ABSTRACT This study assessed biotin nutritional status longitudinally during pregnancy as judged by urinary excretion of biotin and biotin metabolites and by serum concentration of biotin. 3-Hydroxyisovaleric acid excretion was also assessed because increased excretion of that acid reflects decreased tissue activity of the biotin-dependent enzyme, methylcrotonyl-CoA carboxylase. Thirteen women provided untimed urine samples during both early and late pregnancy. Twelve nonpregnant women served as controls. Biotin and metabolites were determined by a combined HPLC/avidin-binding assay. 3-Hydroxyisovaleric acid was determined by gas chromatography/mass spectrophotometry. Significance of changes from early to late pregnancy was tested by paired t test; to compare nonpregnant controls with early and late pregnancy, ANOVA was used. During early pregnancy, biotin excretion was not significantly different than controls; however, 3-hydroxyisovaleric acid excretion was significantly increased relative to controls \((P < 0.0001)\) and was greater than the upper limit of normal in 9 of 13 women. From early to late pregnancy, biotin excretion decreased in 10 of 13 women \((P < 0.01)\); by late pregnancy, biotin excretion was less than normal in six women. During late pregnancy, 3-hydroxyisovaleric acid remained significantly increased relative to controls \((P < 0.0001)\). Serum concentrations of biotin were significantly greater than those of controls during early pregnancy \((P < 0.0001)\) and decreased in each woman from early to late pregnancy \((P < 0.0001)\). These data provide evidence that biotin status decreases during pregnancy. J. Nutr. 127: 710±716, 1997.

KEY WORDS: • humans • pregnancy • avitaminosis • biotin • 3-hydroxyisovaleric acid

Biotin is a water-soluble vitamin generally classified with the B complex. Biotin was discovered initially in experiments that demonstrated the presence in many foodstuffs of a watersoluble factor capable of curing the scaly dermatitis, hair loss and neurologic signs induced in rats fed dried egg white as the sole source of protein. A similar dermatitis, alopecia, and similar neurological findings are characteristic of human biotin deficiency. Because symptomatic biotin deficiency has never been reported during human gestation, relatively little effort has been focused on the risks of biotin deficiency during pregnancy. However, degrees of biotin deficiency that produce no obvious physical findings in pregnant animals are teratogenic in several species. 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). However, the plasma concentration of biotin is probably not an early or sensitive indicator of biotin status (Mock et al. 1997). In the study reported here, we sought to determine whether biotin status is decreased during normal human gestation as judged by the serum concentration of biotin and by indices that may more accurately reflect biotin status. These are the urinary excretion of biotin and 3-hydroxyisovaleric acid (3-HIA), an organic acid excreted in increased quantities in response to decreased activity of the biotin-dependent enzyme, methylcrotonyl-CoA carboxylase. To assess whether pregnancy accelerates biotin biotransformation into inactive metabolites, the two principal metabolites, bisnorbiotin (BNB) and biotin sulfoxide (BSO), were measured as well.

SUBJECTS AND METHODS

Subjects. The study protocol was reviewed and approved by the University of Iowa Committee on Research Involving Human Subjects.

Sixteen healthy women, 24–34 y of age, with a history of normal current pregnancy completed the study. Women consuming a vitamin supplement containing biotin were excluded prospectively. Women consuming a diet containing any food substantially fortified with biotin, such as certain breakfast cereals, were also excluded prospectively.
BIOTIN STATUS LONGITUDINALLY DURING PREGNANCY

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weight cut-off Spectra/Por membrane (Spectrum, Houston, TX) while rotating at 30 rpm. After 3 h, the contents on each side of the membrane were removed by syringe aspiration. The radioactivity in the saline and the serum was quantitated by liquid scintillation. The percentage of reversibly bound biotin was calculated as the difference of the concentration of total (free plus bound) radioactivity in serum and the concentration of free biotin in saline over the total. A complete description of the technique is provided in the Spectrument manual.

Urinary concentrations of 3-HIA were determined by gas chromatography/mass spectrophotometry as described previously (Mock and Mock 1992). To obtain high precision and accuracy, authentic unlabeled 3-HIA and deuterated 3-HIA were used as external and internal standards, respectively (Mock and Mock 1992).

