GOOD ARTICLE FOR ILLUSTRATIVE EXAMPLES



# Multivitamin/Folic Acid Supplementation in Early Pregnancy Reduces the Prevalence of Neural Tube Defects

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We examined the relation of multivitamin intake in general, and folic acid in particular, to the risk of neural tube defects in a cohort of 23 491 women undergoing maternal serum α-fetoprotein screening or amniocentesis around 16 weeks of gestation. Complete questionnaires and subsequent pregnancy outcome information was obtained in 22 776 pregnancies, 49 of which ended in a neural tube defect. The prevalence of neural tube defect was 3.5 per 1000 among women who never used multivitamins before or after conception or who used multivitamins before conception only. The prevalence of neural tube defects for women who used folic acid–containing multivitamins during the first 6 weeks of pregnancy was substantially lower—0.9 per 1000 (prevalence ratio, 0.27; 95% confidence interval, 0.12 to 0.59 compared with never users). For women who used multivitamins without folic acid during the first 6 weeks of pregnancy and women who used multivitamins containing folic acid beginning after 7 or more weeks of pregnancy, the prevalences were similar to that of the nonusers and the prevalence ratios were close to 1.0.

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REVIEW of the etiologies of neural tube defects (NTDs) reveals a remarkably heterogeneous spectrum varying from known single-gene and chromosomal disorders to putative environmental causal associations.[1,2] Dietary deficiencies associated with an increased rate of NTDs had been noted in both postwar Germany[3] and Holland,[4] and in England the same association was found for the lower social classes.[5]

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Recognition that aminopterin, a folic acid antagonist, was a human teratogen[6] and that such agents caused NTDs in mice, cats, and rats[7] focused attention on the possible etiologic role of this nutrient. Twenty-five years ago, Hibbard and Smithells[8] demonstrated significantly lower blood folic acid levels at term in women having babies with NTDs compared with controls. Subsequently, Smithells et al[9] observed significant low levels of red blood cell folate, serum vitamin A, white blood cell vitamin C, and red blood cell riboflavin in 900 poor women studied during the first trimester of pregnancy, and even lower levels of red blood cell folate and white blood cell vitamin C in 7 of these women who had babies with NTDs.

Serial reports (reviewed elsewhere[1]) by Smithells et al[10] of multivitamin (MV) supplementation prior to and during pregnancy for women at risk of having another child with an NTD yielded recurrence rates of 0.7% (3 offspring) among 454 women taking vitamin supplements and 4.6% (24 offspring) among 519 women not taking them. Although these were intervention studies, they did not employ randomization, a placebo control group, or double-blind design.

Laurence et al[11] found, in a very small controlled, randomized, double-blind trial of women with a previous child with an NTD, a nonsignificant protective effect of folic acid supplementation given before conception and during early pregnancy. In another small study, Holmes-Siedle et al[12] also found a reduction in the recurrence rate of NTDs in infants of women similarly treated. Seller and Nevin,[13] in comparing the effectiveness of periconceptional vitamin supplementation in areas of high and low NTD prevalence, also observed results consistent with a protective effect of MV supplementation.

Recently, in a case-control study of 347 cases of NTD, Mulinare et al[14] reported the results on 181 case-persons and comparable control-persons whose mothers either never used MVs in the 6 weeks before or after conception or used MVs both before and after conception. Mothers were interviewed up to 16 years after the relevant pregnancy regarding their use of vitamin supplements in the periconceptional period. The relative risk estimate comparing women who used MVs during the period 3 months before and after conception with those who did not was 0.40.

In the current study, we prospectively examined the relation of MV intake in general and folic acid intake in particu-

lar to the risk of NTDs in a cohort of over 22 000 women who underwent amniocentesis or had a serum α-fetoprotein assay at about 16 weeks of pregnancy during the period October 1984 through June 1987.

