Folate Intake and the Risk of Neural Tube Defects: An Estimation of Dose-Response

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Background. Studies have shown that folic acid supplementation in early pregnancy markedly reduces the risk of neural tube defects (NTDs). Investigation of the relation between relative dose of supplemental folic acid or total folate intake and NTD risk is limited.

Methods. We used data from 23,228 women, predominantly from the northeastern United States, enrolled between October 1984 and June 1987 in a prospective study of early prenatal exposures and pregnancy outcomes. Diet and vitamin intake data were gathered in the early second trimester. NTDs were ascertained through prenatal testing and by report of the delivering physician. Data analyses included multiple logistic regression and restricted spline regression modeling.

Results. For each additional 500 dietary folate equivalents consumed per day, the prevalence of NTDs decreased by 0.78 cases (95% confidence interval [CI] = 0.47-1.09) per 1,000 pregnancies. Compared with women having the lowest total folate intakes (0-149 folate equivalents per day), the prevalence of NTDs declined by 34%, 30%, 56% and 77% among the offspring of those women consuming 150-399, 400-799, 800-1199 and ≥ 1200 folate equivalents per day, respectively (P-value for linear trend = 0.016).

Conclusions. Our results suggest that NTD risk declines markedly with modest increases of total folate in early pregnancy. Total folate dose, rather than supplemental folic acid alone, should be considered in formulating public health guidelines for NTD prevention.

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Key words: folate, folic acid, neural tube defects, pregnancy outcome, cohort study.

Case-control studies during the 1980s and 1990s suggested that women who took multivitamins containing folic acid during the periconceptional period had a lower risk of neural tube defects (NTDs) in their offspring.5-3 In a large prospective study, we found a 64% reduced risk of a NTD in the offspring of women using multivitamins during the first 6 weeks of pregnancy compared with those who did not; the offspring of those whose multivitamin contained folic acid had a 73% reduced risk, whereas the offspring of those whose multivitamin did not contain folic acid had only a 7% reduced risk.6 The epidemiological evidence was strengthened by a nonrandomized intervention study carried out in China.7 Periconceptional use of daily supplements containing 400 µg of folic acid led to a 79% reduction in NTD risk in northern China (where there were higher baseline rates) and a 41% reduction in the South (with much lower baseline rates).

Some randomized clinical trials have also demonstrated a protective effect of folic acid supplements on NTDs.8-12 Although none of these trials looked directly at the effects of different doses of folic acid, a high-dose trial carried out by the Medical Research Council (MRC) provided strong evidence for an overall reduction in NTD risk associated with folic acid use among women with a previous pregnancy affected by an NTD.11 That trial used a supplementation regime of 4000 µg per day in all folic acid intervention arms. A factorial design allowed the assessment of folic acid alone, multivitamins alone (without folic acid) and folic acid plus multivitamins, compared with control group women who were given a supplement with iron and calcium alone. The occurrence of NTDs was similar in both folic acid arms of the trial, with an overall reduction in risk of 72% associated with the use of these supplements. The MRC
and China studies have recently been criticized on methodological grounds, but most observers agree that the overall evidence strongly supports a protective effect of folic acid-containing supplements during the periconceptional period. Questions remain, however, about the relative effectiveness of various folic acid doses.15-17

A few of the previously published case-control studies examined folic acid dose and NTD risk, but only one of them found a clear dose-response relation. No prospective study or clinical trial has evaluated NTD risk according to the maternal dose of folic acid supplements or total folate intake. To address this question, we have used detailed dosage data from our earlier prospective study to examine the effect of folic acid dose during the first 5 weeks of pregnancy.

Methods

In the mid-1980s, 24,559 women in the early second trimester of pregnancy were asked to participate in a prospective study of early pregnancy exposures and pregnancy outcome. The women were predominantly from the northeastern United States, and all had either a serum alpha-fetoprotein screening test or an amniocentesis. Recruitment details have been published previously.6 The current analyses are based on data from the 23,228 women who agreed to participate and who had complete interview and outcome information for all variables of interest. The study was approved by the Institutional Review Board of the Boston University Medical Center, and all study subjects gave informed consent for participation. All interviews were carried out by telephone by a nurse interviewer generally between the 15th and 20th gestational week. Each woman was asked to provide detailed information about her use of multivitamins and other vitamin supplements, including folic acid and yeast (which contains folic acid). The woman was asked for the brand of any supplement she took and the number of times it was taken per week. She was also asked when she had started taking the supplement and when and if there had been any change in its use.

