Lymphocyte propionyl-CoA carboxylase and its activation by biotin are sensitive indicators of marginal biotin deficiency in humans\textsuperscript{1–3}

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ABSTRACT

Background: Marginal biotin deficiency may be a human teratogen. A biotin status indicator that is not dependent on renal function may be useful in studies of biotin status during pregnancy. A previous study of experimental biotin deficiency suggested that propionyl-coenzyme A carboxylase (PCC) activity in peripheral blood lymphocytes (PBLs) is a sensitive indicator of biotin status.

Objective: We examined the utility of measuring PCC activity and the activation of PCC by biotin in detecting marginal biotin deficiency.

Design: Marginal biotin deficiency was induced in 7 adults (3 women) by egg-white feeding for 28 d. Blood and urine were obtained on days 0, 14, and 28 (depletion phase) and 44 and 65 (repletion phase). PBLs were incubated with (activated) or without (control) biotin before PCC assay. The activation coefficient of PCC is the ratio of PCC activity in activated PBLs to that in control PBLs. The significance of differences for all measurements was tested by repeated-measures analysis of variance with Fisher’s post hoc test and Bonferroni correction.

Results: Changes in the urinary excretion of biotin and of 3-hydroxyisovaleric acid confirmed that marginal biotin deficiency was successfully induced. By day 14, PCC activity had decreased ($P < 0.0001$) to below the lower limit of normal in all subjects. By day 28, the activation coefficient of PCC had increased significantly ($P = 0.003$) and was above the upper limit of normal in 6 of 7 subjects.

Conclusion: PCC activity is the most sensitive indicator of biotin status tested to date. In future pregnancy studies, the use of lymphocyte PCC activity data should prove valuable in the assessment of biotin status. Am J Clin Nutr 2006;84:384–8.

KEY WORDS Biotin, biotin deficiency, humans, lymphocytes, propionyl-CoA carboxylase, activation coefficient

INTRODUCTION

Clinical studies from our group and others have shown that marginal biotin deficiency is common in certain circumstances, including pregnancy and long-term anticonvulsant therapy (1–4). Because maternal marginal biotin deficiency is clearly teratogenic in several animal species (5), recent reviews expressed concern that maternal marginal biotin deficiency may also be teratogenic in humans (5). Even more recently, we reported evidence of marginal biotin deficiency in adult female smokers (6), an observation that expands on teratogenic concerns and also raises the possibility that smoking and marginal biotin deficiency may have synergistic deleterious effects on the developing fetus. However, concerns about teratogenesis related to biotin deficiency must be evaluated in light of the following caveat: most of the current conclusions about biotin status in pregnancy are based on urinary indicators of biotin status (2, 3), and renal function is affected by pregnancy per se. Accordingly, an indicator of biotin status that is largely independent of renal function would be useful, particularly in testing conclusions from previous studies.

We recently published an initial evaluation of a blood-based indicator of biotin status (7). That study of 11 subjects who were made biotin-deficient by egg-white feeding found that propionyl-coenzyme A carboxylase (PCC) activity in peripheral blood lymphocytes (PBLs) is an early and sensitive indicator of marginal biotin deficiency (7). In the current study, we sought to confirm or refute those findings in a new cohort of subjects and to examine the specificity of the PCC activity findings in those subjects. We also sought to test the validity of an additional blood-based indicator of biotin status—the biotin activation coefficient (AC) of PCC in PBLs.

SUBJECTS AND METHODS

Subjects, study design, and diet

Inclusion criteria included good health without a history of renal disease severe enough to be likely to lead to chronic impairment of renal function. Exclusion criteria included smoking and consumption of either dietary supplements known to contain biotin or certain medications (eg, certain anticonvulsants known to accelerate biotin catabolism; 4).

Eight healthy adult volunteers (3 women) completed the study. One man admitted that he did not comply with the study protocol; his data are excluded. The 7 remaining subjects ranged...
in age from 19 to 43 y. Their body mass index (BMI; in kg/m²) ranged from 22.2 to 28.4. Their racial-ethnic distribution was white (n = 3), Asian (n = 2), Hispanic (n = 1), and African American (n = 1).

Written informed consent was obtained from all subjects. The study was approved by the institutional review board of the University of Arkansas for Medical Sciences (UAMS).