Urinary concentrations of creatinine were determined by the picric acid method of Jaffe (O’Brien et al. 1968). Excretion rates were estimated by calculating the concentration ratios of biotin, BNB, BSO and 3-HIA to creatinine.

RESULTS

Figure 1A depicts the 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, but excretion rates in 4 of the 13 pregnant women were greater than the upper limit of normal. In late pregnancy, biotin excretion was significantly decreased whether compared with control (P < 0.033) or early pregnancy (P < 0.003).

The same data are shown in Figure 1B with each point representing biotin excretion by an individual pregnant woman; a line connects the early and late excretion rates for the same woman. Biotin excretion decreased in 10 of the 13 women; biotin excretion did not change or increased in 3. This decrease in urinary biotin was significant by paired t test (P = 0.012). By late pregnancy, biotin excretion rates for 6 of the 13 women decreased to less than the lower limit of normal.

Figure 2A depicts the serum concentrations of biotin for the control women and for the pregnant women. During early pregnancy, the mean serum concentration of biotin was significantly greater than that of the control group (P < 0.0001). By late pregnancy, the mean serum concentration of biotin was significantly less than in early pregnancy (P < 0.0001) and was not significantly different from that of the control group. The serum concentration of biotin decreased in each pregnant woman and was less than the lower limit of normal in 2 of the 13 women (Fig. 2B); the decrease was significant by paired t test (P < 0.0001).

To test whether the increase in the serum concentration of biotin during early pregnancy could be attributed to an increase in reversibly bound biotin, we measured the proportion of biotin free and reversibly bound to serum macromolecules (presumably proteins) using 1H-biotin and equilibrium dialysis. Mean biotin binding was 9.1 ± 37 wk. pregnancy were tested by paired Student’s
t

Experimental design. Subjects were enrolled at an early prenatal
visit; blood and untreated urine samples were obtained on two occasions, once in early pregnancy and once in late pregnancy. For the early samples, the median duration of gestation was 10 wk as calculated from the last normal menstrual period; at the time of the early sample, duration of gestation ranged from 6 to 16 wk. Individuals were followed throughout pregnancy by personal contact on at least two occasions to reinforce the admonition to avoid biotin supplements. For the late samples, the median duration of gestation was 36 wk, with range of 27 – 42 wk.

Chemicals and reagents. Biotin, biocytin (Nε-biotinyl-L-lysine) and triluoroacetic acid were purchased from Sigma (St. Louis, MO). Acetonitrile and water were HPLC grade (Fisher, Itasca, IL). T>8.9-
H-Biotin (DuPont, New England Nuclear, specific activity 1.65 TBq/mmol) and t-carbonyl-13C-biotin (Amersham, Arlington, IL, specific activity 2.11 GBq/mmol) were used to synthesize 3H-biotin sulfoxide and 13C-bisnorbiotin as previously reported (Chastain et al. 1985, Mock et al 1992). Purity was at least 95% for all radiolabeled compounds used in this study, determined by HPLC as described previously (Mock et al 1992 and 1995).

Sample preparation and analysis. Particulate-free urine was obtained by centrifugation at 1000 × g for 10 min; the supernatant was stored at –70°C. Samples were thawed at 37°C for 30 min for use. Biotin and biotin metabolites were determined by a combination of HPLC and avidin-binding assay as described previously (Mock 1997, Mock and Horowitz 1990; Mock et al. 1993). This combined assay provides sensitive, chemically specific determinations of both (unbound) biotin, BNB, and BSO (Mock et al. 1993). The chromato-

graphic separation precludes interference between biotin and its metabolites in the avidin-binding assay. The sequential, solid-phase avidin-binding assay is sensitive to 10 pmol/L or 0.1 mL of fluid containing at least 1 fmol and is not affected by millimolar quantities of certain amino acids and lipoic acid derivatives with avidin-binding properties (Zempleni et al. 1996). Chromatographic fractions were initially assayed in quadruplicate for avidin-binding activity, as previ-

ously described, using avidin-linked horseradish peroxidase as the reporter (Mock et al. 1995). After identification of each peak by retention time and the avidin-binding assay, total metabolite in each peak was determined by reassembly of individual chromatographic fractions against a standard curve constructed from the pure authentic metabolite. Metabolite specific standard curves are required because the avidin-binding affinity of biotin and its metabolites varies.

