Marginal biotin deficiency during normal pregnancy1–3

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ABSTRACT

Background: Biotin deficiency is teratogenic in several mammalian species. Approximately 50% of pregnant women have an abnormally increased urinary excretion of 3-hydroxyisovaleric acid (3-HIA), which probably reflects decreased activity of the biotin-dependent enzyme methylcrotonyl-CoA carboxylase. However, increased 3-HIA excretion could result from pregnancy per se (eg, from an effect of pregnancy on renal handling of organic acids).

Objective: We tested the hypothesis that biotin supplementation significantly decreases 3-HIA excretion in pregnant women with abnormally increased 3-HIA excretion.

Design: Twenty-six pregnant women with increased 3-HIA excretion were studied in a randomized, placebo-controlled trial; 10 women were studied during early pregnancy (6–17 wk gestation) and 16 women during late pregnancy (21–37 wk gestation). Urine samples were collected before and after 14 d of supplementation with 300 μg (1.2 μmol) biotin/d or placebo.

Results: In the early-pregnancy group, 3-HIA excretion decreased (P < 0.006) by 11.7 ± 3.6 mmol/mol creatinine (mean ± SEM) in the 5 women who received biotin supplements, whereas 3-HIA excretion increased by 1.6 ± 0.6 mmol/mol creatinine in the 5 women who received placebo. In the late-pregnancy group, 3-HIA excretion decreased (P < 0.002) by 7.1 ± 1.2 mmol/mol creatinine in the 8 women who received biotin supplements, whereas 3-HIA excretion increased by 0.9 ± 1.8 mmol/mol creatinine in the 8 women who received placebo.

Conclusions: This study provides evidence that the increased excretion of 3-HIA seen frequently in normal pregnancy reflects reduced biotin status. The conclusion that marginal biotin deficiency occurs frequently in the first trimester further raises concern about potential human teratogenicity. Am J Clin Nutr 2002;75:295–9.

KEY WORDS Pregnancy, teratogenicity, biotin deficiency, 3-hydroxyisovaleric acid, women

INTRODUCTION

As reviewed recently, biotin deficiency is a potent teratogen in some animal species, and the resulting malformations are similar to certain human birth defects (1). We have speculated that biotin deficiency may also be teratogenic in humans (1).

The findings of 2 clinical studies (2, 3) suggest that mild degrees of biotin deficiency are common in normal pregnancy. Urinary excretion of 3-hydroxyisovaleric acid (3-HIA) increases as a result of the decreased activity of the biotin-dependent enzyme methylcrotonyl-CoA carboxylase. Methylcrotonyl-CoA carboxylase catalyzes an essential step in the degradation of the branch-chained amino acid leucine. An increased urinary excretion of 3-HIA is an early and sensitive indicator of reduced biotin status (4). Combined data from 2 studies indicate that 3-HIA excretion was abnormally increased in 26 of 29 women studied early in pregnancy and in 21 of 26 women studied late in pregnancy (2, 3). However, urinary excretion of biotin was normal in most women early in pregnancy and decreased to abnormal values late in pregnancy in only a few women. Thus, these 2 indicators of biotin status conflict. The increased 3-HIA excretion may not have reflected impaired biotin status but instead may have been the result of pregnancy itself. For example, the increased 3-HIA excretion might have resulted from an effect of pregnancy on the renal handling of organic acids. Alternatively, pregnancy may have increased protein turnover or otherwise altered amino acid catabolism, causing an increased influx of intermediates through the leucine catabolic pathway that thereby increased 3-HIA excretion. This study tested the hypothesis that abnormal 3-HIA excretion reflects biotin deficiency and, accordingly, that biotin supplementation causes a decrease in 3-HIA excretion. The change in urinary excretion of 3-HIA was the primary outcome variable.

SUBJECTS AND METHODS

This study was approved by the Human Research Advisory Committee of the University of Arkansas for Medical Sciences.

Pilot study

To assess whether supplementation with 300 μg (1.2 μmol) biotin/d for 14 d would substantially increase biotin excretion and decrease 3-HIA excretion, 2 pregnant women in their third

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trimester of pregnancy were tested in a pilot study with the same design as that of the randomized control trial. Both women had abnormally increased 3-HIA excretion (36 and 19 mmol/mol creatinine, respectively; normal range: 5.1–10.7 mmol/mol creatinine). In addition, both women had normal biotin excretion (6.0 and 3.2 μmol/mol creatinine; normal range: 2.1–8.0 μmol/mol creatinine). These subjects did not participate in the randomized control trial, and their data are not included in the results. Their data are consistent with the central hypothesis of the study and also provided evidence that the dose and duration of biotin would be sufficient to substantially alter biotin excretion (and presumably biotin status).