Details of the experimental induction of biotin deficiency were published previously (8). In the current study, to normalize biotin status at the start of the depletion phase (day 0), subjects completed a biotin-loading and -washout phase before the initiation of egg-white feeding. Subjects were housed as inpatients in the UAMS General Clinical Research Center (GCRC) from day 0 to day 28 and were provided a dietitian-monitored eucaloric diet. Subjects consumed an egg-white drink with each meal. As described in a previous report from this study (9), the egg white contained sufficient avidin to bind 7 times the subjects’ controlled daily dietary intake of biotin (9). Energy intake, biotin intake, and egg-white drink consumption were monitored daily by the GCRC research dietitian. On day 28, subjects were discharged from the GCRC, and thereafter they consumed a self-selected mixed general diet. During this repletion phase, the biotin status of the subjects was monitored for 38 d.

Despite the rigorous control of the GCRC environment, one subject admitted to noncompliance with the dietary regimen; this subject both consumed foods obtained from outside the GCRC and failed to consume most of the egg-white beverage as instructed. As expected, this subject’s urinary excretion of biotin and 3-hydroxyisovaleric acid (3HIA) confirmed that marginal biotin deficiency was not induced. That subject’s data were excluded from statistical analyses.

Blood and urine sample collection

Blood was collected in syringes (to which a stock solution of heparin was added to result in a concentration of 15 U heparin/mL whole blood to prevent coagulation) on days 0, 14, and 28 during the depletion phase of the study and on days 44 and 65 during the repletion phase. PBLs were separated from whole blood by density gradient centrifugation and stored as previously described (7). Urine collections were obtained for the 24-h period just before blood collections and processed as described previously (7).

Urinary biotin and 3-hydroxyisovaleric acid

Urinary biotin was quantitated first by HPLC separation and then by an avidin-binding assay performed as described previously (10). Urinary 3HIA was quantitated by gas chromatography–mass spectroscopy as described previously (9).

Measurement of lymphocyte propionyl-CoA carboxylase activity and the activation coefficient of propionyl-CoA carboxylase

The 14CO2 incorporation assay for measurement of PCC activity in PBLs was completed in triplicate for each blood sample as described previously (7, 11). Activity was normalized by protein concentration of the PBL preparation as determined by the AC of PCC, the suspended PBLs, prepared as described previously (7). For the AC of PCC, the suspended PBLs, prepared as described above, were incubated at 25 °C for 30 min with (activated) or without (control) 10 nmol biotin/L before the protein and PCC assays. ACs were the ratio of PCC of activated PBLs to PCC of control PBLs.

Statistical analysis

For definitions of normal ranges for urinary biotin, 3HIA, and PCC activity in PBLs, the upper and lower limits were chosen as the 10th and 90th percentiles, because the distributions were not normal. For urinary excretion of biotin, the normal range was based on 54 subjects from several previous studies and included the day 0 values of the 3 egg white–feeding studies (2, 3, 6, 9, 12–15). For urinary excretion of 3HIA, the normal range was based on 68 subjects and included the day 0 values from the same studies (2, 3, 6, 9, 15). For PCC activity, the normal range was based on 18 subjects and included the day 0 values in the current study and the same values from 1 previous egg white–feeding study (7). For the AC of PCC, the normal range was based on the day 0 values in the 7 subjects in the current study.

The significance of changes in urinary biotin, urinary 3HIA, PCC activity, and the AC of PCC during the depletion phase (day 0 to day 28) was tested by one-way analysis of variance (ANOVA) with repeated measures. The significance of changes in each variable during the repletion phase (day 28 to day 65) was tested by one-way ANOVA with repeated measures. When ANOVA significance was P < 0.05, Fisher’s post hoc test was used to ascertain the significance of differences between time points. The P values given are the least significant of the Fisher’s comparison P values after Bonferroni correction for multiple comparisons. For all analyses, STATVIEW software (version 5.01; SAS Institute, Cary, NC) was used.