For the studies of endogenous biotin metabolites, collection of 0.25- to 1.0 mL fractions does not consistently separate the d- and l-isomers of BSO. These two isomers will simply be referred to as BSO hereafter.

Biotin binding. In serum, the proportion of biotin free and reversibly bound to macromolecules (presumably proteins) can be determined with tracer 1H-biotin and ultrafiltration as previously described (Mock and Lankford 1990). We have determined that separation of free biotin by equilibrium dialysis instead of ultrafiltration is equally valid (unpublished). Briefly, a tracer amount of 1H-biotin was added to serum and allowed to incubate at room temperature for 30 min. Serum (1.0 mL) was dialyzed against PBS (1.0 mL) using a Spectrum rapid equilibrium device (Laguna Hills, CA). The 1H-biotin was al-

typically. Approximately midway through pregnancy (range, wk 15–

29), untreated urine samples were provided by all participants. If the urinary excretion of biotin was substantially greater than the upper limit of a normal reference population, this was taken as evidence of failure to comply with our request to avoid biotin supplementation. Such values were identified in 3 of the 16 participating women, and these individuals were excluded from the study. The reference population was composed of 46 normal men and women combined from several studies including the 12 controls in this study (Mock and Heird 1997, Mock et al. 1993 and 1997).

Controls. Twelve healthy women, 23–43 y of age, who were not pregnant, served as controls. The subjects provided blood and untreated urine samples. None were taking any medications including oral contraceptive medications or biotin supplements, as noted above. Controls were not selected for or characterized by phase of the menstrual cycle. Data from a different study are provided in the table for comparison.

Statistical analysis. All statistical analyses and graphics were done with StatView 4.5 (1996) for Macintosh. For box whisker plots, the central tendency is depicted as the median, the box borders are depicted as the 75th and 25th percentiles, and the whiskers (error bars) depict the 5th and 95th percentiles; outliers are depicted as individual points. Population distributions of values were roughly normal; conclusions concerning significance were not changed by log transformations of the data. Significance of changes from early to late pregnancy were tested by paired Student’s t test or Wilcoxon’s Signed Rank test depending on whether distributions were approximately normal (biotin, BSO and 3-HIA) or not (BNB). To compare non-pregnant controls to early and late pregnancy, ANOVA with post-

hoc testing using Fisher’s multiple range test was used.

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We investigated whether biotransformation of biotin into BNB was significantly altered in early pregnancy. We investigated further whether increased biotransformation of biotin into BNB would persist in late pregnancy despite biotin deficiency. For each of the 13 pregnant subjects, we calculated the urinary excretion ratio of BNB to biotin in early and late pregnancy. We calculated the same excretion ratio for the 12 control subjects in this study. Dramatic differences were not apparent (Table 1); ANOVA revealed no significant differences.

We further sought to determine if this index of biotin catabolism would have any predictive value for the subgroup of six women for whom biotin excretion decreased to abnormal values in late pregnancy (Fig. 5). Decreased biotin status in late pregnancy did not appear to be the consequence of reduced biotin status in early pregnancy, at least as judged by the observation that urinary excretion of biotin was normal in early pregnancy (panel A). As expected, the decrease from early to late pregnancy remained significant ($P < 0.0013$). In contrast to the complete group of 13 subjects, BNB excretion in early pregnancy for the subgroup of six subjects was significantly greater than control (panel B); the decrease in BNB excretion from early to late pregnancy remained significant for the subgroup. Of particular note, the BNB to biotin excretion ratio was significantly increased in late pregnancy, suggesting that these women were not able to down-regulate biotin break-

**FIGURE 1** Urinary excretion of biotin in 13 pregnant women in early and late pregnancy and 12 control women. (A) Shown are box whisker plots of population distributions. The central tendency is depicted as the median, the box borders are depicted as the 75th and 25th percentiles, and the whiskers (error bars) depict the 5th and 95th percentiles; outliers are depicted as individual points. a = b; b $\neq$ c at $P < 0.003$; a $\neq$ c at $P < 0.033$. (B) Individual values from the same subject are connected by a line. Open symbols denote individuals whose values decreased. NR = normal range. Differences between means were significant at $P < 0.012$ by paired $t$ test.

study group in early pregnancy and 9.0 $\pm$ 1.4% in the same five women in late pregnancy. There were no significant differences among these groups.

