MINIREVIEW

Marginal Biotin Deficiency Is Teratogenic

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Abstract. **Recent studies of biotin status during pregnancy provide evidence that a marginal degree of biotin develops in a substantial proportion of women during normal pregnancy. Several lines of evidence suggest that, although the degree of biotin deficiency is not severe enough to produce the classic cutaneous and behavioral manifestations of biotin deficiency, the deficiency is severe enough to produce metabolic derangements in women and that characteristic fetal malformations occur at a high rate in some mammals. Moreover, our analysis of data from a published multivitamin supplementation study provide significant albeit indirect evidence that the marginal degree of biotin deficiency that occurs spontaneously in normal human gestation is teratogenic. Investigation of potential mechanisms provides evidence that biotin transport by the human placenta is weak. Further, proliferating cells accumulate biotin at a rate five times faster than quiescent cells; this observation suggests that there is an increased biotin requirement associated with cell proliferation. Perhaps this requirement arises from the need to synthesize additional biotin-dependent holocarboxylases or provide additional biotin as a substrate for biotinylation of cellular histones. Reduced activity of the biotin-dependent enzymes acetyl-CoA carboxylase and propionyl-CoA carboxylase can cause alterations of lipid metabolism and might theoretically lead to alterations of polyunsaturated fatty acid and prostaglandin metabolism that derange normal skeletal development.** [P.S.E.B.M. 2000, Vol 223]

Maternal and fetal vitamin status in general and biotin status in particular have been areas of interest and concern for many decades. To determine whether biotin deficiency is a significant risk factor in pregnancy, one must assess the severity and prevalence of biotin deficiency in pregnancy. Some of the early studies of biotin status in pregnancy detected significantly decreased concentrations of biotin in maternal plasma (1, 2); others did not (3). Given the demonstration that substantial amounts of biotin catabolites are present in normal plasma (4, 5), it is not surprising that these previous studies using bioassays and avidin-binding assays have not produced uniform results. Moreover, the plasma concentration of biotin is not a very early or sensitive indicator of marginal biotin deficiency (6). Overall, the implications of these studies concerning maternal biotin status are not clear.

Biotin Status in Pregnancy

We assessed maternal biotin status during normal human gestation in two recent studies (7, 8). Four indicators of biotin status were measured: (*i*) urinary excretion of 3-hydroxyisovaleric acid [biotin deficiency causes reduced activity of biotin-dependent β-methylcrotonyl-CoA carboxylase (E.C. 6.4.1.4); the associated metabolic block in leucine metabolism leads to shunting of β-methylcrotonyl-CoA to

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an alternate metabolic pathway, causing an increased urinary excretion of 3-hydroxyisovaleric acid (6)]; (*ii*) urinary excretion of biotin; (*iii*) serum concentration of biotin; and (*iv*) urinary excretion of biotin metabolites (bisnorbiotin, biotin-*d,l*-sulfoxide).

One of these two studies in pregnant women was a longitudinal study (8). Blood and untimed urine samples were collected on two occasions, once in early pregnancy and once in late pregnancy ($n = 13$). For the early and late samples, median durations of gestation were 10 and 36 weeks, respectively. In addition, 12 nonpregnant women who were not receiving oral contraceptives served as controls. The urinary excretion of 3-hydroxyisovaleric acid was significantly increased ($P < 0.0001$) in both early and late pregnancy (Fig. 1). Excretion of 3-hydroxyisovaleric acid was significantly greater than the upper limit of normal in 9 of the 13 women in early and in late pregnancy. For unexplained reasons, the serum concentration of biotin was greater in early pregnancy than in the nonpregnant controls; the serum concentration of biotin decreased significantly in late pregnancy and reached values that were below the lower limit of normal in some individuals (Fig. 2, upper panel). The increased serum concentration of biotin early in pregnancy was not caused by an increase of a biotin binding protein or by a change in the proportion of biotin that is bound to a biotin binding protein (8). The urinary excretions of biotin and its inactive catabolite bisnorbiotin were significantly less in late pregnancy than in early pregnancy; biotin excretion was significantly decreased compared to normal controls (Fig. 2, middle and lower panel). Taken together, these findings are consistent with the hypothesis that biotin status decreases in normal pregnancy. The ratio of urinary bisnorbiotin to biotin was significantly increased both early and late in pregnancy. This observation concerning the ratio is similar to that observed in rats treated with steroid hormones (9). Together these observations in humans and animals are consistent with the hypothesis that biotin catabolism is accelerated as a result of steroid-accelerated β-oxidation. Based on the magnitude of the catabolism, we speculate that accelerated catabolism contributed importantly to reduction of biotin status.