Dietary folate intake was assessed by means of a 50-item food frequency questionnaire that was adapted from a previously validated standardized food frequency instrument.14 The 50-item questionnaire was developed by the same investigators who designed and tested the original instrument. Food items on the checklist were specifically chosen to provide complete and valid estimates of dietary folate and included such items as liver, dark green leafy vegetables, legumes (eg, beans, lentils), other vegetables such as brussel sprouts, broccoli and peas, pizza, beef, orange juice, tomato sauce and juice, wheat germ and breakfast cereals. The woman was asked about her intake of each food item during the first 8 weeks of pregnancy. Nutrient composition was obtained for each food using a database provided by the Center for Clinical Computing at Harvard University.

The presence of fetal and congenital malformations was determined using data from the amniocentesis and ultrasound test results as well as a questionnaire sent to the delivering physician at the time of expected delivery. The questionnaire asked for details of any other prenatal studies, maternal illnesses during the pregnancy, other pregnancy complications, occurrence of fetal or neonatal deaths, newborn complications and congenital malformations. All congenital anomalies were coded using the 6-Digit Code List for Reportable Congenital Anomalies published by the Centers for Disease Control and Prevention.19

Statistical Analysis

To account for differences in bioavailability of folate from different sources, we converted all reported folate intake to dietary folate equivalent (DFE) units. Conversions were done as follows: (1) 1.0 μg folate from food = 1 DFE, (2) 0.5 μg folate from supplements = 1 DFE and (3) 0.6 μg folate from fortified grains = 1 DFE. Thus, for example, 400 μg of naturally occurring folate from food would provide 400 DFEs, a 400 μg vitamin supplement of folic acid would provide 800 DFEs and 400 μg of folic acid from supplemented breakfast cereals would provide about 668 DFEs.

We estimated folate dose from vitamin supplements by combining vitamin composition information from a computer file (which had detailed vitamin and mineral composition data from the mid-1980s for approximately 1,500 vitamin brands) with the reported information on frequency of intake. We used questionnaire data on starting and stopping weeks for each supplement to calculate supplemental folate dose during each week of the first trimester. Folate intake from foods (including supplemented foods) was estimated using data on usual portions and reported frequency of consumption. Thus, we were able to estimate average folate intake from foods during the first 2 months of pregnancy, whereas for supplemental folate we were able to derive estimates of intake for each individual week during the first trimester. After converting all intakes to standard DFE units, we combined weekly data from vitamin supplements with average folate intake from foods to estimate total daily folate intake during the first 8 weeks since the last menstrual period.

We examined the prevalence of NTDs according to the daily dose of folate from foods, supplements and the two combined (total folate). We conducted multiple logistic regression analyses to estimate the relative risk (prevalence ratio) of NTDs, adjusting for potential confounding by the following factors: history of NTD in any first degree relative of the offspring, maternal age, edu-
TABLE 1. Characteristics of Subjects by Supplemental Folate Intake Weeks 1–5

<table>
<thead>
<tr>
<th>Daily Folate Intake (DFEs/Day)</th>
<th>Age &lt;30 years (N = 13,431)</th>
<th>Age 30–39 years (N = 6,605)</th>
<th>Age ≥40 years (N = 297)</th>
<th>Education</th>
<th>First trimester exposures</th>
<th>Maternal history/characteristics</th>
<th>Previous diabetes mellitus</th>
<th>Obesity (BMI ≥30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>N 6,529</td>
<td>N 6,605</td>
<td>N 297</td>
<td>None</td>
<td>Cigarette use (any)</td>
<td>Medical history of NTD*</td>
<td>Previous diabetes mellitus</td>
<td>786</td>
</tr>
<tr>
<td>1-399 DFEs/Day*</td>
<td>N 1,179</td>
<td>N 1,264</td>
<td>N 46</td>
<td>College or higher</td>
<td>8,867</td>
<td>Multivitamins (prior)</td>
<td>1,111</td>
<td>66</td>
</tr>
<tr>
<td>400-799 DFEs/Day*</td>
<td>N 749</td>
<td>N 1,031</td>
<td>N 34</td>
<td>First trimester exposures</td>
<td>2,776</td>
<td>Maternal history of NTD*</td>
<td>64</td>
<td>&lt;1</td>
</tr>
<tr>
<td>≥800 DFEs/Day*</td>
<td>N 2,139</td>
<td>N 3,202</td>
<td>N 153</td>
<td>Family history of NTD*</td>
<td>281</td>
<td>Maternal history of NTD*</td>
<td>102</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>N 4,260</td>
<td>N 778</td>
<td>N 4,328</td>
<td>Previous diabetes mellitus</td>
<td>1,264</td>
<td>Maternal history of NTD*</td>
<td>786</td>
<td>6</td>
</tr>
</tbody>
</table>