Randomized control trial

Subjects
Twenty-six healthy pregnant women with a median age of 30 y (range: 20–39 y) were enrolled in the study. Subjects were excluded from the study if they were taking a vitamin or supplement containing biotin or if previous children were not normal at birth. Subjects were included in the study only if they were under the care of a physician, were receiving a maternal vitamin that provided recommended vitamin intakes, and had an abnormally increased urinary excretion of 3-HIA, ie, a ratio of 3-HIA to creatinine >10.7 mmol/mol creatinine in an untimed urine sample (the screening sample). Potential study subjects provided written, informed consent before the screening sample was collected. Subjects were studied in either early or late pregnancy. The median duration of gestation was 13.5 wk (range: 6–17 wk) in the early-pregnancy group and 30.5 wk (range: 21–37 wk) in the late-pregnancy group. To assess the effect of biotin supplementation on 3-HIA excretion in the absence of pregnancy, we studied 5 healthy nonpregnant women (control group) with a median age of 40 y (range: 31–43 y). None of the women were using oral contraceptives. The phase of the menstrual cycle was not assessed.

Experimental design
Subjects were randomly assigned to receive either a biotin supplement or a placebo with the use of a random-number-generator program that generated a balanced stratification between the supplement and placebo subgroups in both the early- and late-pregnancy groups.

Urinary excretion of biotin, 3-HIA, and creatinine
Complete 24-h urine samples were collected on the day before initiating the biotin supplement or placebo as previously described (4). After completing the first urine collection, the women ingested 1 capsule/d for 14 d. The capsules contained either 300 μg (1.2 μmol) biotin (supplement subgroups) or an equal volume of powdered lactose (placebo subgroups). The placebo was visually indistinguishable from the biotin capsule. The women were blinded as to capsule identity. On the last day of supplementation, the women collected a second 24-h urine specimen. The nonpregnant women received the biotin supplement and followed the same collection protocol.

The excretion rates of biotin and 3-HIA were expressed per mole of creatinine. We previously observed that the urinary excretion of 3-HIA and biotin are early and sensitive indicators of biotin status whether normalized by time or by mass of creatinine (4). Therefore, the change in the urinary excretion of biotin was quantitated with the use of an avidin binding assay after HPLC separation of biotin from biotin metabolites as described previously (5) to assess protocol compliance. In addition, 3-HIA was quantitated with deuterated 3-HIA and unlabeled 3-HIA as standards by using gas chromatography–mass spectrometry as described previously (6).

The completeness of the urine collection was determined by calculating the creatinine excretion. Creatinine was measured with the picric acid method (7) by using a Beckman creatinine analyzer (Beckman Instruments, Palo Alto, CA). The creatinine excrections for all timed urine collections were within the sex-specific normal range (8), with a single exception. The creatinine excretion in a pretreatment, timed urine sample from one subject in the placebo subgroup (early-pregnancy group) was 31% less than her posttreatment creatinine excretion, which was normal. This discrepancy suggests an incomplete collection. Because the results are expressed per mole of creatinine, however, we conclude that this incomplete collection should have little effect on the interpretation of the data. Thus, the data for this subject are included in all analyses; exclusion of these data did not change the statistical significance of any analysis.

The normal range for 3-HIA excretion (5.1–10.7 mmol/mol creatinine) was chosen as the 10th–90th percentiles of the values for 21 nonpregnant women. The normal range for biotin excretion (2.1–8.0 μmol/mol creatinine) was chosen as the 10th–90th percentiles of the values for 23 nonpregnant women.

Statistical methods
Significance was set at P < 0.05. The significance of the differences in the mean change in 3-HIA excretion between the supplement and placebo subgroups was tested by using Student’s unpaired t test with a hypothesized difference of 0 and STATVIEW 5.0 (9). The significance of the differences between the supplement and placebo subgroups in 3-HIA excretion and biotin excretion before treatment was tested in a similar fashion.

RESULTS

Pilot study
After biotin supplementation, urinary 3-HIA excretion decreased in both subjects (to 15 and 10 mmol/mol creatinine, respectively); urinary biotin excretion increased substantially in both subjects (to 64 and 38 μmol/mol creatinine, respectively).