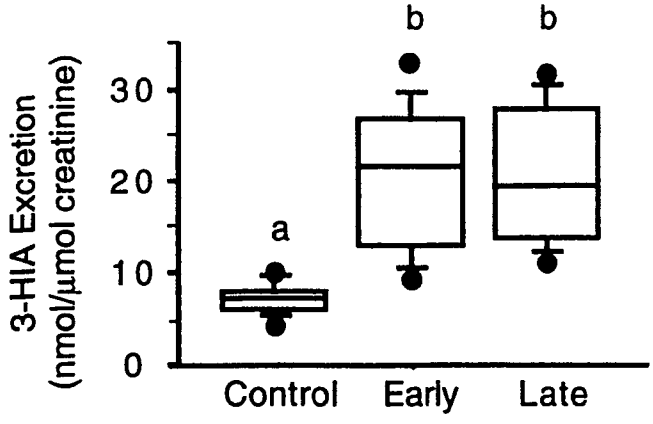

Figure 1. Urinary excretion of 3-hydroxyisovaleric acid (3-HIA) in 13 pregnant women in early and late pregnancy and 12 control women. Shown are box whisker plots of population distributions. The central tendency is depicted as the median, the box borders are depicted as the 75th and 25th percentile, and the whiskers (error bars) depict the 5th and 95th percentile; outliers are depicted as individual points. a ≠ b at $P < 0.0001$. (With permission from (8).)

