Biotin Status: Which Are Valid Indicators and How Do We Know?1,2

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ABSTRACT Although estimated average requirements for biotin have been proposed, the human requirements for biotin in specific populations and at various ages remain uncertain, in part because indicators of biotin status have not been validated. With the use of improved methods for measuring biotin and metabolites, a recent study indicated that decreased urinary excretion of biotin and bisnorbiotin is an early and sensitive indicator of biotin deficiency, but decreased serum concentration of biotin is not. Increased urinary excretion of 3-hydroxyisovaleric acid (3-HIA), a leucine metabolite that is excreted in increased quantities with deficiency of the biotin-dependent enzyme ß-methylcrotonyl-CoA carboxylase, is also an early and sensitive indicator of biotin deficiency. When these indicators were assessed longitudinally in 13 pregnant women, biotin excretion was not significantly decreased early in pregnancy but did decrease significantly from early to late pregnancy. Excretion of 3-HIA was abnormally increased in about three-fourths of the women studied in both early and late pregnancy. Thus, each indicator detected biotin deficiency late in pregnancy, but assessment of biotin status for the two indicators conflicted early in pregnancy. Preliminary results from a trial assessing response of 3-HIA excretion to biotin treatment indicate that biotin status is indeed impaired both early and late in pregnancy. J. Nutr. 129: 498S–503S, 1999.

KEY WORDS: • biotin • pregnancy • teratology • birth defects • 3-hydroxyisovaleric acid

This paper summarizes the following three recent studies from our laboratory: 1) a study of the experimental induction of biotin deficiency that examined the validity of putative indicators of biotin status; 2) a study that used the validated indicators to examine biotin status longitudinally in pregnancy; and 3) an ongoing study designed to resolve a conflict in interpreting results of the longitudinal pregnancy study. This study presents preliminary results concerning the response to biotin supplementation in pregnancy.

Although safe and adequate intakes for the water-soluble vitamin biotin have been recommended (NRC 1989) and estimated average requirements have been proposed (Mock and Guilarte 1998, NRC 1998), the human requirement for biotin in specific populations and at various ages remains uncertain. This surprising gap in modern nutritional knowledge arises at least in part from technical problems with indicators of biotin status. From the absence of studies validating the indicators of biotin status during progressive, experimental biotin deficiency. The importance of valid indicators is emphasized by recent reports that reduced biotin status may not be rare. Several studies have reported biotin deficiency in patients receiving parental nutrition without biotin supplementation (Mock et al. 1985, Mock 1986), in individuals with the inborn error of biotinidase deficiency (Wolf et al. 1985), in patients receiving long-term therapy with certain anticonvulsants (Krause et al. 1982a and 1982b, Mock and Dyken 1997, Mock et al. 1998), in children with severe protein-energy malnutrition (Velazquez et al. 1988) and in a substantial proportion of women with otherwise normal pregnancies (Mock and Stadler 1997, Mock et al. 1997), as discussed below.

The speculation that the human biotin requirement can always be met by the contribution of biotin produced by gut bacteria (Schanler 1997) conflicts with a recent report of an infant who developed biotin deficiency while consuming a biotin-free, elemental formula (Higuchi et al. 1996).

VALIDATION OF INDICATORS OF BIOTIN STATUS

To interpret correctly studies such as those referenced above, indicators of biotin nutritional status should be validated under controlled conditions designed to cause progressive biotin deficiency (Mock et al. 1997). We have used egg white to induce experimentally a marginal, asymptomatic degree of biotin deficiency.

Study design and analytic methods. Subjects (n = 10) not receiving a biotin supplement for at least the previous month were housed in a clinical research center and received an egg-white diet for 3 wk. The egg white was provided in a blended beverage containing sufficient avidin to bind >7

1 Presented at the symposium “Nutrition, Biochemistry and Molecular Biology of Biotin” as part of Experimental Biology 98, April 18–22, 1998, San Francisco, CA. The symposium was sponsored by the American Society for Nutritional Sciences and was supported in part by an educational grant from Roche Vitamins and Fine Chemicals. Published as a supplement to The Journal of Nutrition. Guest editor for the symposium publication was Donald Mock, University of Arkansas for Medical Sciences, Arkansas Children’s Hospital, Little Rock, AR.

2 Supported in part by National Institutes of Health (grant DK 36823) and the March of Dimes (grant 6-FY98-0640).

022-3166/99 $3.00 © 1999 American Society for Nutritional Sciences.
times the dietary biotin intake. Blood and urine collections were made twice weekly. At the end of the study, the subjects were discharged and received a biotin supplement.

To measure the specific concentrations of biotin and biotin metabolites, the HPLC/avidin binding assay was used for quantitation against authentic standards as described in the preceding symposium paper (Mock 1990, Mock et al. 1995, Zempleni and Mock 1999). Urinary excretion of 3-hydroxyisovaleric acid (3-HIA) was determined by gas chromatography-mass spectrometry as previously reported (Mock et al. 1989).

