

Has mandatory folic acid supplementation of foods increased the risk of asthma and allergic disease?

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Folate (the naturally occurring form) and folic acid are forms of a water-soluble B vitamin (B9) that were first synthesized in 1945.¹ Folate functions as an important cofactor in the transfer and use of 1-carbon moieties, primarily methyl groups.² An important advance in understanding subclinical folate deficiency came in 1991 with the demonstration that folic acid supplementation before and during pregnancy dramatically reduced the risk of neural-tube defects in newborns.³ Folate supplementation of women before and during the first trimester of pregnancy has a dose-response effect in preventing neural-tube defects, ranging from a 23% reduction with 200 µg to an 85% reduction with 5000 µg of folic acid per day.³ The strong evidence demonstrating reduced risks of neural-tube defects led to mandatory folic acid fortification of cereal grain products in the United States by January 1, 1998. Fortification of foods with folic acid in the United States costs about \$1,000 per neural-tube defect prevented.³ Even with all the information on the benefits of folate, many studies show inadequate folate intake among young women.⁴ Adequate folate levels have also been associated with reduced risks of coronary artery disease, colorectal cancer, and dementia.^{2,5,6}

In contrast to these well-documented beneficial effects, there are now 2 studies, 1 in mice and 1 in human subjects, suggesting that folic acid supplementation during early pregnancy increases the risk of asthma, wheezing, and respiratory disease. The elegant study in mice by Hollingsworth et al⁷ demonstrated that a diet supplemented with methyl donors, including folate, increased the severity of allergic disease. These investigators used a mouse model of allergic asthma in which mice are immunized intraperitoneally with ovalbumin (OVA) to produce OVA-specific IgE and then challenged through the respiratory tract with OVA to induce respiratory and histologic changes similar to those found in human asthma. OVA challenge of pups from mothers receiving supplemented diets compared with control diets induced greater airway hyperreactivity, higher levels of eosinophils and IL-13

in lung lavage fluid, higher total serum IgE levels, increased concentrations of OVA-specific IgE, and more prominent histologic changes associated with allergic airway disease. The pups of mothers given the high methyl donor diets had higher ratios of CD4⁺/CD8⁺ cells and produced increased levels of IL-4 and the chemokine ligands CCL4 and CCL5, which is typical of the T_H2 bias found in allergic human subjects.

Hollingsworth et al⁷ further demonstrated that these changes were associated with increased methylation and therefore reduced protein expression in several genes (*Runx3*, *Nfact1*, *Jak2*, *Rcor3*, and *Ube2j1*). The runt-related transcription factor 3 gene (*Runx3*) has been shown to regulate CD4⁺/CD8⁺ T-lymphocyte development by reducing CD8⁺ cell development.⁸ These changes in DNA methylation are a form of epigenetic change, meaning that the DNA changes can be transmitted to the next generation. Importantly, supplementation of the pups' diets with methyl donors from birth onward did not produce the same proallergic effects as supplementing the maternal diet during pregnancy.⁷

The second study suggesting that increased levels of folate might increase human respiratory disease comes from Håberg et al,⁹ who analyzed data from the Norwegian Mother and Child Cohort study. They compared the outcomes of wheezing and lower respiratory tract infections during the first 18 months of life in 32,077 children born between 2000 and 2005 in relationship to maternal reported intake of 400 µg of folic acid and 5 mL of cod liver oil. In mothers who took folate supplements in the first trimester, the relative risks in their infants were 1.06 (95% CI, 1.03-1.10) for wheezing, 1.09 (95% CI, 1.02-1.15) for lower respiratory tract infections, and 1.24 (95% CI, 1.09-1.41) for hospitalizations associated with lower respiratory tract infections. These relatively small relative risks are statistically significant in the very large study population. A unique aspect of this study is the absence of mandatory folate fortification of foods in Norway. The absence of food fortification makes it easier to estimate folic acid intake in women before and during pregnancy.

Collectively, the studies of Hollingsworth et al⁷ and Håberg et al⁹ raise an important public health policy question. What are the relative risks and benefits of efforts to increase folate intake, whether by means of fortification of food or supplemental vitamins, especially in women of childbearing potential? The study by Matsui and Matsui¹⁰ in this issue of the *Journal* provides evidence contrary to the risks suggested by the Hollingsworth et al⁷ and Håberg et al⁹ studies. These investigators found that higher serum folate levels are associated with a lower risk of high total serum IgE concentrations, atopy, and wheezing. In addition to an apparent dose-response relationship between higher serum folate levels and lower risks of these outcomes, the associations appeared to be independent of age, sex, race/ethnicity, and poverty. Serum folate levels were also inversely related to the risk of doctor-diagnosed asthma, but this relationship did not reach statistical significance. The most important strength of this study is that the data analyzed came from the

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2005-2006 National Health and Nutrition Examination Survey, which provided a population of 8,083 subjects 2 to greater than 85 years of age who were selected to represent the total noninstitutionalized population of the United States. Individuals participating in the 2005-2006 National Health and Nutrition Examination Survey were evaluated in a standardized fashion, and minority groups were oversampled, allowing estimates of possible racial/ethnic differences. However, this study is cross-sectional and does not directly examine the effects of maternal folate intake on fetal development for asthma or other conditions.

National asthma surveillance data in the United States show that nearly all of the increased prevalence of asthma among children in the United States occurred before 1996, which was before mandatory folic acid supplementation of foods was initiated in 1998.¹¹ Unfortunately, the questions used by the Centers for Disease Control and Prevention to assess asthma were changed in 1997, making it somewhat challenging to directly compare the prevalence of asthma before and after this change.

Unfortunately, many questions are left. Although the mouse studies of Hollingsworth et al⁷ were very well done, they were performed in a single inbred strain of mice. It is possible that the particular mouse strain chosen by these investigators is uniquely susceptible to the effects of methyl donors, that the dose of folate and other methyl donors was pharmacologic and not physiologic for the mice, or that DNA methylation responses in mice are different from those in human subjects. The study by Håberg et al⁹ has the strength of a very large population, but it does not answer the question of whether the increased wheezing during the first 18 months associated with folate supplementation will result in more asthma later in childhood.

Currently, there is abundant scientific evidence showing that folate supplementation of women during the first trimester of

pregnancy prevents neural-tube defects and limited evidence suggesting an increased risk of respiratory disease. However, it is always important to consider the possibility of unintended consequences. Studies are now needed to examine whether folic acid supplementation of pregnant women is associated with patterns of DNA methylation in their children similar to those found in the mice pups and whether these DNA changes are also associated with increased childhood asthma.

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