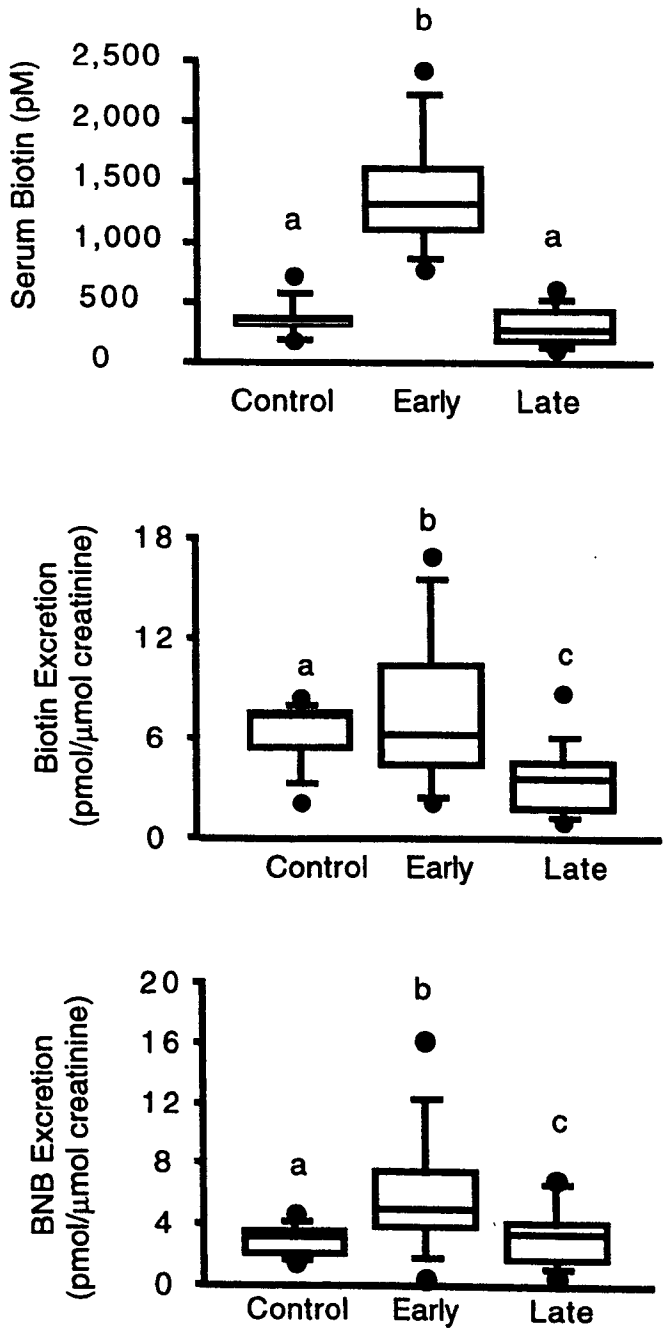

Figure 2. (Upper panel) Serum concentrations of biotin in 13 pregnant women in early and late pregnancy and 12 control women. Shown are box whisker plots of population distributions. Symbols as per Figure 1. a ≠ b at $P < 0.0001$. (Middle panel) Urinary excretion of biotin in 13 pregnant women in early and late pregnancy and 12 control women. a = b; b ≠ c at $P < 0.003$; a ≠ c at $P < 0.033$. (Lower panel) Urinary excretion of bisnorbiotin (BNB) in 13 pregnant women in early and late pregnancy and 12 control women. a ≠ b at $P < 0.008$; b ≠ c at $P < 0.02$; a = c. (With permission from (8).)

Our second study in pregnant women was a cross-sectional study (7). We collected untimed urine samples

either at an early prenatal visit (n = 16) or at a late prenatal visit (n = 13). For the early and late groups, median durations of gestation were 17 and 36 weeks, respectively. In addition, 12 nonpregnant women served as controls. The urinary excretion of 3-hydroxyisovaleric acid was increased in pregnant women in both early and late pregnancy. This finding confirms the findings from the longitudinal study (8) and provides further evidence that biotin status decreases during pregnancy. However, in this cross-sectional study, the urinary excretion of biotin, bisnorbiotin, and biotin-*d,l*-sulfoxide increased during late pregnancy (7). Thus, the data from the cross-sectional and the longitudinal studies partially conflict. It is possible that the apparent conflict arose from inadvertent supplementation in late pregnancy by the cross-sectional study subjects. However, this is not an attractive explanation because the supplementation would have had to occur in a way that produced high biotin excretion into the urine yet allowed preceding excretion of abnormal amounts of 3-hydroxyisovaleric acid into the same bladder accumulation. At a minimum, there would have to have been a time lag in which altered flux in the leucine pathway or relative metabolic deficiency of β-methylcrotonyl-CoA carboxylase at the tissue level persisted despite a normal or increased plasma biotin level.

In an ongoing study, alternate interpretations are being investigated. It remains theoretically possible that the increased excretion of 3-hydroxyisovaleric acid might have arisen from altered kidney function caused by pregnancy *per se* rather than from biotin deficiency. To assess these alternate possibilities, we measured urinary excretion of 3-hydroxyisovaleric acid before and after biotin supplementation in pregnancy. If the women were indeed biotin-deficient, urinary excretion of 3-hydroxyisovaleric acid should decrease with biotin supplementation; no change in 3-hydroxyisovaleric acid excretion would be expected if the increase in 3-hydroxyisovaleric acid arises from an effect of pregnancy *per se*.

Pregnant women were recruited and assigned to one of four groups in a stratified, randomized design: biotin and placebo in early pregnancy (8–18 weeks) and biotin and placebo in late pregnancy (30–35 weeks). Initially, women were screened for reduced biotin status by 3-hydroxyisovaleric acid excretion in an untimed urine. A dietary questionnaire was obtained to screen for intended or inadvertent biotin supplementation. Each participant who did not supplement with biotin and who had increased 3-hydroxyisovaleric acid excretion provided a baseline 24-hr urine collection. Daily for the next two weeks, each participant consumed either a supplement containing 300 μg of biotin or a placebo. Each participant again provided a 24-hr urine collection during the 24 hr starting with the last biotin dose.