**Results.** The mean urinary biotin excretion for the group declined from d 0 through d 20 and was significantly different from d 0 by d 3 (Fig. 1, upper panel). Mean urinary biotin excretion fell below the lower limit of normal by d 14. Although the urinary excretion of biotin declined from the baseline value at d 0 in every subject, biotin did not decrease to less than the lower limit of normal in two subjects.

As depicted in the middle panel of Figure 1, the mean excretion of bisnorbiotin (BNB, the principal biotin metabolite) also decreased. On d 14, BNB excretion was less than the lower limit of normal in 8 of 10 subjects; on d 20, BNB excretion was less than the lower limit of normal in six subjects.

Did biotin deficiency progress to the point of interfering with intermediary metabolism because of reduced biotin-dependent enzyme activity? We measured the urinary excretion of 3-HIA, an organic acid that appears in urine as a by-product of the build-up of 3-methylcrotonyl-CoA, secondary to deficient activity of the biotin-dependent enzyme β-methylcrotonyl-CoA carboxylase (EC 6.4.1.4). Mean excretion of 3-HIA increased to above the upper limit of normal by d 7 and reached more than twice the upper limit of normal by d 20 (Fig. 1, lower panel). However, in one subject, 3-HIA was still not consistently increased from d 14 to 20. In this subject, biotin excretion was not consistently below the lower limit of normal late in the study, suggesting that 3 wk of egg-white feeding was not enough to induce biotin deficiency in this individual.

**Figure 2** provides data to address a longstanding controversy in biotin nutrition: what is the value of serum biotin concentration in assessing biotin status? As indicated in Figure 2 (upper panel), the mean serum biotin concentration did not decrease significantly (P < 0.2 by ANOVA). Serum biotin concentrations did show a decrease over the 20 d for four subjects (lower panel); even for these subjects, only three of the four concentrations were less than the lower limit of normal by d 20. These data indicate that serum biotin is not a particularly good indicator of early or marginal biotin deficiency, but increased urinary 3-HIA and decreased urinary biotin are early and sensitive indicators of marginal biotin deficiency. We speculate that, unlike most of the other water-soluble vitamins, the tissue pools of biotin do not bear a very reliable relationship to fasting plasma concentrations.

**LONGITUDINAL STUDY OF BIOTIN STATUS IN PREGNANCY**

Because symptomatic biotin deficiency has never been reported during human gestation, relatively few studies have focused on the risks of biotin deficiency during gestation (Baker et al. 1975, Bhagavan 1969, Dostalova 1984). However, degrees of biotin deficiency that produce no obvious physical findings in pregnant animals are teratogenic in several species (Balnave 1977, Watanabe and Endo 1990 and 1991, Watanabe 1993, Watanabe et al. 1995, Whitehead 1978).

Concern about fetal and maternal health effects of biotin deficiency led to studies of biotin status during human gestation. Some of these studies detected decreased plasma concentrations of biotin during pregnancy (Bhagavan 1969, Dostalova 1984); others did not (Baker et al. 1975). As presented above, the plasma concentration of biotin is probably not an
early or sensitive indicator of biotin status (Mock and Heird 1997). Thus, the conflicting results of previous studies should not be surprising. In this study, we sought to determine whether biotin status decreases during normal human gestation as judged by urinary and serum biotin and by urinary 3-HIA as well as to assess whether pregnancy accelerates biotin biotransformation into the inactive metabolite BNB.

We studied 13 women early in pregnancy (median gestation time of 10 wk) and again late in pregnancy (median gestation time of 36 wk). Twelve healthy women of approximately the same age who were not pregnant and who were not receiving oral contraceptives served as controls.

![Figure 3](upper panel) depicts the mean biotin excretion rates for the control group and for the study group during early and late pregnancy. There was no significant difference in biotin excretion between early pregnancy and the controls. In late pregnancy, biotin excretion was significantly decreased whether compared with control or early pregnancy. The same data are provided in Figure 3 (lower panel) but are depicted with each point representing biotin excretion by an individual subject; a line connects the early and late excretion rates for the same woman. Biotin excretion decreased in 10 of the 13 women, \( P < 0.012 \) by paired \( t \) test. By late pregnancy, biotin excretion rates for 6 of the 13 women had decreased to less than the lower limit of normal. These results suggest that biotin status decreased during gestation and was frankly abnormal in about half of women by late pregnancy.

To assess whether the decrease in urinary excretion of biotin was associated with a depletion of biotin at the tissue level and decreased biotin-dependent enzyme activity, we measured the urinary excretion of 3-HIA. As depicted in Figure 4 (upper panel), mean excretion of 3-HIA was significantly greater than mean excretion of controls in both early and late pregnancy and was not significantly different between early and late pregnancy (lower panel). In both early and late pregnancy, 3-HIA excretion rates were greater than the upper limit of normal in >8 of the 13 women. These findings provide further evidence that a substantial proportion of normal women become at least marginally biotin deficient during early pregnancy and remain so late in pregnancy.