Randomized control trial
For both the early-pregnancy and late-pregnancy groups, pretreatment urinary excretion of 3-HIA was not significantly different between the supplement (Figure 1, top) and placebo (Figure 1, bottom) subgroups. Likewise, for both groups, pretreatment urinary excretion of biotin was not significantly different between the supplement (Figure 2, top) and placebo (Figure 2, bottom) subgroups.

The urinary excretion of 3-HIA decreased in every woman who was treated with biotin (Figure 1). In the early-pregnancy group, 3-HIA excretion decreased (P < 0.006) by 11.7 ± 3.6 mmol/mol creatinine (mean ± SEM) in the 5 women who received biotin supplements, whereas 3-HIA excretion increased by 1.6 ± 0.6 mmol/mol creatinine in the 5 women who received placebo. In the late-pregnancy group, 3-HIA excretion decreased (P < 0.002) by 7.1 ± 1.2 mmol/mol creatinine in the 8 women who received biotin supplements, whereas 3-HIA excretion increased by
0.9 ± 1.8 mmol/mol creatinine in the 5 women who received placebo. In both the early- and late-pregnancy groups, the decrease in 3-HIA excretion in the supplement subgroups was significantly different from zero. In both the early- and late-pregnancy groups, the change in 3-HIA excretion in the placebo subgroups was not significantly different from zero. However, for the placebo subgroups studied early in pregnancy, the increase in 3-HIA excretion was nearly significant \( (P < 0.058) \), consistent with a gradual decrease in biotin status during the 14 d of the study.

The effect of biotin supplementation on the urinary excretion of biotin is shown in Figure 2. By the last day of supplementation, biotin excretion in the supplement subgroups had increased substantially (mean increase: >10-fold) and significantly \( (P < 0.0001) \). The smallest increase for a subject receiving biotin supplementation was 70%. Biotin excretion did not change significantly in subjects receiving placebo in either early or late pregnancy.

The 3-HIA excretion in the 5 nonpregnant control women decreased substantially (Figure 3). However, the magnitude of the decrease was 50% of that observed in the pregnant women who received biotin supplementation. The difference in the mean decrease in 3-HIA excretion was significantly different between the pregnant and the nonpregnant women.
nonpregnant women (P < 0.05, Mann-Whitney U test). Note that the biotin excretion of one control woman was above the normal range before supplementation; the 3-HIA excretion of that subject before supplementation was the lowest of the 5 control women, and the change in 3-HIA excretion was the smallest. These observations suggest that her biotin intake may have been modestly greater than that of the other nonpregnant control women.

DISCUSSION

This study provides evidence that biotin supplementation decreased 3-HIA excretion to normal values in most pregnant women with an increased urinary excretion of 3-HIA. This result is consistent with our hypothesis that an increased urinary excretion of 3-HIA reflects marginal biotin deficiency. Because previous studies reported that >50% of the pregnant women studied had abnormally increased 3-HIA excretion (2, 3), we interpret the results of the present study as indicating that marginal biotin deficiency is common during normal human gestation. However, other interpretations of our results are possible. One could argue that pregnancy causes increased flux through the leucine catabolic pathway, resulting in a relative deficiency of methylcrotonyl-CoA carboxylase activity; accordingly, biotin supplementation may reduce 3-HIA excretion by increasing the expression or specific activity of methylcrotonyl-CoA carboxylase above normal levels. Note that methylcrotonyl-CoA carboxylase covalently binds one molecule of biotin via the usual amide bond to the epsilon amino group of a specific lysyl residue. Methylcrotonyl-CoA carboxylase also binds a second molecule of biotin noncovalently; both molecules of biotin are catalytically competent (10). This feature is unique to methylcrotonyl-CoA carboxylase among the 5 mammalian biotin-dependent carboxylases.

Maternal and fetal vitamin status in general and biotin status in particular have been areas of interest and concern for many decades. Some early studies of biotin status in pregnancy detected significantly lower biotin concentrations in the plasma of pregnant women (11, 12) than in that of nonpregnant control women; others did not (13). A striking decrease from early to late pregnancy was reported in the plasma concentration of biotin, but plasma biotin decreased to frankly abnormal concentrations in only a few women (2). The differences in the results between the studies may have arisen from methodologic differences in biotin assays (1, 14). Moreover, the plasma concentration of biotin is not a very early or sensitive indicator of marginal biotin deficiency (4).