Urinary excretion of 3-hydroxyisovaleric acid decreased in the 11 women who received biotin treatment (Fig. 3); the difference in the mean excretion before treatment and after was significant at P = 0.0001 by a paired t test. Urinary 3-hydroxyisovaleric acid did not decrease in

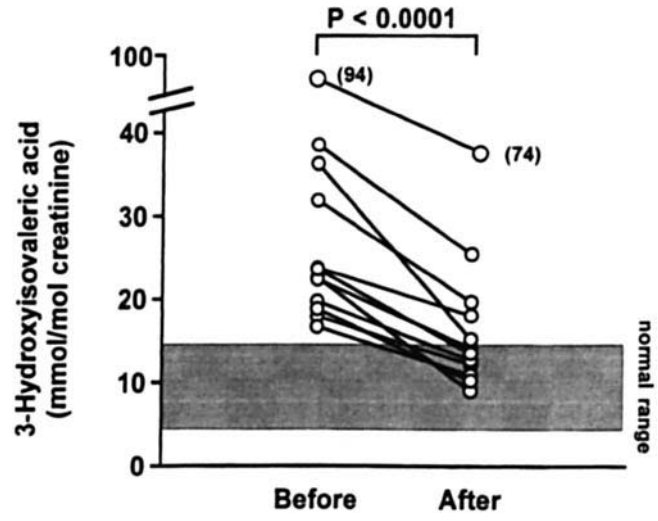

Figure 3. Biotin supplementation of pregnant women causes a decrease of 3-hydroxyisovaleric acid excretion in urine. Excretion of 3-hydroxyisovaleric acid was measured before and after 2 weeks of biotin supplementation (300 μg/day). $P < 0.0001$ by paired t test (n = 11).

the four women who received placebo treatment. These preliminary data provide evidence that pregnant women do become biotin-deficient during pregnancy, and this deficiency can be at least partially reversed by 300 μg of biotin taken orally for 2 weeks.

Compliance with the supplementation protocols was determined by measurement of urinary biotin excretion. Measurement of urinary biotin showed that 10 of the 11 women receiving biotin treatment had increased excretion of biotin relative to baseline values. The one subject whose urinary biotin excretion did not increase did not collect her urine 24 hr after completing the supplementation period (contrary to the protocol). None of the four women receiving placebo showed any evidence of inadvertent biotin supplementation.

Teratogenic Effects of Biotin Deficiency

Biotin deficiency is teratogenic in several animal species at degrees of deficiency that produce no obvious findings in the pregnant animal. Hens with marginal biotin deficiency produce eggs with higher embryonic mortality, reduced hatchability, chronodystrophy ("parrot beak" deformity), perosis (an abnormality of bone tendon formation that results in a deformity similar to "club foot"), micromelia, and syndactyly (10–13). Similar effects on hatchability and viability have been reported in turkey poults (14). In some strains of mice, biotin deficiency during pregnancy causes substantial increases in fetal malformations and mortality (15–21). For example, Watanabe induced biotin deficiency in mice by feeding raw egg white (containing the biotin-binding protein avidin) to render dietary biotin unabsorbable (17–20). Although the pregnant dams showed no specific signs of biotin deficiency and gained 101% of the weight of pregnant controls that had free access to a biotin-sufficient diet, the rate of fetal malformations was high.

Ninety-four percent of all pups were malformed. Multiple malformations were common; 94% had cleft palate, 85% had micrognathia, and 41% had micromelia. These examples of teratogenic effects at marginal degrees of biotin deficiency are of particular concern because our studies suggest that biotin status may be reduced during human pregnancy.

Differences in teratogenic susceptibility among rodent species have been reported, and corresponding differences of biotin concentrations in fetal liver were observed. This led Watanabe and co-workers to propose that differences in teratogenic susceptibility among rodent species are caused by differences in biotin transport from the mother to the fetus (17). Studies from our laboratories (22, 23) and others (24) have provided evidence that the transport of biotin across the human placenta is not rapid and does not generate a substantial fetal-to-maternal gradient, even though a biotin transporter is present in the brush-border membrane. In contrast, a recent study of fetal and maternal plasma concentrations of biotin at 18–24 weeks gestation of normal human pregnancies reported a fetal-to-maternal biotin ratio ranging from 3 to 17:1 (25). However, biotin was measured using an assay that does not distinguish between biotin and inactive biotin catabolites in serum (4), and production of catabolites increases during pregnancy (8). Thus, inadvertent detection of metabolites may have led to an overestimate of transport into the fetus. Overall, the possibility remains that maternal deficiency may translate into fetal deficiency and may predispose to human fetal biotin deficiency.

