symptoms observed in young untreated children with the disorder.

Various abnormalities on computerized tomography and magnetic resonance imaging have been reported in children with biotinidase deficiency [12, 13,14•]. These findings included cerebral edema, low attenuation of white matter, diffuse cerebral atrophy, and basal ganglia calcifications. Children who initially had low attenuation of white matter shortly after onset of their first neurologic symptoms improved neurologically after biotin treatment. Significant recovery is possible even after the appearance of marked neurologic deficits in some children, but not in others.

All children with profound biotinidase deficiency are treated with pharmacologic doses of biotin (5 to 20 mg daily). Essentially, all symptomatic individuals improve clinically. Seizures usually resolve within hours to days, and the cutaneous manifestations usually resolve within weeks. Depending on the severity and frequency of episodes of metabolic and neurologic compromise, many children with developmental delay rapidly achieve new milestones or regain those that were lost. Children with biotinidase deficiency, who cease biotin therapy, accidentally or deliberately, develop symptoms within several weeks to months.

Two asymptomatic adults with profound biotinidase deficiency were ascertained because their biotinidase-deficient children were identified by newborn screening [15]. These adults were homozygous for two different mutations, which resulted in different abnormal enzymes. There was no evidence of an increased dietary intake of biotin to explain why they have remained asymptomatic. Although these adults may still be at risk for developing symptoms, they may represent a small group of individuals with profound biotinidase deficiency that will never develop clinical problems. Their lack of symptoms suggests that there are epigenetic factors that protect some enzyme-deficient individuals from developing symptoms.

Neonatal screening for biotinidase deficiency is available using the same samples of whole blood spotted on filter paper [16,17]. Currently, at least 25 states in the US and 25 other countries screen their newborns for biotinidase deficiency [18]. The estimated incidence of biotinidase deficiency is about 1 in 60,000.

OTHER FUNCTIONS OF BIOTINIDASE

Untreated children with biotinidase deficiency excrete elevated concentrations of biotin and biocytin, suggesting that biotinidase acts as a biotin-binding protein [19]. However, results of studies examining biotinidase or other serum proteins as biotin-binding proteins are equivocal. Equilibrium dialysis studies of biotinidase with radioactive biotin at pH 7.4 suggest that biotinidase noncovalently binds biotin and functions as a biotin carrier protein in plasma [20]. Other studies performed with plasma dialyzed against distilled water (evidently at pH 6 or below depending on the anticoagulant used) found that most of the biotin in serum is not protein bound [21]. Biotinylation of biotinidase in the presence of biocytin, but not biotin, occurs at neutral or alkaline pH [22]. Incubation with nucleophilic

acceptors, such as ethanolamine, hydroxylamine, or mercaptoethanol, with biocytin and biotinidase results in loss of avidin reactivity.

Binding of biotin to the enzyme is likely to occur through a thioester, involving the thiol group of cysteine in the active site and the carboxyl group of biotin, formed during the cleavage of biocytin [23]. These findings possibly explain how biotinidase functions as a biotin-binding protein in plasma. These results would also explain why previous investigations have failed to detect significant noncovalent binding of biotin to biotinidase. Mechanistically, researchers propose that biotinidase cleaves biotin from biocytin, releases the lysine, and binds the biotin as an acyl enzyme. Biotin is released slowly at alkaline pH when water is the acceptor. Some nucleophiles are better acceptors than water, and biotinidase transfers the biotin to these compounds. More substrate is cleaved in the presence of such nucleophiles.

If biotinidase acts as the biotin carrier protein in plasma, it may be responsible for specifically transporting biotin into cells through a specific cell membrane receptor. Because biotinidase is highly sialylated, this may occur through desialylation of the enzyme and subsequent reaction with a galactose receptor on the cell membrane resulting in internalization of the biotinyl enzyme. Another possibility is that the biotinyl-biotinidase reacts with a specific biotin receptor on the cell membrane [24]. Once the biotinyl-biotinidase enters the cell, the biotin can be released by hydrolysis or transferred to appropriate cellular acceptors, depending on the pH of the compartment.