Although frank, symptomatic biotin deficiency has never been documented in normal human gestation, biotin deficiency that is too mild to cause specific signs in pregnant animals is a potent teratogen in some mammalian species (1, 15, 16). Thus, the absence of symptoms of biotin deficiency during normal human gestation does not exclude the possibility that biotin deficiency may cause human birth defects. In mice, biotin deficiency causes malformations at severities of metabolic disturbance similar to those frequently observed in normal pregnancy. For example, the rates of cleft palate and limb shortening were 100% in mice rendered mildly biotin deficient by the feeding of egg whites (16); at the appropriate critical period of embryogenesis, the 3-HIA excretion had increased only ≈60% compared with biotin-sufficient controls (DM Mock, NI Mock, C Stewart, J LaBorde, and D Hansen, unpublished observations, 2001). The relative increases in 3-HIA excretion observed in human gestation are greater; 2 clinical studies have detected 2–3-fold increases in the first trimester (2, 3).

The decrease in mean 3-HIA excretion observed in the supplement subgroups but not in the placebo subgroups cannot be ascribed to differences in biotin status before supplementation (ie, failure of randomization). As judged both by 3-HIA excretion and by biotin excretion, the biotin statuses of the supplement and placebo subgroups were equal before treatment. This was true for both the early- and late-pregnancy groups.

One inclusion criterion was abnormally increased 3-HIA excretion in a screening urine sample. At least 2 wk was required for the measurement of 3-HIA and creatinine concentrations in the screening sample; study enrollment and scheduling further delayed collection of the 24-h pretreatment urine sample. For this...
sample, 3-HIA excretion had returned to a normal value in 4 subjects. The cause of this change in 3-HIA excretion is not clear. Inadvertent or deliberate supplementation with biotin during the time interval between screening and initiation of the presupplementation urine collection may have been the cause, particularly in view of the extensive discussion of vitamin nutrition and birth defects provided as part of the initial screening and informed consent process. However, the biotin excretion of these 4 subjects was not significantly greater than that of the other study subjects. One subject was in the placebo subgroup studied early in pregnancy, 1 subject was in the supplement subgroup studied late in pregnancy, and 2 subjects were in the placebo subgroup studied late in pregnancy. If biotin supplementation did occur, inclusion of the subjects in the placebo subgroup could theoretically bias our results in favor of accepting the null hypothesis. Alternatively, inclusion of the subject in the supplement subgroup could theoretically bias our results in favor of rejecting the null hypothesis. However, statistical analysis of the data excluding these 4 subjects did not appreciably change any of the statistical results. The final analyses cited in the Abstract and Results include data from all subjects in the randomized trial.

We previously observed that supplementation with biotin in amounts substantially greater than those consumed in a mixed general diet (50–100 μg/d, or 0.2–0.4 μmol/d) reduces the urinary excretion of 3-HIA in normal subjects (17). We observed a similar reduction in the control women who were not pregnant. This reduction suggests that methylcrotonyl-CoA carboxylase can be rate limiting in the leucine degradation pathway, even in persons with normal biotin status. However, both the increased absolute 3-HIA excretion and the greater relative decrease in 3-HIA excretion seen in the pregnant women who received biotin supplementation are consistent with the hypothesis that the pregnant women studied were indeed biotin deficient.

Of particular interest is the observation that the urinary excretion of 3-HIA did not decrease to the normal range in 3 of the 13 pregnant women who received biotin supplementation; yet, their biotin excretion rates in the same postsupplement urine samples were all substantially greater than normal. One interpretation of this finding is that biotin status was not restored to normal by the dose and duration of biotin supplementation used in this study. Accordingly, this would imply that an increased urinary excretion of biotin is not a reliable indicator of biotin status during biotin repletion. In children with mild biotin deficiency caused by long-term anticonvulsant therapy, we observed persistent abnormal excretion of 3-HIA despite biotin supplementation that increased urinary biotin to normal or greater than normal values (18). An alternative interpretation is that urinary excretion of biotin is paradoxically increased in pregnancy; this interpretation is consistent with previous observations concerning the conflict between these 2 indicators of biotin status (3).

Although the findings of the present study are unambiguous, they do not justify widespread biotin supplementation during pregnancy. Additional steps in the causal link between biotin deficiency and deleterious effects on the fetus or mother must be established. If possible, the presence of marginal biotin deficiency should be confirmed by a complementary, validated index of biotin status that does not depend on either maternal renal function or on methylcrotonyl-CoA carboxylase activity. Furthermore, a link between biotin deficiency and deleterious effects on the fetus or mother must be directly established. Finally, the therapeutic efficacy of biotin supplementation at an appropriate dose and at an appropriate time with respect to conception should be demonstrated in a randomized, controlled trial.

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REFERENCES