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Marginal biotin deficiency is teratogenic in mice and perhaps humans: a review of biotin deficiency during human pregnancy and effects of biotin deficiency on gene expression and enzyme activities in mouse dam and fetus☆

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Abstract

Recent studies of biotin status during pregnancy provide evidence that a marginal degree of biotin deficiency 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 may be teratogenic. In studies of mice, a similar degree of biotin deficiency induces characteristic fetal malformations at a high rate. Fetal hepatic biotin content and PCC activity decrease indicating that the fetuses also become biotin deficient. Fetal hepatic acetyl-CoA carboxylase, pyruvate carboxylase, propionyl-CoA carboxylase and β-methylcrotonyl-CoA carboxylase abundances determined by Western blotting decreased more than the dam holocarboxylase abundances (10% of sufficient vs. 50% of sufficient); however, hepatic mRNA for the carboxylases and for HCS did not change significantly in either dams or fetuses. These observations suggest that maternal biotin deficiency results in a lack of adequate biotin to biotinylate apocarboxylases in the fetus despite the normal expression of genes coding for the apocarboxylases and holocarboxylase synthetase.
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Maternal biotin status and fetal biotin status have been areas of interest and concern for several decades. Three recent studies from our laboratory investigating biotin status during pregnancy provide evidence that a marginal degree of biotin deficiency develops in a substantial proportion of women during normal pregnancy.

Four indicators of biotin status were initially validated in normal nonpregnant women in whom marginal biotin deficiency was induced by egg white feeding while residing at a General Clinical Research Center [1,2]: (i) urinary excretion of 3-hydroxyisovaleric acid (3HIA) that reflects reduced activity of the biotin-dependent enzyme 3-methylcrotonyl-CoA carboxylase (E.C. 6.4.1.4); (ii) urinary excretion of biotin; (iii) serum concentration of biotin; and (iv) urinary excretion of biotin metabolites (bisnorbiotin, biotin-*d*,*l*-sulfoxide). Urinary 3HIA and biotin were the best indicators.

We first investigated maternal biotin status during pregnancy in two studies [3,4]—cross-sectional and longitudinal. In the initial cross-sectional study [3], untimed urine samples were collected either at an early prenatal visit (median duration of gestation=17 weeks) or at a late prenatal visit (median duration of gestation=36 weeks). The urinary excretion of 3HIA was increased in pregnant women in both early and late pregnancy. However, the urinary excretion of biotin increased during late pregnancy [3] for unknown reasons.

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In the longitudinal study [4], blood and untimed urine samples were collected on two occasions—during early pregnancy (median duration of gestation=10 weeks) and late pregnancy (median duration of gestation=36 weeks). The urinary excretion of 3HIA was significantly increased ($P<.0001$) in both early and late pregnancy, confirming the results of the cross-sectional study. Moreover, excretion of 3HIA was significantly greater than the upper limit of normal in 9 of the 13 women in both early and late pregnancy. Surprisingly, the serum concentration of biotin was greater in early pregnancy than in the nonpregnant controls; however, the serum concentration of biotin decreased significantly in late pregnancy and reached values that were below the lower limit of normal in some women. 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 [4]. The urinary excretion of biotin was significantly less in late pregnancy than in early pregnancy or normal controls.

The urinary ratio of the inactive catabolite bisnorbiotin to biotin was significantly increased in both early and late pregnancy suggesting accelerated biotin catabolism. In rats, treatment with steroid hormones causes an increased bisnorbiotin/biotin ratio [5]. These observations suggest that biotin catabolism may be increased as a result of accelerated β-oxidation in response to a gestational increase in steroid hormone concentrations. Based on the magnitude of the biotin catabolism, we have speculated that accelerated biotin catabolism contributes importantly to reduction of biotin status in pregnancy.

We further investigated this marginal biotin deficiency by supplementing biotin during pregnancy [6]. Twenty-six healthy pregnant women with increased 3HIA excretion were studied in a randomized, placebo-controlled trial of biotin supplementation stratified by early and late pregnancy. Timed urine samples were collected before and at the end of 2 weeks of supplementation with 300 μg (1.2 μmol) biotin. In the 10 women (5 per group) studied early in pregnancy (gestational week 6 to 17), biotin supplementation significantly ($P<.003$) decreased 3HIA excretion (11.7±3.6 μmol/mol creatinine, X±S.E.M.) compared to placebo (1.6±0.6). In 16 women (8 per group) studied late in pregnancy (week 21 to 37), biotin supplementation significantly ($P<.001$) decreased 3HIA excretion (7.1±1.2) compared to placebo (0.9±1.8). These decreases in response to biotin supplementation provide evidence that the increased excretion of 3HIA seen frequently in normal pregnancy does reflect reduced biotin status.