## METHODS

All women in the study were identified and recruited when they had either a maternal serum α-fetoprotein (MSAFP) screen or an amniocentesis. They were receiving prenatal care and routine MSAFP screening in the practices of over 100 participating obstetricians; 96% of the pregnancies were between 15 and 20 weeks' gestation. All of the MSAFP samples and 81% of the amniotic fluid samples were analyzed at the Center for Human Genetics of Boston University School of Medicine. The remaining amniocenteses were performed and analyzed at other genetic centers throughout the country and the results were made available to us. Thirty-three percent of subjects lived in the Boston area, 48% elsewhere in Massachusetts, 5% elsewhere in New England, and 14% in states outside New England.

Trained nurse interviewers contacted the pregnant women by telephone at the time their prenatal test was received by the laboratories. The large majority of the interviews took place before test results were known to either the patient or the interviewer; 508 (7%) of the patients undergoing amniocentesis knew their test results at the time of interview.

Interviews focused on family, medical, and genetic history with special emphasis on diet, medication, and illness during the first trimester of pregnancy. Questions also covered environmental and occupational exposures prior to conception. Interviewers contacted 24 559 women; 1068 (4%) refused to participate. Of 23 491 interviewed women, we have complete questionnaires and pregnancy outcome information on 22 776 pregnancies; 686 women could not be located for follow-up, and 29 interviews were not completed. Interviews of mothers pregnant a second or third time during the study were excluded.

### Maternal Multivitamin Use

As part of the questionnaire, each woman was asked to provide detailed information on her use of vitamins and other nutritional supplements. First, they were asked, "In the 3 months prior to pregnancy, did you take a multivitamin?" If a woman answered yes, the interviewer asked for the brand name of the vitamin and how many times per week the vitamin was taken. The women were then asked if, in the 3 months prior to pregnancy, they used any of the following supplements: vitamin A, vitamin C, vitamin E, yeast, folic acid, selenium, zinc, iron, or any other nutrients. If yes, the dose and frequency of use of each supplement were recorded.

Next, all women were asked, "Did you take a multivitamin in the first 3 months of pregnancy?" If yes, the brand name of the vitamin, the week of pregnancy in which the vitamin was started, and the frequency of its use were recorded. Similar information was obtained on the aforementioned specifically named supplements (with the addition of dosage) when applicable.

Estimation of which week of pregnancy that supplementation began depended on self-reported pregnancy duration, in practice based on the date of the woman's last menstrual period. More precise estimation would have required systematic ultrasound studies.

In a random sample of 150 MV users, 143 women used MVs containing folic acid. The daily dose of folic acid ranged from 100 to 1000 μg, with 17% of the users having 100 μg; 22%, 400 μg; 23%, 300 μg; and 45% of the users receiving 1000 μg in their MVs.

### Diet

Another section of the questionnaire provided information on the diets of these women during their first 8 weeks of pregnancy. For a list of 50 foods, they were asked, "How often, per day, per week, or per month, did you eat one serving of the following foods during the first 8 weeks of your pregnancy?" Servings were clearly defined for each food item (eg, one cup of milk, one slice of cheese, half a grapefruit). For cold breakfast cereals, the patients were asked to provide the brand and type for up to three cereals they ate most often. Dietary folic acid intake was calculated from responses to the food frequency questionnaire, using published composition values.[15,16]

### Pregnancy Outcome Information

Pregnancy outcome was ascertained from a brief questionnaire mailed to the delivering physicians near the expected date of delivery, or subsequently to the mothers themselves when physicians did not respond. Information requested included results of prenatal tests, presence of any birth defects or chromosome abnormalities, complications of the pregnancy or delivery, complications in the newborn, and perinatal maternal illnesses. A case of NTD was defined as any occurrence of spina bifida, anencephaly, or encephalocele alone or in combination with other defects. Physicians provided 76.5% of the outcome data received; the remaining 23.5% of the outcome questionnaires were completed by mothers.

### Data Analysis

A woman was considered an MV user if she reported taking at least one MV per week. We evaluated the relations among periconceptional use of MVs, diet, and the occurrence of NTDs with the following analyses:

1. We compared the prevalence of NTDs in pregnancies of women who did not use MVs at any time with that in women who used MVs only in the first trimester and with that in women who used MVs both before and after conception.