Our examination of the data from the classic study of Czeizel and Dudás (26) further increases concern that biotin deficiency may be teratogenic in humans. Czeizel and Dudás observed that folic acid supplementation prior to and during pregnancy reduced the incidence of neural tube defects from 6 in the roughly 2000 women of the mineral supplement control group to zero in the roughly 2000 women who received folic acid in a multivitamin supplement. This reduction was significant; $P = 0.02$ by Fisher's test.

The vitamin supplement also included biotin. In analogy with grouping of all neural tube defects based on studies showing that folate deficiency causes a spectrum of neural tube defects in animals, we were guided by our mouse studies to group isolated cleft palate with limb shortening. Multivitamin supplementation reduced the combined incidence of cleft palate and limb shortening from seven to one. This reduction approaches significance by Fisher's test; $P = 0.07$.

Lipid Metabolism in Biotin Deficiency

Abnormal lipid metabolism is a mechanism by which biotin deficiency might cause fetal malformations. In mammals, holocarboxylase synthetase (E.C. 6.3.4.10) catalyzes the covalent binding of biotin to the ε-amino group of lysine in four different apocarboxylases to form the active holocarboxylases (27). Two carboxylases are linked directly to lipid metabolism. (*i*) Acetyl-CoA carboxylase (E.C. 6.4.1.2) is located in the cell cytosol and catalyzes the incorporation of bicarbonate into acetyl-CoA to form malonyl-CoA. This is a key reaction in fatty acid synthesis (elongation). Recently, a mitochondrial form of acetyl-CoA carboxylase (acetyl-CoA carboxylase β) was identified (28, 29). Kim has proposed that acetyl-CoA carboxylase β controls fatty acid catabolism in mitochondria and this effect is mediated through malonyl-CoA, an inhibitor of fatty acid transport into mitochondria (30).

(*ii*) Propionyl-CoA carboxylase (E.C. 6.4.1.3) is located in mitochondria and catalyzes the incorporation of bicarbonate into propionyl-CoA to form methylmalonyl-CoA. This reaction is an essential step in the metabolism of the cholesterol side chain and odd-chain fatty acids. Propionyl-CoA carboxylase also catalyzes steps in the catabolism of isoleucine, valine, methionine, threonine, and organic acids formed by breakdown of dietary carbohydrates by intestinal microorganisms.

Studies in biotin-deficient animals suggest a role for biotin in polyunsaturated lipid metabolism. Although specific findings vary from study to study and among biotin-deficient individuals, some valuable generalizations can be made. Biotin deficiency causes alterations of the fatty acid profile in liver, skin, and serum of several animal species (31–35); fatty acid composition of the brain is less affected by biotin deficiency than the liver is (33). In particular, biotin deficiency causes an increase in the percentage of odd-chain fatty acids (33, 34), suggesting that odd-chain fatty acid accumulation may be a marker for reduced propionyl-CoA carboxylase activity in biotin deficiency. This is consistent with observations that activities of mitochondrial carboxylases in biotin-deficient rats (e.g., propionyl-CoA carboxylase) decreased to <18% of control values (35), whereas cytosolic acetyl-CoA carboxylase decreased only about 50% (35, 36).

With regard to the role of acetyl-CoA carboxylase, supplementation of biotin-deficient rats with corn oil (high in ω6 unsaturated fatty acids) or Intralipid (high in linoleic acid, 18:2ω6) delays the onset or prevents the cutaneous findings of biotin deficiency (37, 38).

Biotin deficiency also causes abnormalities in fatty acid composition in humans. In patients who developed biotin deficiency during parenteral alimentation, the percentage of odd-chain fatty acids (15:0, 17:0) in serum increased for each of the four major lipid classes: cholesterol esters, phospholipids, triglycerides, and free fatty acids (39). However, changes in composition for these four classes of lipids have not always been consistent among various studies (33, 40–42).