* Intake as DFEs (dietary folate equivalents); 0.5 µg (micrograms) of folic acid equals 1 DFE.
† Used multivitamins in 3 months before conception.
‡ Family history of neural tube defect in any first-degree relative of the baby.
§ Includes Type 1, Type 2, and prior gestational diabetes.

Finally, to depict the dose-response relation of total folate intake from each source with NTD risk, we used a multivariable restricted regression spline analysis, with second-order terms (quadratic) added to smooth the curves across the dose distribution. A spline analysis fits separate curves for segments of the dose distribution, which allows the overall curve to reflect more accurately the shape of a dose-response trend.

For most analyses, we have restricted our examination of folate intake to the first 5 weeks since the last menstrual period. The reason for this restriction was to increase the likelihood that the woman was taking the reported dose before closure of the neural tube (between the 26th and 29th gestational day, which roughly translates to between the 40th and 43rd day since the last menstrual period). There is undoubtedly error in the precise reporting of weeks since last menstrual period, and some women’s report of pregnancy duration may also have been influenced by the knowledge of ultrasound gestational age. Finally, in this cohort, week 6 was the median reported week in which prenatal vitamins were started by women who had not previously been taking multivitamins. Given the potential for error in reporting pregnancy duration, we chose to use week 5 as the cut-off point for folate intake before closure of the neural tube.

**Results**

Women using the highest dose supplements (≥800 DFEs per day) in the first 5 weeks of pregnancy were older, more likely to be college-educated and more likely to have been using multivitamins before conception. They were also more likely to have a history of diabetes mellitus or a family history of NTDs compared with women in other dose categories. Those using no folic acid had lower education levels, were slightly more likely to be obese and were more likely to have smoked cigarettes during the first trimester.

First we examined the effect of folate intake from supplements alone. Table 2 shows the prevalence of NTDs according to the daily dose of folic acid from vitamin supplements during weeks 1–5 of the pregnancy. There was no evidence of a dose-response relation according to intake from supplements alone. The offspring of women using supplemental folate had

**TABLE 2. Prevalence and Relative Risk of Neural Tube Defect (NTD) According to Daily Folate Intake from Supplements**

<table>
<thead>
<tr>
<th>Daily Folate Intake (DFEs/Day) (Weeks 1–5)</th>
<th>N</th>
<th>No. of Defects</th>
<th>Prevalence/1,000</th>
<th>PR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>13,431</td>
<td>17</td>
<td>2.8</td>
<td>1.0</td>
</tr>
<tr>
<td>1–399</td>
<td>2,489</td>
<td>2</td>
<td>0.8</td>
<td>0.29</td>
</tr>
<tr>
<td>400–799</td>
<td>1,812</td>
<td>2</td>
<td>1.1</td>
<td>0.41</td>
</tr>
<tr>
<td>≥800</td>
<td>5,494</td>
<td>8</td>
<td>0.3</td>
<td>0.56</td>
</tr>
</tbody>
</table>

DFEs = dietary folate equivalents; PR = prevalence ratio; CI = confidence interval.

* Adjusted for age, education (college or higher), body mass index, history of NTD in any first-degree relative of baby, any diabetes history, parity (previous pregnancy lasting ≥22 weeks), spermicide exposure after conception, first trimester fever, alcohol intake (drinks per week in first trimester), first trimester smoking, and intake of supplemental zinc (µg/day) during the first 8 gestational weeks.
TABLE 4. Prevalence and Relative Risk of Neural Tube Defect (NTD) According to Daily Folate Intake from Food