**FIGURE 3A** depicts 3-HIA excretion rates for control, early and late pregnancy. Excretion of 3-HIA was significantly greater than controls in both early and late pregnancy ($P < 0.0001$) and was not significantly different between early and late pregnancy. In both early and late pregnancy, 3-HIA excretion rates were greater than the upper limit of normal in 9 of the 13 women (Fig. 3B).

In early pregnancy, BNB excretion was significantly greater than controls ($P < 0.008$, Fig. 4A). By late pregnancy, BNB excretion had decreased significantly compared with early pregnancy ($P < 0.02$) and was not significantly different than that of controls. From early to late pregnancy, BNB excretion decreased substantially for four women, decreased modestly for six women and remained unchanged or increased for four women (Fig. 4B). By late pregnancy, BNB excretion was less than the lower limit of normal in two women.

Previous studies in healthy, nonpregnant adults indicated that the urinary excretions of biotin and BNB decrease roughly in parallel during progressive biotin deficiency (Mock et al. 1997). This finding suggests that biotin biotransformation is down-regulated roughly in the same proportion as up-regulation of renal biotin reabsorption. We investigated whether biotransformation of biotin into BNB was significantly altered in early pregnancy. We investigated further whether increased biotransformation of biotin into BNB would persist in late pregnancy despite biotin deficiency. For each of the 13 pregnant subjects, we calculated the urinary excretion ratio of BNB to biotin in early and late pregnancy. We calculated the same excretion ratio for the 12 control subjects in this study. Dramatic differences were not apparent (Table 1); ANOVA revealed no significant differences.

We further sought to determine if this index of biotin catabolism would have any predictive value for the subgroup of six women for whom biotin excretion decreased to abnormal values in late pregnancy (Fig. 5). Decreased biotin status in late pregnancy did not appear to be the consequence of reduced biotin status in early pregnancy, at least as judged by the observation that urinary excretion of biotin was normal in early pregnancy (panel A). As expected, the decrease from early to late pregnancy remained significant ($P < 0.0013$). In contrast to the complete group of 13 subjects, BNB excretion in early pregnancy for the subgroup of six subjects was significantly greater than control (panel B); the decrease in BNB excretion from early to late pregnancy remained significant for the subgroup. Of particular note, the BNB to biotin excretion ratio was significantly increased in late pregnancy, suggesting that these women were not able to down-regulate biotin break-

**FIGURE 2** Serum concentration of biotin in 12 control women and 13 pregnant women in early and late pregnancy. (A) Symbols and analysis as per Figure 1A, a $\neq$ b at $P < 0.0001$. (B) Symbols and abbreviations as per Figure 1B. Serum concentration of biotin decreased in every woman from early to late pregnancy. Differences between means were significant at $P < 0.0001$. 
the pregnant dams showed no specific signs of biotin deficiency and gained 87% of the weight of freely feeding biotin-sufficient pregnant controls, the rate of fetal malformation was 94%. Multiple malformations and severe malformations were common; 85% had micrognathia, 85% had cleft palate, and 82% had micromelia.

Concern about the health effects of biotin deficiency led to three reports of biotin status during pregnancy. One study used the \textit{Lactobacillus plantarum} bioassay; nine samples were obtained from four women during the course of pregnancy (4 from one woman, 2 from two others, and 1 from one woman). Mean blood concentrations of biotin were about half of normal concentrations, and the difference was statistically significant. Concentrations of samples taken sequentially from the same subject decreased (Bhagavan 1969). A second study used the \textit{Ochromonas danica} bioassay to determine biotin concentrations in plasma obtained early in labor from 174 women; no values were less than the lower limit of normal (Baker et al. 1975). The third study used the \textit{L. plantarum} bioassay to determine biotin concentrations in plasma obtained from 76 women in the first stage of labor; 16% of the values were less than the lower limit of the normal range (Dostalova 1984).

