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Is impaired folate absorption a factor in neural tube defects?[1,2]

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Neural tube defects (NTDs) are among the most prevalent debilitating birth defects worldwide. By 1992, after a series of trials and intervention studies, the scientific world was finally convinced that NTDs could be substantially prevented by maternal periconceptional supplementation with folic acid. Nearly a decade has elapsed since then, but despite vigorous research efforts it is fair to say that little progress has been made in answering the question of how folic acid actually affords this protection. Phenotypically, there are few clues as to which folate-related process is not functioning properly in NTD-affected pregnancies. However, it is widely acknowledged that the etiology of NTDs falls into the broad category of an interaction of multiple genes superimposed on a high-risk environment. The key topics to address, therefore, are which interactive or folate-related genes confer the genetic predisposition and what constitutes the high-risk environment. A good starting point has been to ask whether low maternal folate status provides the high-risk environment and whether this low status is itself driven by genetic factors.

Maternal folate status has been assessed in several studies in relation to NTDs. Although it seems clear that clinical folate deficiency is not a major cause of NTDs (1), there is some evidence of disordered folate metabolism in women with an NTD-affected pregnancy. Yates at al (2) found depressed red cell folate concentrations that could not be attributed to lower dietary intake in women with a history of NTD-affected pregnancy. Kirke et al (1) found lower maternal plasma and red cell folate concentrations in NTD-affected pregnancies than in normal pregnancies. The differences were subtle and represented an ≈20% reduction in folate status compared with that in control subjects. A defect in folate absorption might explain these observations and also provide a hypothesis for how folic acid supplementation could overcome a similar defect inherited by the fetus.

Folate absorption involves a complex mixture of enzymes and binding proteins, including a polyglutamate hydrolysis step catalyzed by pteroyl-poly-γ-glutamate hydrolase (conjugase, or γ-Glu-X carboxypeptidase). The method used to assess absorption has to overcome difficulties posed by a variety of factors. These include inherent differences in luminal or mucosal binding or transport of the various folate cofactor and polyglutamate forms, inhibition of folate conjugase or folate transport by substances present in food or the pH of the milieu, and differential uptake into tissues depending on the initial folate status of subjects. In addition, any method applied to the study of NTDs must be able to detect the relatively subtle changes that might result in the lower blood status observed in the population studies described above.

Several groups addressed the question of whether folate absorption is impaired in women with NTD-affected pregnancies (3–5). Bower et al (3) examined the absorption of a test meal containing 4.5 mg yeast folate polyglutamates after an initial dose of 5 mg folic acid to saturate the tissues. The measurement index was the increase in serum folate over a 3-h period. No significant differences were detected between women with a history of NTD-affected pregnancy and matched control subjects. By contrast, when using the same measurement index, Schorah et al (4) found a significantly lower response to food folates in orange juice in women with a history of NTD-affected pregnancy, but did not take the tissue status of the subjects into consideration. Neuhauser et al (5) also found a lower serum response to both folic acid alone and to food folates in orange juice in women with a history of NTD-affected pregnancy but the effect was significant only for folic acid. It is difficult to interpret these results because of the different methodologic approaches. Clearly, more work is needed in this area.

In this issue of the Journal, Boddie et al (6) report on the use of their previously developed stable-isotope technique to reexamine the issue of folate absorption in women with a history of NTD-affected pregnancy. This stable-isotope method has several advantages over previously used methods. It allows for a high degree of specificity in the type of compound administered along with the capability of mixing 2 different stable isotopes in the same dose. In addition, the principle of the method, which involves estimating 48-h urinary excretion rather than serum response, offers a novel approach to the problem. Boddie et al estimated the urinary excretion over 48 h of a single test dose containing both [^{2}H]pteroylpentaglutamate and [^{13}C]pteroylmonoglutamate, after a prolonged supplementation period to saturate the tissues. Although this is a preliminary study, the results showed a significantly lower recovery of both polyglutamate and monoglutamate folate forms in case mothers than in controls in the first 24 h postdose despite exceptionally high total folate excretion in both cases and controls. The total excretion in 48 h was similar in both groups but this would not necessarily be inconsistent with a less efficient process in cases than in controls.

If these results can be validated in a larger study, there will be good evidence to suggest that abnormalities in folate absorption may contribute to low folate status in women with an

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NTD-affected pregnancy. The results of studies conducted to date give no indication that a problem occurs at the conjugase step because the drop in absorption efficiency is seen for both the polyglutamate and monoglutamate forms (5, 6). This, however, leaves a wide range of candidates to be considered.

It is likely that nutrient status is under considerable genetic control (7). The discovery of the thermolabile variant of 5,10-methylenetetrahydrofolate reductase (the C-to-T substitution at nucleotide 677, or C677T) has provided one example of genetic variance affecting folate status (8). The mutation is a risk factor for NTDs in some populations, accounting for ≈12% of the NTD-attributable risk (9). This is about one-sixth of the NTDs that are thought to be folate responsive. Although individuals who are homozygous for this variant (*TT*) may have a folate status that is ≈20% lower than expected (8), this does not account for the low folate status seen in many mothers with an NTD-affected pregnancy (10). None of the subjects in the study conducted by Boddie et al were homozygous for the *TT* mutation. The implications of Boddie et al's results would therefore include the presence of other less efficient polymorphisms in folate-related enzymes involved in the absorption process. Although the effect on maternal status might be quite small, the embryonic risk for having inherited such a variant could be substantial.

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