Thus, several lines of evidence suggest that marginal biotin deficiency develops frequently during normal gestation and that the deficiency is severe enough to produce metabolic derangements in women. However, the degree of deficiency is not severe enough to produce the classic cutaneous and behavioral manifestations of biotin deficiency and goes unrecognized. The clinical significance of marginal deficiency remains unclear, but animal studies raise concern about teratogenicity.

We investigated the teratogenicity of marginal biotin deficiency in two mouse studies (ICR, Harlan Sprague–Dawley and CD-1, Charles Rivers). In the first study, we characterized the relationships among maternal biotin status, fetal biotin status and fetal malformations [7]. Biotin status was controlled by feeding diets with varying egg white content. In dams and fetuses, biotin status was assessed by hepatic biotin content and hepatic activity of the biotin-dependent enzyme propionyl-CoA carboxylase; in dams, status was also assessed by urinary excretion of biotin and 3HIA. Although no overt signs of deficiency appeared in the dams, metabolic disturbances caused by biotin deficiency were detectable. Biotin excretion decreased and 3HIA excretion increased with increasing egg white. Fetal biotin status correlated significantly ($P<.001$) with maternal biotin status as judged by hepatic biotin and PCC activity: fetal vs. dam hepatic biotin, $r=.671$; fetal vs. dam PCC activity, $r=.70$. PCC activity in deficient fetuses was reduced to about 10% of sufficient fetuses. The rates of cleft palate and limb hypoplasia were strikingly dependent on egg white concentration. Three control diets were used: (1) mouse chow, (2) 0% egg white and (3) a high egg white diet supplemented with enough biotin to occupy all the biotin-binding sites of avidin and still provide excess free biotin. All three groups had similar low rates of malformations (<3%). Thus, the teratogenic effects observed in these studies were not attributable either to a teratogen present in the egg white diet or to some other micronutrient deficiency. We concluded that marginal maternal biotin deficiency caused fetal biotin deficiency and speculated that the fetal malformations were primarily the consequence of fetal biotin deficiency [7].

In the same mouse strain, we investigated the mechanism of reduced carboxylase activity in fetuses and dams [8]. We tested the hypothesis that the decreased carboxylase activity observed in deficient dams and their offspring is mediated by decreased abundance of biotinylated carboxylases, decreased expression of their mRNAs or both. During gestation, CD-1 mice were fed either a diet that induced biotin deficiency (5% egg white) or a biotin-sufficient diet (5% egg white plus biotin). On gestational day 17, gravid uteri were removed, and each live fetus was examined grossly for defects. The biotin-deficient diet produced the expected high incidence of malformations; for example, the incidence of cleft palate was 83% in the deficient group. Maternal and fetal hepatic holocarboxylase abundances were determined for hepatic acetyl-CoA carboxylase, pyruvate carboxylase, propionyl-CoA carboxylase and β-methylcrotonyl-CoA carboxylase by Western blotting. Although the maternal biotin deficiency produced no overt signs of deficiency in the dams, biotinylated carboxylase mass was only half that of control animals. Marginal maternal biotin deficiency had even greater effects on biotinylated enzyme mass in the fetuses. The

mass of biotinylated carboxylases in deficient fetuses was less than 10% of the biotin-sufficient fetal controls. Expression of mRNAs for acetyl-CoA carboxylase, propionyl-CoA carboxylase and β-methylcrotonyl-CoA carboxylase, and holocarboxylase synthetase were determined by real-time RT-PCR. In contrast to the reduced holocarboxylase mass, the amounts of mRNA encoding the hepatic carboxylases in the deficient dams and fetuses were not different from the biotin-sufficient controls. Likewise, expression of the gene encoding holocarboxylase synthetase did not change significantly. The observed reductions in biotinylated carboxylase activity and mass coexisting with normal gene expression for the carboxylases support the hypothesis that maternal biotin deficiency results in a lack of adequate biotin to biotinylate apocarboxylases in the fetus despite the normal expression of genes coding for the apocarboxylases and holocarboxylase synthetase. The relative preservation of maternal carboxylase activities suggests that the limited amount of biotin available to biotinylate proteins is sequestered in the dam liver. Further, the proportional reduction carboxylase activity and holocarboxylase mass in the fetus suggests that the holocarboxylases present in the deficient fetuses are normally active [8].

In summary, our studies in this mouse model support and extend the pioneering observations of Watanabe [9] that marginal and maternal biotin deficiency are highly teratogenic, at least in this strain of mouse. The pathogenesis appears to involve biotin deficiency in the fetus; unlike several other micronutrients, the deficiency in the fetus is greater than the deficiency in the dam. Fetal biotin deficiency has a profound effect on the abundance of holocarboxylases, but the suppression of holocarboxylases synthetase and apocarboxylases gene expression is minimal.

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