Results of these studies should be interpreted in the light of more recent observations concerning biotin in serum and plasma. In a study of three children who were profoundly biotin deficient as a result of short gut syndrome and parenteral nutrition without biotin supplementation, plasma concentra-

**FIGURE 3** Urinary excretion of 3-hydroxyisovaleric acid (3-HIA) in 12 control women and 13 pregnant women in early and late pregnancy. (A) Symbols and analysis as per Figure 1A. \(a \neq b\) at \(P < 0.0001\). (B) Symbols and abbreviations as per Figure 1B. Urinary excretion of 3-HIA was increased above the upper limit of normal for 9 of 13 women in early pregnancy and 8 of 13 women in late pregnancy. The two groups were not different by paired \(t\) test.

Biotin sulfoxide excretion rates were not significantly different between the control group and subjects in early or late pregnancy. Only 2 of 13 pregnant women excreted less than the lower limit of normal BSO early in pregnancy and 1 of 13 in late pregnancy (data not shown).

**DISCUSSION**

In some species of rodents (Watanabe 1993, Watanabe and Endo 1989b, 1990 and 1991, Watanabe et al. 1995) and fowl (Balnave 1977, Whitehead 1978), egg-white feeding during pregnancy caused an increase in fetal malformations and mortality. Although questioned at one point (Heard and Blevins 1989), subsequent studies (Watanabe and Endo 1989a and 1990, Watanabe et al. 1995) in mice and tissue culture of fetal mouse cells provided evidence that the teratogenic effects of the egg-white diet were caused by biotin deficiency. Because overt biotin deficiency has never been reported during human gestation, the risk of human teratogenesis caused by biotin deficiency could be low or nonexistent. However, in some animal species, fetal malformations can be caused by degrees of biotin deficiency too mild to cause any specific findings in the pregnant animal (Watanabe 1993, Watanabe and Endo 1989b, 1990 and 1991). For example, Watanabe induced biotin deficiency in ICR mice by egg-white feeding (Watanabe and Endo 1989b, 1990 and 1991, Watanabe 1993). Although

**FIGURE 4** Urinary excretion of bisnorbiotin (BNB) in 12 control women and 13 pregnant women in early and late pregnancy. (A) Symbols and analysis as per Figure 1A. \(a \neq b\) at \(P < 0.008\); \(b \neq c\) at \(P < 0.02\); \(a \neq c\). (B) Symbols and abbreviations as per Figure 1B. Mean urinary excretion of BNB was significantly greater in early pregnancy than in late pregnancy (\(P < 0.011\), Wilcoxon Signed Rank test).
tions of biotin measured by the *O. danica* bioassay were normal in two of the three, but urinary biotin was strikingly less than normal in all three. This observation suggests that plasma concentrations of biotin determined by the *O. danica* assay might not reflect biotin status in some circumstances. Moreover, we recently showed that BSO accounts for ~15% of the molar total of biotin, BNB and BSO in human serum (Mock et al. 1995). The *L. plantarum* organism grows as well on the d-isomer of BSO as on biotin. Thus, some lack of chemical specificity might be expected from the *L. plantarum* bioassay. Finally, in a study of marginal biotin deficiency induced experimentally in adult volunteers (Mock et al. 1997), the serum concentration of biotin was not an early or sensitive indicator of decreased biotin status. The mean serum concentration of biotin did not decrease significantly, although concentrations decreased to values less than the lower limit of normal in 5 of 10 subjects by d 20 of feeding. Given the lack of chemical specificity of bioassays, the absence of validation of plasma biotin concentrations as an indicator of biotin status, and the conflicting results of previous studies, biotin status during normal human gestation remains unknown.