<table>
<thead>
<tr>
<th>DFEs/Day (Weeks 1–5)</th>
<th>Prevalence of NTD</th>
<th>Relative Risk of NTD*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Defects</td>
<td>Prevalence/1,000</td>
</tr>
<tr>
<td>0–99</td>
<td>744</td>
<td>3</td>
</tr>
<tr>
<td>100–199</td>
<td>6,011</td>
<td>12</td>
</tr>
<tr>
<td>200–299</td>
<td>6,095</td>
<td>14</td>
</tr>
<tr>
<td>300–399</td>
<td>4,019</td>
<td>11</td>
</tr>
<tr>
<td>≥400</td>
<td>3,669</td>
<td>9</td>
</tr>
</tbody>
</table>

DFEs = dietary folate equivalents; PR = prevalence ratio; CI = confidence interval.

* Adjusted for some variables as in Table 2.

We found that the prevalence of NTDs decreased by 0.78 cases (95% confidence interval [CI] = 0.47–1.22) per 1,000 pregnancies for each additional 500 DFEs per day.

Discussion

In this study, we combined folate intake data from vitamin supplements and...
and foods. We converted folate intake from all sources to DFE units to account for the differing bioavailability of folate from different sources. We found that NTD risk generally declined with increasing total daily folate intake during the first 5 weeks since the last menstrual period (Table 4 and Figure 1). This effect was even stronger for those consuming more than 600 DFEs per day of total folate at the time of conception. There was no progressive dose-response relation for either vitamin supplements alone or folate intake from foods alone with regard to NTD risk. This result for supplements alone is not surprising because the use of vitamin supplements containing folic acid might well have a different effect among women whose diet is already rich in folate, compared with women whose dietary folate intake is very low. Because the diet questionnaire targeted average intake in the first 8 weeks of pregnancy, the absence of a dose-response relation for food may have resulted from imprecise estimation of folate consumed from food sources specifically in the weeks before neural tube closure. It may also be that diets that are low in naturally occurring folate may be less harmful for women using a folic acid-containing multivitamin at the time of conception.

For the most part, folate dose information in this prospective study was gathered before the outcome of the pregnancy was known. However, 19 of the 49 women with a NTD offspring were aware of the prenatal test results before the interview. Nonetheless, the study was carried out before widespread publicity about the potential protective effects of folic acid, making biased reporting less likely. In addition, the close proximity of the interview to the exposure period would have enhanced the accuracy of the subject’s report of exposure information. The study is further strengthened by the fact that we gathered detailed information on folic acid supplement use as well as intake of folate-containing foods, allowing us to estimate total folate dose for each week during early pregnancy.

Few women were taking high-dose folic acid supplements in this study, and so we are unable to evaluate the effects of high-dose supplementation levels. In fact, only five women in this cohort took supplements of 4 mg per day (8,000 DFEs) during the first 5 weeks, as used in the MRC study. The relative reduction in risk associated with total folate doses of approximately 800 DFEs per day in our study was similar in magnitude to that seen with the higher-dose supplements used in the MRC trial. However, this comparison is complicated by the fact that this latter study targeted a high-risk population (ie, those with previous NTD-affected pregnancy).

In 1996, the weight of evidence linking folate intake with NTD risk led the United States Food and Drug Administration to embark on a program designed to add 100 µg (167 DFEs) of supplemental folate per day to the diets of all Americans by adding 140 µg of folic acid to every 100 g of grain. Similar programs are being considered in the United Kingdom and elsewhere. In the meantime, the debate continues about the optimal levels of fortification, with some arguing that the current level of fortification in the U.S. is too low and others calling for restraint until the efficacy of the current program is tested and more data on safety of high-dose folate in the general population are available. Recent data from the Centers for Disease Control suggest that since the onset of mandatory food fortification in the U.S., there has been a 13% decline in the occurrence of NTDs among the offspring of women who received only late (third trimester) or no prenatal care. There is evidence that the current levels of food fortification have dramatically raised blood levels of folate in the population at large, leading to additional concerns about the safety of still higher levels of fortification. And at the same time, there is some disappointment that the NTD risk reduction has not been greater.

In summary, there are still not enough data to determine the optimal periconceptional folate intake needed to prevent NTDs. However, this study provides important additional evidence that NTD risk declines steeply with increasing intake of modest amounts of total folate in the early weeks of pregnancy. These results highlight the need to consider total folate dose rather than supplemental folate intake alone in establishing public health guidelines for NTD prevention.

Acknowledgments
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References