Researchers have shown that biotinidase in the presence of biocytin, but not biotin, can specifically biotinylate histones (Fig. 1) [25]. The K_m value for hydrolysis of biocytin by biotinidase is in the µM range. In addition, although the pH of serum is 7.4, biotinidase hydrolysis of biocytin occurs optimally at pH 5.5 to 6, with a precipitous decrease in activity above pH 7. Conversely, biotinylation of histones by biotinidase occurs at physiologic pH, and at physiologic concentrations of biocytin, in the nM range [25]. Therefore, biotinyl-transferase activity is likely a major function of biotinidase.

MOLECULAR CHARACTERIZATION OF THE BIOTINIDASE GENE AND MUTATIONS CAUSING BIOTINIDASE DEFICIENCY

The complementary DNA that encodes normal human serum biotinidase has been cloned and sequenced [26]. Northern blot analyses have demonstrated biotinidase message in human liver, kidney, pancreas, lung, skeletal muscle, heart, brain, and placenta. The biotinidase gene has been mapped to chromosome 3p25 [27], and the genomic structure of the human biotinidase gene has been determined [28].

There are now 79 known mutations that cause profound biotinidase deficiency [29•,30,31]. The two most common mutations found in the US are 98-104del7ins3 and 538R>C. The 98-104del7ins3 mutation occurs in a region of the gene that encodes the putative signal peptide, and results in a frame shift and premature termination of the enzyme [32]. Fifty percent of symptomatic children have this mutation in one of their alleles. The second most common mutation 538R>C is a missense mutation in a CpG dinucleotide near the terminal end of the gene [33]. This mutation is found in at least one allele in 30% of symptomatic children. Aberrant biotinidase protein was not detectable in extracts of fibroblasts from a child who is homozygous for the 538R>C mutation, but was present in less than normal concentration in identical extracts treated with beta-mercaptoethanol. Because there is no detectable biotinidase protein in sera from children who are homozygous for the 538R>C mutation and in combination with the deletion/insertion mutation, the 538R>C mutation likely results in inappropriate intra- or intermolecular disulfide bond formation, more rapid degradation of the aberrant enzyme, and failure to secrete the residual aberrant enzyme from the cells into blood. Serum samples from symptomatic children had no detectable cross-reactive material (CRM) to antibody to normal purified biotinidase, reduced quantities of CRM, or normal quantities of CRM in serum. All of these mutations result in complete absence of biotinyl-transferase activity in serum.

The most common cause of profound biotinidase deficiency in children ascertained by newborn screening in the US is the missense mutation 456Q>H [34]. This mutation is found in at least one allele in 51% families or 28% alleles studied. This mutation was not identified in normal adults or in normal newborns. In addition, a child homozygous for 456Q>H has very low biotinyl-hydrolase activity, lacks biotinyl-transferase activity, and no CRM in serum. The second most common mutation that causes profound biotinidase deficiency in children identified on newborn screening in the US is a double mutation, 171A>T and 444D>H [35]. The double mutations are inherited from a single parent as a double mutation allele. Sera of children homozygous for the double mutation allele have very low CRM, and the aberrant enzyme has very low biotinyl-hydrolase activity and no biotinyl-transferase activity. The 444D>H mutation alone causes a 52% loss of activity in the aberrant enzyme from that allele [35]. The single 444D>H mutation was found in DNA from randomly selected, anonymous dried blood spots. The frequency of this allele in the general population is estimated at 0.039. In contrast, the single 171A>T mutation was not identified in any of these normal blood spot samples.

Researchers compared the mutations in children with profound biotinidase deficiency ascertained by

newborn screening in the US with those of symptomatic children. Of the mutations identified in the two populations, four mutations comprise 59% of the disease alleles studied. Two of these mutations occur in the populations, but at a significantly greater frequency in the symptomatic group. The other two common mutations occur only in the newborn screening group. The newborn screening population, therefore, is a group from which complete ascertainment of all mutations is possible, whereas clinical ascertainment leads to the identification of only a subgroup of mutations. Because two common mutations do not occur in the symptomatic population, it is possible that individuals with certain mutations in the newborn screening group may have a decreased risk for developing symptoms if left untreated.