Several studies of biotin-deficient patients have indicated that increased urinary excretion of 3-HIA can indicate reduced tissue activity of methylcrotonyl-CoA carboxylase. This has been the case whether the deficiency was related to total parenteral nutrition, egg-white feeding or inborn errors of biotin metabolism. In the rat model of biotin deficiency, the expected decrease in hepatic methylcrotonyl-CoA carboxylase activity was directly demonstrated (Mock 1990). In human studies, tissue deficits were demonstrated in carboxylase activities of lymphocytes (Velazquez et al. 1986) and inferred from carboxylase activities in cultured fibroblasts (Wolf 1995). The study of marginal biotin deficiency cited above provided evidence that increased urinary excretion of 3-HIA was an early and sensitive indicator of decreased biotin status (Mock et al. 1997). Decreased urinary excretion of biotin was also an early and sensitive indicator of decreased biotin status.

On this basis, the urinary excretions of biotin and 3-HIA were chosen for assessment of biotin status in a longitudinal study of healthy pregnant women. In addition, to relate our findings to previous studies and to assess biotin metabolism, the serum concentration of biotin and the urinary excretion of BNB and BSO were also measured. The study presented here provides evidence that biotin status decreases from early to late pregnancy. The most striking changes were decreased biotin excretion, decreased BNB excretion, and decreased serum concentration of biotin. The excretion of 3-HIA was already greater than that of controls in early pregnancy and did not further increase in late pregnancy.

The study described here provides some evidence that biotin status was abnormal in late pregnancy in some women. In about half of the women, biotin excretion was less than the lower limit of normal; in about three fourths of the women, 3-HIA excretion was increased to greater than the upper limit of normal. We speculate that the increased 3-HIA excretion reflected biotin depletion at the tissue level, at least in some of these women. The observation that the serum concentration of biotin was less than normal for only a few women is not an argument against decreased biotin status; we observed a decrease in the serum concentration of biotin to obviously abnormal values in only about half of subjects by d 20 of feeding an egg-white diet (Mock et al. 1997). Rather, the observed relative decrease of serum biotin concentration further strengthens the conclusion that biotin status is reduced in some women in late pregnancy.

In serum, biotin exists in free, reversibly bound and covalently bound forms. Because only free biotin is detected by the method described here, one might question whether a shift in the equilibrium between free and reversibly bound biotin could be the explanation for the striking decrease in serum concentration of biotin from early to late pregnancy. Because reversibly bound biotin accounts for only 8% of the total (Mock and Lankford 1990), this is not a likely explanation. Notwithstanding, we empirically determined that the percentage of bound and free biotin in early pregnancy and late pregnancy did not change. Assuming as we have before that 3H-biotin is an appropriate tracer for endogenous biotin, we conclude that the observed differences in the concentrations of free biotin are not explained by changes in the distribution of biotin between the free and reversibly bound forms.

In normal adults, covalently bound biotin accounts for ~12% of total serum biotin (Mock and Malik 1992). Any change in covalently bound biotin would not be detected by the method used in this study. Because the bioassays used in previous studies detect the total of free + reversibly bound + covalently bound biotin (Mock 1989), differences in results theoretically could arise from covalently bound biotin.

Many teratogenic events occur at critical times in the first trimester of pregnancy. Accordingly, we were particularly interested in assessing biotin status in early pregnancy. Excretion

<table>
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<th>Range</th>
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<td>Late pregnancy</td>
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<td>1.3</td>
<td>0.21–4.3</td>
</tr>
</tbody>
</table>

1 Ten normal adults in a study of experimental biotin deficiency (Mock et al. 1997).
2 Experimental biotin deficiency subjects before initiation of diet.
3 Experimental biotin deficiency subjects after 20 d of egg-white feeding.

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**TABLE 1**

Comparison of the urinary excretion ratios of bisnorbiotin to biotin in early and late pregnancy to the control group and to a group in a study of experimental biotin deficiency.
of 3-HIA is significantly increased, suggesting reduced biotin status. However, the serum concentration of biotin is significantly increased and urinary excretion of biotin is normal, suggesting adequate biotin status. The apparent conflict can be resolved by two alternate hypotheses. First, some degree of compromise of biotin status occurs in early pregnancy but is not yet reflected in biotin excretion. For example, some factor might reduce renal reabsorption of biotin. The observed increased excretion of BNB could also result from such a factor.