Some studies provide evidence that the alterations in fatty acid metabolism caused by biotin deficiency might cause the fetal malformations described in biotin deficiency. Bain *et al.* (43) reported changes in bone growth in biotin-deficient chicks. These investigators have proposed that the teratogenic and postnatal defects in skeletal development

caused by biotin deficiency are related to derangements of polyunsaturated fatty acid metabolism, particularly reduced metaphyseal prostaglandin E_2, a product of arachidonic acid (20:4ω6). Arachidonic acid, its immediate precursor (20:3ω6), and eicosapentaenoic acid (20:3ω3) are all decreased in cortical bone in biotin-deficient chicks (44). These investigators have hypothesized that reduced prostaglandin E_2 causes decreased replication and differentiation of periosteal preosteoblasts. Such effects of prostaglandin E_2 have been reported in culture (45) and in direct injection experiments in growing rats (46).

Mutant *Schizosaccharomyces pombe* that lack acetyl-CoA carboxylase exhibit a defect in nuclear division that causes cell death during mitosis. Only nondividing cells retain viability (47). This finding is consistent with the observation that profound acetyl-CoA carboxylase deficiency has not been reported and the subsequent hypothesis that this biotin-dependent carboxylase is essential to maintain normal proliferation of fetal cells.

Biotin Transport into Proliferating Cells

Fetal development is characterized by phases of rapid cell proliferation and differentiation (48–50). Theoretically, the cellular requirement for biotin might increase during such phases. For example, proliferating cells might need biotin to synthesize new biotin-dependent carboxylases (51) or to biotinylate histones, particularly if the biotinylation state of histones plays a role in regulating transcription and replication of DNA (52, 53).

In recent studies in our laboratory, we sought to determine whether the rate of biotin transport into human cells is affected by cell proliferation. We used human lymphocytes as a model system for the following reasons: 1) Lymphocytes can be isolated in sufficient quantities from human blood without requiring biopsy; 2) lymphocyte viability was at least 96% even during extended periods of incubation (54–57); 3) unlike in immortal human tumor cell lines, chromosomes in freshly isolated lymphocytes are not deranged; and 4) most importantly, proliferation of lymphocytes can be induced easily and reproducibly by incubation with mitogens.

First, we investigated biotin transport in freshly isolated, nonproliferating human lymphocytes. We found that biotin is co-transported with sodium into lymphocytes by a saturable, energy-dependent process (54). The transporter is specific for biotin; lipoic acid, pantothenic acid, bilirubin, and hexanoic acid do not compete for binding (54, 55). In studies using biotin analogs as inhibitors, we inferred that the thiophane portion of the biotin molecule is important for binding to the transporter. In contrast, modifications of the ureido portion or valeric acid side chain of the biotin molecule do not affect the ability of a biotin analog to compete with biotin for binding to the transporter (54), suggesting that this portion of biotin does not interact strongly with the putative biotin-binding site on the transporter.

We infer that the same transporter that mediates biotin uptake into lymphocytes also accounts for biotin efflux out of lymphocytes. Evidence for this conclusion arises from experiments in which biotin efflux was stimulated by increasing concentrations of extracellular biotin and biotin analogs. This appears to be a classic countertransport mechanism (56).

We next investigated the effects of cell proliferation on cellular biotin uptake. In these studies, we induced lymphocyte proliferation by either pokeweed lectin, concanavalin A, or phytohemagglutinin (57). After 3 days of stimulation with mitogens, biotin uptake into proliferating lymphocytes was 300%–700% of uptake into nonproliferating cells. Figure 4 provides typical data for stimulation of biotin by pokeweed lectin; effects of concanavalin A and phytohemagglutinin were similar. These findings are consistent with the hypothesis that cellular accumulation of biotin increases in proliferating human cells.

Theoretically, the increased uptake of biotin into mitogen-stimulated lymphocytes could be mediated by either an increased number of biotin transporters on the cell surface or an increased affinity of transporters for biotin (or both). To examine these two potential mechanisms, we incubated pokeweed-stimulated and unstimulated lymphocytes with [^{3}H]biotin at concentrations that varied from 238–2850 p*M* (57). The maximal transport rates in pokeweed-stimulated lymphocytes and in unstimulated controls were 9126 ± 6641 amoles/(10^6 cells × 30 min) and 2328 ± 1627 amoles/(10^6 cells × 30 min), respectively ($P < 0.05$). In contrast, the Michaelis constants of the transporter were not significantly different in pokeweed-stimulated lymphocytes and unstimulated controls (2.4 ± 1.7 n*M* vs 3.7 ± 3.4 n*M*, respectively). These findings provide evidence that the number of biotin transporters per cell is greater in pokeweed-stimulated lym-