RESULTS

Induction of marginal biotin deficiency

Urinary excretion rates for biotin and for 3HIA were previously validated as indicators of marginal biotin deficiency (9, 15). Increased excretion of 3HIA reflects decreased hepatic activity of the biotin-dependent enzyme methylcrotonyl-CoA carboxylase (16–20). As intended, none of the overt signs or symptoms of biotin deficiency developed in these subjects during the study. However, on the basis of urinary biotin and 3HIA, marginal biotin deficiency was successfully induced in these 7 subjects (Figure 1). By day 28, urinary biotin excretion had decreased significantly (P = 0.0002) and was below the lower limit of normal in all 7 subjects (Figure 1A). With repletion by consumption of a mixed general diet, biotin excretion rates increased significantly from day 28 to day 65 (P = 0.011) and were above the lower limit of normal in 3 of 7 subjects. By day 28, urinary 3HIA excretion had increased significantly (P = 0.0003) and was above the upper limit of normal in 6 of 7 subjects (Figure 1B). With repletion, 3HIA excretion decreased significantly from day 28 to day 65 (P < 0.0001) and was normal in 5 of 7 subjects.

PCC activity

In the depletion phase of the study, the activity of PCC in PBLs decreased progressively in each compliant subject (Figure 2). By day 14, PCC activity was below the lower limit of normal for all subjects, and mean PCC activity had decreased to 46% of the mean day 0 value (P < 0.0002). By day 28, mean PCC activity had decreased to only 23% of day 0 activity (P < 0.0002). The
diagnostic sensitivity was 100% on both days 14 and 28. PCC activities slowly but significantly recovered with repletion (P < 0.0001). After 15 d of repletion, the PCC activity in only 1 of 7 subjects had returned to the normal range (P = 0.07), but, after 36 d of repletion, the PCC activity in 5 of 7 subjects had returned to the normal range (P < 0.0002).

Activation coefficient of propionyl-CoA carboxylase

The AC of PCC (Figure 3) did not increase significantly (P = 0.003) until day 28. By day 28, the AC of PCC in 6 of 7 subjects had increased to above the upper limit of normal, and thus the diagnostic sensitivity was 86%. After 12 d of repletion, the AC of PCC had decreased significantly (P < 0.002) and was within the normal range in all 7 subjects. The AC of PCC remained normal for the duration of the repletion phase (P < 0.002).

DISCUSSION

In the current study, we observed a highly significant decrease in the PCC activity in the PBLs of healthy adults who were experimentally made marginally biotin-deficient by being fed a diet high in egg white. The findings from this study confirm the high diagnostic sensitivity of PCC activity in PBLs that was observed in a previous study (7). Moreover, we found that the AC of PCC in PBLs is also a sensitive indicator of marginal biotin deficiency in humans. However, PCC activity decreased more quickly than AC of PCC activity increased. The diagnostic sensitivity of PCC activity was 100% on day 14, and that of AC of PCC was only 14%. This observation suggests that PCC activity is an earlier marker of biotin depletion than is AC of PCC. Consistent with this interpretation is the observation that PCC activity returned to the normal range more slowly than did the AC.
of PCC; after 14 d of repletion, 86% of AC of PCC values were within the normal range, but none of the PCC activity values had returned to normal. On the basis of this analysis, we conclude that the AC of PCC activity as measured here is not as sensitive an indicator of marginal biotin deficiency as is PCC activity.

However, confidence in this conclusion is limited by our arbitrary choice of conditions for incubation of the PBLs with biotin. The generally accepted mechanism for activation is the intracellular accumulation of apoenzyme during deficiency. The apoenzymes are thought to be activated when the deficient vitamin is provided in vitro. For this study, we propose that apopropionyl-CoA carboxylase α chains accumulate in PBLs and are biotinylated by holocarboxylase synthetase when biotin is transported into the PBLs during in vitro incubation with biotin. The observed activation also implies that adequate amounts of apopropionyl-CoA carboxylase β chain and holocarboxylase synthetase are present intracellularly. We did not conduct optimization experiments for time, temperature, or extracellular concentration of biotin because the PBLs available under our institutional review board limitations were insufficient. Furthermore, Baez-Saldana et al (21) recently reported the effects of in vivo biotin supplementation on carboxylase activities. Persons with and without type 2 diabetes who were not biotin deficient were supplemented with 5 mg biotin 3 times/d for 28 d. Activities of the biotin-dependent enzymes PCC, pyruvate carboxylase, and acetyl-CoA carboxylase in PBLs increased ∼3-fold. This observation indicates that the activity of biotin-dependent carboxylases could increase substantially more with chronic in vivo supplementation than we observed in the current study. Accordingly, there is a distinct possibility that the transport into the lymphocytes was not optimal in restoring biotin status and that sufficient time and temperature were not allowed for maximal carboxylation of the accumulated apopropionyl-CoA carboxylase. With an optimized in vitro biotin incubation, we speculate that the AC of PCC may have increased sooner or to a greater extent. Nevertheless, the incubation of concentration for biotin was 20 times the physiologic concentration, and the absolute magnitude of the AC of PCC activity we observed were similar to the ACs observed by others for other vitamins (22), which suggests that adequate biotin was transported and incorporated.