Eventually, this biotin wasting should reduce plasma concentrations of biotin and filtered load of biotin, and finally, renal excretion of biotin. However, how quickly this might evolve during pregnancy is not clear. Moreover, the relationship between the serum concentration of biotin and total body pools of biotin is not known. Even if this hypothesis and explanation are correct, the mechanism(s) leading to increased serum concentrations of biotin are not clear.

Alternatively, we can hypothesize that the increase in 3-HIA excretion does not reflect biotin deficiency at the tissue level at all. Rather, we could propose that a factor (or factors) associated with gestation (e.g., increased protein synthesis) increases organic acid metabolism or impairs renal reabsorption of organic acids, causing an increase in 3-HIA excretion that has nothing to do with biotin status. The observed normal to increased excretion of biotin and increased serum concentration of biotin in early pregnancy are consistent with this second hypothesis. If this second alternative is correct, a decrease of both the urinary excretion of biotin and the serum concentration of biotin into the normal range might be expected as a consequence of the simple avoidance of biotin supplementation and fortification. These trends were observed. However, this second alternative does not explain the observed decrease of biotin excretion to obviously abnormal values in more than half of the subjects by late pregnancy. In summary, at this point, the data do not permit a firm conclusion either way concerning biotin status in early pregnancy.

The design of this study did not specifically address the mechanism(s) by which biotin status decreases during pregnancy. We speculate that an accelerated rate of biotin biotransformation into the inactive metabolite BNB, renal wasting of biotin, or accretion of biotin by the fetus/products of conception (or some combination of these factors) contributed to decreased maternal biotin status. In the subgroup of six women with abnormally reduced biotin excretion in late pregnancy, BNB to biotin ratios were increased in late pregnancy despite substantially reduced excretion of both BNB and biotin. Our study of biotin deficiency induced in normal subjects indicated that the BNB to biotin excretion ratio reflects the rate of biotransformation, when normalized for the effects of biotin deficiency. If the BNB to biotin excretion ratio also reflects biotransformation in pregnancy when normalized for deficiency, the data from this study indicate the following: 1) biotransformation of biotin to BNB is accelerated in early pregnancy, and 2) the accelerated biotransformation persists in late pregnancy despite the effects of deficiency (Fig. 5C).

Unfortunately, currently available information does not permit a comparison of the magnitude of urinary excretion of biotin and metabolites with biotin that is available to the woman. The metabolic requirement for biotin is not known. Even if this hypothesis and explanation are correct, the mechanism(s) leading to increased serum concentrations of biotin are not clear. The design of this study did not specifically address the mechanism(s) by which biotin status decreases during pregnancy. We speculate that an accelerated rate of biotin biotransformation into the inactive metabolite BNB, renal wasting of biotin, or accretion of biotin by the fetus/products of conception (or some combination of these factors) contributed to decreased maternal biotin status. In the subgroup of six women with abnormally reduced biotin excretion in late pregnancy, BNB to biotin ratios were increased in late pregnancy despite substantially reduced excretion of both BNB and biotin. Our study of biotin deficiency induced in normal subjects indicated that the BNB to biotin excretion ratio reflects the rate of biotransformation, when normalized for the effects of biotin deficiency. If the BNB to biotin excretion ratio also reflects biotransformation in pregnancy when normalized for deficiency, the data from this study indicate the following: 1) biotransformation of biotin to BNB is accelerated in early pregnancy, and 2) the accelerated biotransformation persists in late pregnancy despite the effects of deficiency (Fig. 5C).

In conclusion, this study provides evidence that biotin status decreases in normal pregnancy. On the basis of increased 3-HIA excretion and decreased biotin excretion, about half of a small group of pregnant women appeared to become at least marginally biotin deficient late in pregnancy. The potential mechanisms for this disturbance in biotin nutrition included an increased biotin biotransformation to inactive catabolites, reduced renal retention of biotin or fetal accretion of biotin. The diagnosis of biotin deficiency could be confirmed by a biotin intervention study in which pregnant women with...
increased 3-HIA are provided oral supplements of biotin and 3-HIA response is assessed.

LITERATURE CITED