Almost all individuals with partial biotinidase deficiency have the 444D>H mutation in combination with a second mutation for profound biotinidase deficiency on the second allele [36].

HEARING LOSS IN BIOTINIDASE DEFICIENCY

Initial surveys of symptomatic children with biotinidase deficiency revealed that 20% to 30% had hearing loss [37,38]. Based on these findings, the author recommends that hearing be evaluated in all children with the disorder. Because many of these children were very young at the time of diagnosis, hearing was not assessed. In those who had hearing loss diagnosed early in the course of their disorder, the hearing loss was evident before biotin therapy [38]. Hearing loss usually was determined by abnormal auditory brain stem response studies in infants and occasionally by audiograms in young children, and was always described as sensorineural in nature.

In a preliminary study to determine the occurrence of hearing loss in children with biotinidase deficiency, the author obtained data on 33 children with profound biotinidase deficiency from 29 families [39•]. All of the children had profound biotinidase deficiency based on serum enzyme determinations in laboratory. All of these children were diagnosed after they exhibited one or more symptoms of biotinidase deficiency, including seizures, hypotonia, ataxia, poor feeding, breathing problems, alopecia, and skin rash. Hearing loss was evident in 76% children. Two thirds of the children with hearing loss required hearing aids. The mean age of diagnosis of hearing loss was 32.5 months, which is well into the critical period for normal speech and language development. There were no statistical differences between the mean age of onset of symptoms, mean age of diagnosis, and mean age from the onset of symptoms to diagnosis in the children with and without hearing loss. In addition, there was a high incidence of speech and learning problems in the children with hearing loss (78% and 70%, respectively) and without hearing loss (62% and 50%, respectively). The involvement of the auditory system in these problems is unknown.

From the reports and recordings of audiograms and auditory brain stem response studies that had been performed on these children, researchers have found that all of the children with hearing loss had sensorineural loss. The typical audiometric configuration is of moderate to severe sloping sensorineural hearing loss. Once such a large number of children with hearing loss were discovered, the author pushed the recommendation that the other enzyme-deficient children who were thought to have normal hearing also be tested. Subsequently, two children who did not report hearing loss were tested and found to have moderate sensorineural hearing loss. A few of the children with hearing loss have had serial hearing evaluations, whereas most have not. In those individuals who were studied serially, the hearing loss typically does not appear progressive. A small number of children have shown slight, but continual deterioration in hearing, and one child showed improvement in hearing with time and biotin therapy. It also is possible that hearing loss is more likely to occur in children with specific mutations, especially those with mutations that completely abolish biotinylhydrolase and transferase activities.

The preliminary immunohistochemical findings suggest that biotinidase and biotin have a major role(s) in the structural or functional processes in cochlea and retrocochlear sites [40]. The role of biotinidase and biotin in the auditory system and as a cause of hearing loss in biotinidase deficiency is unknown.

IS THERE A DIFFERENCE IN THE DEGREE OF PROFOUND BIOTINIDASE DEFICIENCY?

Recently, it was suggested that a distinction should be made between children with profound deficiency who have less than 1% and those with greater than 1% enzyme activity [41], and that those with greater than 1% activity may not need treatment. To further examine this question, the author studied a series of 31 consecutive symptomatic children with profound biotinidase deficiency. They had a mean serum enzyme activity of 2.3%, ranging from undetectable to 10%. Of these children, 13 had activities less than 1% and 18 had activities between 1% and 10%. In addition, if one considered the genotype of these children, 17 had non-sense mutations, deletions, and deletion/insertion mutations, or mutations that result in failure to secrete the enzyme from the cell into the serum; all resulting in the absence of enzyme protein and, therefore, total deficiency of serum enzyme activity. The remainder had missense mutations that may or may not result in total loss of serum enzyme activity. A group of children with less than 1% activity did not develop symptoms until they were many months or years of age and a group with between 1% to 10% of mean normal activity initially exhibited symptoms during the first few