Comparison of the PBL PCC data with 3HIA excretion data provides preliminary evidence that PBL PCC activity is a better indicator of marginal biotin deficiency than is 3HIA excretion. On the basis of PBL PCC activity, 7 of 7 subjects were marginally deficient by day 14. In contrast, 1 subject never excreted abnormal amounts of 3HIA. This observation is consistent with our previous studies of 3HIA in which marginal biotin deficiency was not induced (data not shown). In those studies (9, 15), ≈10% of subjects did not show an increase in 3HIA excretion. We speculate that the 3HIA pathway for the metabolic disposition of intracellular accumulation of 3-methylcrotonyl-CoA is not active in those particular persons. We also speculate that the direct quantification of PCC activity may prove to be both more sensitive and more specific for detecting marginal biotin deficiency than is urinary excretion of 3HIA.

Available data indicate that the lower expression of the genes for the α and β chains of PCC contributes little if at anything to the dramatic reduction in PCC activity. In a previously published study in these same 7 subjects (8), we observed that the mRNA in PBLs for the α chain of PCC decreased to 75% of the day 0 value by day 28 (P = 0.027), and that for the β chain of PCC decreased to 86% of the day 0 value (P = 0.067). Thus, a decrease in gene expression could explain at most only one-fourth of the decrease in PCC activity.

A related study of biotin deficiency in our pregnant mouse model (23) provides additional data consistent with the proposed mechanisms for a decrease in PCC activity and an increase in AC of PCC. In this study, mouse dams and fetuses were rendered biotin-deficient by egg-white feeding. We found that a decrease in carboxylase activity was mediated by a decrease in the abundance of the biotinylated carboxylases rather than by a decrease in the expression of their mRNA. The abundance of biotinylated carboxylases decreased in both maternal (to ∼48% of control) and fetal (to ∼6% of control) liver in parallel with a decrease in PCC activity. In contrast, mRNA for the α chain of PCC did not change significantly. Moreover, the metabolically less active dam exhibited a smaller decrease than did the metabolically more active fetus.

This study is limited in 2 additional ways. First, the number of persons who successfully completed this rigorous inpatient study is small; thus, extrapolation to larger populations must be cautious. Nevertheless, the results of the current study concerning the utility of PCC activity in PBLs are entirely consistent with the results of a previous study in 11 subjects (7). Second, the normal range for the AC for PCC in PBLs was ascertained in only 7 subjects; no other reports of this index have been published. Although this finding represents a careful initial observation, inferences concerning diagnostic sensitivity must be made with caution.

The in vitro increase in vitamin-dependent enzyme activity with vitamin incubation is frequently used as a measure of the status of several water-soluble vitamins, including thiamine, riboflavin, and vitamins B-6 and B-12 (22, 24–26). These activation coefficients are generally accepted as valid measures of vitamin status (27, 28); however, the functional implications of identified deficiencies remain controversial in most cases. The turnover of biotin in lymphoid tissue, as judged by [14C]biotin degradation (29), is slower than that in liver. Thus, the decrease in PCC activity and the increase in the AC of PCC in lymphocytes may lag behind biotin depletion in more metabolically active tissues such as hepatocytes, and the deleterious effects of deficiency may even precede these early indicators.

Previous studies using urinary excretion of 3HIA as the primary indicator of biotin status indicated that marginal biotin deficiency occurs commonly during normal human gestation. Despite the limitations discussed above, we conclude that the study presented here provides evidence that PCC activity in PBLs will be useful in future studies that attempt to further assess biotin status in normal human gestation. An important subsequent step would be the assessment of the functional implications of the identified biotin deficiency.

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SLS, AB, MMM, and NIM performed various laboratory measurements. AW served as study dietitian. SLS performed statistical analyses, drafted the manuscript, and prepared the figures. DMM served as Principal Investigator and is responsible for the final version of the manuscript, statistics, figures, and figure legends. None of the authors had any personal or financial conflict of interest.
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