In early pregnancy, BNB excretion was significantly greater than controls, (Fig. 5, upper panel). By late pregnancy, BNB excretion had decreased significantly compared with early pregnancy (\( P < 0.02 \)) and was not significantly different from normal range.
From early to late pregnancy, BNB excretion decreased substantially in only four women, decreased modestly for six women, and remained unchanged or increased for four women (Fig. 5, lower panel). By late pregnancy, BNB excretion remained normal in 11 women. Thus, BNB excretion persisted at normal rates late in pregnancy despite the expectation that BNB excretion should have been down-regulated as a result of biotin deficiency per se.

Our study of biotin deficiency induced in normal subjects indicated that the BNB to biotin excretion ratio remained constant despite progressive biotin deficiency. Thus, the ratio may be considered to reflect the rate of biotransformation normalized for the down-regulating effects of biotin deficiency.

We analyzed BNB to biotin ratios in a subgroup of six women who exhibited abnormally reduced biotin excretion in late pregnancy (i.e., the most biotin-deficient subjects) in an attempt to assess whether biotin deficiency late in pregnancy correlated with accelerated biotransformation early in pregnancy. Bisnorbiotin excretion was accelerated in early pregnancy in these six subjects, and the ratio of BNB to biotin was increased in late pregnancy compared with controls despite...
substantially reduced excretion of both BNB and biotin. We speculate that the accelerated biotransformation persists in late pregnancy despite the effects of biotin deficiency and that the increased loss of active vitamin (as well as accrual of late pregnancy despite the effects of biotin deficiency and that speculate that the accelerated biotransformation persists in excretion did not decrease after a placebo.

Lower panel

urinary excretion of 3-HIA.

Upper panel

(3-HIA) excretion in pregnant women who had abnormally increased pregnancy, but the deficiency is not yet reflected in reduced pregnancy. In all eight of the treated subjects, the 3-HIA excretion decreased; in six of eight, 3-HIA excretion normalized completely. Neither of the two placebo subjects showed a decrease in 3-HIA excretion. Rather, the 3-HIA excretion showed only a modest upward trend. (Both of the placebo subjects were studied early in pregnancy.) These data provide strong, though preliminary, evidence that increased 3-HIA excretion does indicate impaired biotin status in pregnancy. These placebo findings suggest that during the 2 wk of treatment, while pregnancy is progressing, there is no predominant time-dependent decrease of 3-HIA excretion that could confound interpretation of the response of the treated subjects.

FIGURE 6 Biotin treatment decreased 3-hydroxyisovaleric acid (3-HIA) excretion in pregnant women who had abnormally increased urinary excretion of 3-HIA. Upper panel: urinary excretion of 3-HIA decreased after biotin supplementation. Lower panel: urinary 3-HIA excretion did not decrease after a placebo.

ONCING BIOTIN INTERVENTION STUDY IN PREGNANCY

Because many teratogenic events occur at critical times in the first trimester of pregnancy, we were particularly interested in assessing biotin status in early pregnancy. As noted above, excretion of 3-HIA was significantly increased in early pregnancy, providing evidence of reduced biotin status. However, the urinary excretion of biotin was normal, suggesting adequate biotin status early in pregnancy. The apparent conflict might cause an increase in 3-HIA excretion that is not related to biotin status.

Study design. To test these alternate hypotheses, we treated women who had increased 3-HIA excretion with biotin and observed the change in 3-HIA excretion. We propose that, if the women were indeed biotin deficient, 3-HIA excretion should decrease or even completely normalize with biotin treatment. However, if 3-HIA excretion remained unchanged, despite biotin treatment, this would be taken as evidence that the women were not deficient; instead we would infer that the women were biotin sufficient and the increased 3-HIA excretion was the result of a nonspecific effect of pregnancy.

In either early or late pregnancy, women were screened for high 3-HIA excretion by using an untimed sample urine. If the 3-HIA excretion in the screening sample was increased, a 24-h urine was collected and the subjects randomized to biotin treatment or placebo. At the end of 2 wk of treatment, a 24-h urine was collected. Both samples were analyzed for 3-HIA.

Shown in Figure 6 are the data from the eight treated subjects and two placebo subjects. Four of the eight treated subjects were studied early in pregnancy and four late in pregnancy. In all eight of the treated subjects, the 3-HIA excretion decreased; in six of eight, 3-HIA excretion normalized completely. Neither of the two placebo subjects showed a decrease in 3-HIA excretion. Rather, the 3-HIA excretion showed only a modest upward trend. (Both of the placebo subjects were studied early in pregnancy.) These data provide strong, though preliminary, evidence that increased 3-HIA excretion does indicate impaired biotin status in pregnancy. These placebo findings suggest that during the 2 wk of treatment, while pregnancy is progressing, there is no predominant time-dependent decrease of 3-HIA excretion that could confound interpretation of the response of the treated subjects.

LITERATURE CITED