months of life. Although it is expected that a child with total loss of enzyme activity is at a high risk of developing symptoms, a child with between 1% and 10% activity may be just as likely to develop symptoms. Based on the author's data and knowledge of the unpublished results of others, the author strongly recommends that physicians and parents should not use the percent of residual enzyme activity or genotype information to alter their decision to treat children with profound biotinidase deficiency with biotin. The author recommends that all children with profound biotinidase deficiency be treated with biotin.

IS BIOCYTIN TOXIC?

When researchers first discovered biotinidase deficiency, the possibilities that biocytin is accumulating and that it may have some harmful effects were immediately considered [40]. Biocytin concentrations were elevated in sera and excreted in the urine of symptomatic children [41–43]. The proteolytic degradation of the carboxylases results in the formation of biocytin. Individuals with biotinidase deficiency cannot cleave biocytin. Fortunately, biocytin is water soluble and is easily excreted. Initially, there was concern that the biocytin may cause hearing loss [44]. The concern was that pharmacologic doses of biotin would "fuel" the synthesis of increased quantities of holocarboxylases, that when degraded would form increased amounts of biocytin in these children. The author's 20-year experience in treating individuals with biotin has not identified any obvious effects of biocytin. Whether biocytin or biocytin together with secondary biotin deficiency is toxic remains to be determined.

CONCERNS ABOUT CONFORMATIONAL ENZYME TESTING

A recent major concern has been conformational testing for biotinidase testing in some laboratories. After many years of performing quantitative biotinidase activity determinations in serum/plasma, the author's laboratory learned several important properties about the enzyme that must be considered by all laboratories performing the testing. First, the enzyme in serum/plasma can readily lose activity if the sample is stored at room temperature or at 4°C to -20°C. Stability of activity is retained only if the sample is stored at -70°C to -80°C. There are two times in the processing of samples that this becomes an issue—first, when the blood is drawn, serum/plasma is separated, and it is stored for shipment to the laboratory; and second, when the serum/plasma is received by the laboratory and stored before assay.

The author is aware of several children diagnosed with profound and partial biotinidase deficiency who were ultimately found to have normal activity. This disparity is not because of the complexity of the colorimetric assay, because this procedure is extremely simple and straightforward. It is far more likely that these results are because of the handling and storage of the samples, and that no external controls are concomitantly obtained and assayed for comparison. Incorrect diagnosis results in anxiety to the families. In addition, this may cause the children to be inappropriately treated for a condition that they do not have, and will result in failure to continue pursuing the correct diagnosis. Based on these findings, the author strongly recommends that samples from the parents and an unrelated control are obtained and submitted with the sample from the proband. This will assure comparison of the activity of the child with an individual with normal activity and with the parents who should have activity in the heterozygous range if the child is truly deficient. If the normal external control has lower than expected activity, then it is likely that the samples were inappropriately handled. This procedure should readily eliminate the vast majority of the ambiguous results that have been occurring nationally.

Treatment

Pharmacologic treatment

Biotin

Standard dosage Children with profound biotinidase deficiency are routinely treated with 5 to 20 mg of biotin per day independent of the age. This dose of biotin was determined empirically using the experience of treating children with the various forms of multiple carboxylase deficiency over the past two decades. Keeping the dose constant while the child is growing is done by titrating the dose, because the quantity of biotin per kilogram of body weight is continually decreasing. Some physicians have attempted to use the concentration of plasma biotin as indicator of adequate treatment only to find that the concentration in compliant children is always manifold time higher than normal, and has little or no clinical value. In addition, it is important to keep in mind the method that is used to determine the concentration of biotin. Methods that use avidin binding measure a combination of biotin and biocytin, whereas only some microbiologic methods measure only biotin. Others have used monitoring of urinary organic acids as a means of assessing treatment

efficacy. Finding elevations of those organic acids consistent with multiple carboxylase deficiency while the child is treated with biotin likely indicates the need to increase the dose or provides evidence of noncompliance. Unfortunately, 20% of untreated enzyme-deficient children had normal urinary organic acid secretion when symptomatic [8]. Nevertheless, monitoring of urinary organic acids may be the only useful method of ascertaining whether the dosage of biotin is sufficient.