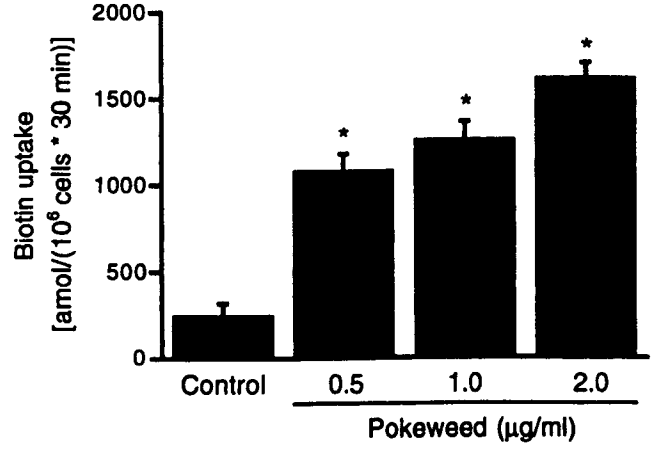

Figure 4. Uptake of [^{3}H]biotin into proliferating human peripheral blood lymphocytes. Proliferation was induced by incubation with pokeweed lectin (0.5, 1.0, or 2.0 µg/ml) at 37°C for 3 days; controls were incubated without pokeweed. Then, lymphocytes were incubated with [^{3}H]biotin at a concentration of 475 p*M* at 37°C for 30 min. After incubation, the lymphocytes were centrifuged and washed three times to remove extracellular [^{3}H]biotin; the lymphocytes were lyzed, and the intracellular [^{3}H]biotin was measured by liquid scintillation counting. Values are means ± SD ($n = 6$). *Significantly different from controls ($P < 0.01$). (With permission from (57).)

phocytes than in controls but the affinities for biotin are similar. Lineweaver-Burk plots of the biotin uptake data for the regression lines of pokeweed-stimulated and unstimulated lymphocytes intersect near the x-axis (Fig. 5), consistent with an increased number of biotin transporters per cell in pokeweed-stimulated lymphocytes.

Next, we sought to determine the time course of stimulation of biotin uptake into proliferating lymphocytes. Also, we sought to determine whether inhibition of protein synthesis by cycloheximide affects stimulation of biotin transport into mitogen-induced lymphocytes (57). In these experiments, we added either pokeweed alone or pokeweed plus cycloheximide to the lymphocyte incubation. At intervals up to 168 hr, rates of [^{3}H]biotin and [^{3}H]thymidine uptake were measured. Stimulation of biotin uptake was maximal 48 hr to 72 hr after addition of pokeweed mitogen (Fig. 6). Of note, the maximal biotin uptake coincided with maximal proliferation as assessed by [^{3}H]thymidine uptake (data not shown). Addition of cycloheximide to the medium completely inhibited the pokeweed-mediated increase of biotin and thymidine uptake. This observation is consistent with our hypothesis that proliferating lymphocytes increase biotin transport by synthesizing new transporters.

Theoretically, it seemed possible that mitogen-stimulated lymphocytes increase biotin accumulation by transcribing a transporter that is different from the biotin-specific transporter in unstimulated lymphocytes, for example, a biotin-lipoic acid-pantothenic acid transporter (58). Thus, we investigated the specificity of the biotin transporter in proliferating lymphocytes (57) and compared the data to our previous studies in nonproliferating lymphocytes (54). The biotin transporters in both proliferating and nonproliferating lymphocytes had similar substrate specificities, suggesting that proliferating lymphocytes transcribe the same biotin transporter as nonproliferating lymphocytes.