Contraindications None known.

Main drug interactions Some anticonvulsants can lower plasma biotin concentrations.

Main side effects The only concern about using an empiric dose of biotin has come from the anecdotal reports of two girls who when going through puberty began to lose their hair. In these cases, the biotin dose was increased to 15 to 20 mg from 10 mg and the hair loss stopped. Currently, the dosage range of biotin of 5 to 10 mg per day appears adequate and effective with consideration of increasing the dosage during puberty. Fortunately, experience continues to indicate that high doses of biotin are not toxic. Studies of the effects and adequacy of long-term biotin therapy are needed.

Special points Biotin is administered in several forms. Biotin can be purchased as a capsule, tablet, or some form of liquid preparation. For an infant or young toddler, the contents of the capsule or a finely crushed tablet is placed in a spoon or children's medicine dispenser, mixed with a small volume of formula, and then "washed" down with additional formula. As the child gets older, the biotin can be combined with or sprinkled on various foods, such as applesauce. When old enough, the child can swallow the capsule or tablet. Many pharmacies formulate liquid preparations of the vitamin. Pure biotin is purchased and is then added to water, other aqueous solutions, or made as syrup. These preparations are obviously more economical, because the pure biotin and the time required to make the preparation is short. However, there are several concerns with these liquid preparations. The visible quantity of biotin to prepare 10 mg per 1 or 5 mL of solution or syrup is exceedingly small. Although biotin is a water-soluble vitamin, it is difficult to solubilize. In the laboratory, biotin must be stirred overnight in alkalotic aqueous solvents to solubize it. This is a procedure rarely performed by pharmacies. Therefore, simply adding the weighed out quantity of biotin to a volume of water, especially if it contains other solutes, is likely to be a suspension, not a solution. The biotin cannot be seen by simply inspecting the liquid to determine if the vitamin is in solution. In addition, it is usually recommended that the liquid preparation is stored in the refrigerator, further decreasing the solubility of the vitamin. Because the biotin is likely in suspension in these preparations and will sediment with time and reduced temperature, even if the liquid is shaken well before each administration, the dose of the biotin delivered is probably inconsistent and suboptimal. Moreover, some preparations, especially syrups, may be prone to bacterial growth with prolonged storage. For these reasons, the author recommends administering the solid vitamin from a capsule or tablet. This method guarantees a consistent and appropriate dose of biotin.

Cost/cost effectiveness Pure biotin is relatively inexpensive. The majority of the cost of various biotin preparations is in the making of the solutions, capsules, and tablets. Even then, the cost of treatment for a year is between $100 and $200.

Additional pediatric considerations

- Treatment of partial biotinidase deficiency (between 10% to 30% of mean normal serum activity) has been controversial. Initially, partial biotinidase deficiency was only diagnosed in children identified by newborn screening. None of these children was ascertained because they developed symptoms. It was uncertain whether these children required treatment with biotin. Therefore, when parents were presented with this information, some chose not to treat their children. As frequently occurs with rare inborn errors of metabolism, it was through anecdotal reporting that several children with partial deficiency exhibited mild symptoms during an infection, such as gastroenteritis [45]. These children were treated with biotin, and their

symptoms resolved. This prompted the recommendation that all children with partial deficiency should be treated with some biotin, usually between 1 to 5 mg of biotin per day. A few metabolic specialists still question the need for treating these children, whereas most insist that because there is no known toxicity of the biotin, they should receive some biotin. Because of the lack of data to suggest toxicity and that some untreated children developed symptoms when stressed, the latter regimen seems the most reasonable and prudent at this time.

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