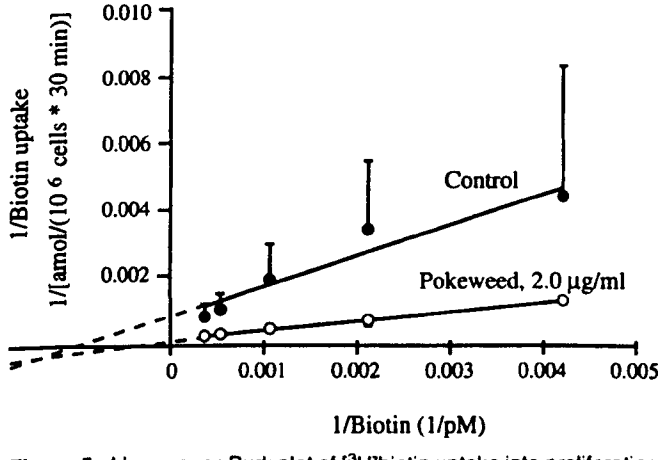

Figure 5. Lineweaver-Burk plot of [^{3}H]biotin uptake into proliferating human peripheral blood lymphocytes and controls. Lymphocytes were incubated with pokeweed mitogen (2.0 µg/ml) at 37°C for 3 days to induce proliferation; controls were incubated without mitogen. Then, lymphocytes were incubated with 238–2850 pM [^{3}H]biotin at 37°C for 30 min. After incubation, the lymphocytes were centrifuged and washed three times to remove extracellular [^{3}H]biotin; the lymphocytes were lyzed, and the intracellular [^{3}H]biotin was measured by liquid scintillation counting (n = 3 subjects; each subject was measured in triplicate). (With permission from (57).)

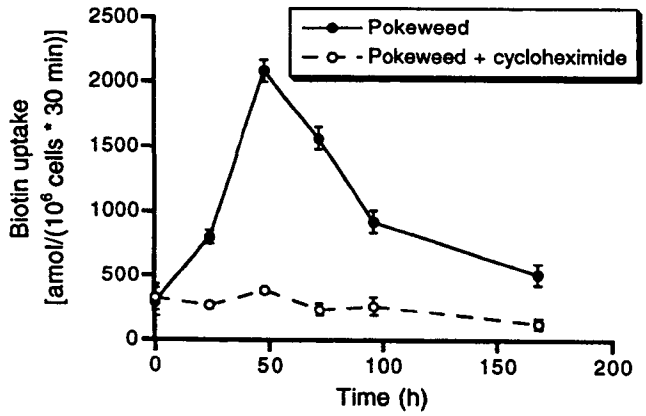

Figure 6. Time course of [^{3}H]biotin uptake into proliferating human lymphocytes. Lymphocytes were incubated at 37°C for up to 168 hr with either pokeweed mitogen alone (2.0 µg/ml) or pokeweed mitogen (2.0 µg/ml) plus cycloheximide (17.8 µM), an inhibitor of protein synthesis. Aliquots of the suspensions were collected at timed intervals during the incubation, and the uptake of [^{3}H]biotin was measured. The values are means ± one standard deviation of triplicate measurements. (With permission from (57).)

We propose that increased uptake is a specific response to increased cellular demand for biotin and that one or both of the following mechanisms generates that increased demand: 1) Proliferation causes increased synthesis of biotin-dependent carboxylases per cell and hence increased demand for biotin (59); 2) proliferation causes increased synthesis of biotinylated histones or increased biotinylation per histone or both. Hymes *et al.* (52, 53) have shown that plasma biotinidase (E.C. 3.5.1.12) catalyzes biotinylation of histones and that histones act as biotinyl acceptors. Biotinidase is ubiquitous in mammalian cells, and 25% of the cellular biotinidase activity is located in the nuclear fraction (60). Given that histones are intimately involved in DNA packaging in nucleosomes and in the transition from condensed to active chromatin, biotinylation of histones suggests that biotin might play some role in DNA transcription and replication. In analogy, transcription and replication of DNA are affected by acetylation, methylation, phosphorylation, or ADP-ribosylation of histones (61–71).

Conclusion

Marginal biotin deficiency is teratogenic in some mammalian species and may be teratogenic in humans. The following factors might predispose humans to fetal malformations caused by biotin deficiency: spontaneous maternal biotin deficiency of marginal degree; weak placental biotin transfer; and an increased biotin requirement of proliferating cells. Alteration of fatty acid metabolism is a potential mechanism for the fetal malformations.

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