2. We then identified women who began their use of MVs after conception and before the start of the seventh week of pregnancy. (This predetermined cutoff point was considered to be biologically significant in that, using the date of the woman's last menstrual period, it is during the first 8 weeks of gestation that the neural tube forms and closes.[17]) We compared the prevalence of NTDs among the fetuses of women who did not use MVs after conception with the prevalence for women who started MV use in the first 6 weeks of pregnancy and the prevalence for women who started MV use after week 6.

3. Having identified nonusers of MVs, users of MVs some time during the first 6 weeks of pregnancy, and users who began their use of MVs after the sixth week, we subdivided users in the first 6 weeks into those who used MVs containing folic acid and those whose MVs contained no folic acid and compared the prevalence of NTDs in each group with that in nonusers.

4. Among the women we identified as having no folic acid supplementation in the first 6 weeks of pregnancy, we estimated folic acid intake from their diet alone. We separated these women into two categories: those with folic acid intake considered to be low (<100 μg/d) and those with folic acid intake considered to be adequate (≥100 μg/d) and compared the prevalence of NTDs in these two groups. One hundred micrograms per day was chosen, before analysis of the data, as a level below which intake might be inadequate, and it is midway between the minimal daily requirement and the recommended dietary intake.[18] Similar analyses were repeated with preformed vitamin A and vitamins C, D, and E as the exposures of interest.

Prevalence ratios (PRs) and their 95% test-based confidence intervals (CIs) were estimated using a program

from Rothman and Boice.[19] We evaluated the potential effect of maternal age, family history of NTD, number of previous pregnancies, smoking, education, and dietary folic acid on the relation of MV folic acid intake to NTDs by multivariate logistic regression analysis.

## RESULTS

The final study population consisted of the 22 776 interviewed women for whom we had complete questionnaires and pregnancy outcome information. Fifteen thousand six hundred seventy-two women underwent MSAFP analysis and 7104 underwent amniocentesis. Forty-four percent were 20 to 29 years of age, 52% were 30 to 39 years of age, 2% were below 20 years of age, and 2% were 40 years of age or above. Ninety-six percent of the study population were white, 93% were married, and 70% had attended college. For 27% of the women, this was their first pregnancy. A positive family history of an NTD was present in 596 women in the cohort. (A positive family history of NTD included affected respondents and husbands, their offspring, half-siblings, siblings, parents, aunts, uncles, nieces, nephews, and first cousins.) For nonmarried women, family history was obtained for the mother's family only. A previous pregnancy with an NTD was reported by 107 women.

Of the 22 776 women with complete interview and pregnancy outcome information, 49 women had an NTD outcome for their pregnancies. Of the 107 women with a previous child with an NTD, 3 (28.0 per 1000) had a baby with an NTD in this study. Among the 489 women with a family history of NTD other than offspring, 2 (4.1 per 1000) had an affected baby. Among the remaining 22 093 women with no family history, 44 women (2.0 per 1000) had an affected pregnancy outcome. (Family history was unknown in 87 women.) Information on MV use was unknown in 61 (0.3%) women, none of whom had an NTD outcome for their pregnancies. Analyses were carried out on the remaining 22 715 women.

Among the 49 case-persons, 6 women gave birth to live infants, 1 woman spontaneously aborted, 34 women had induced abortions, and in 7 instances only a prenatal diagnosis of an NTD was reported. Among the women with live-born infants, 3 were patients with normal serum values of MSAFP, 1 had a high MSAFP concentration (2.2 multiples of the median) and carried the child to term, and 2 were amniocentesis patients with normal fluid α-fetoprotein levels.

The 49 cases of NTD included 13 cases of spina bifida alone, 9 cases of spina bifida with hydrocephalus, 1 case of spina bifida with cleft lip and palate, 3 cases of spina bifida with chromosome abnormalities (a triploidy partial mole, a trisomy 13, and a balanced translocation in an infant whose parents had normal karyotypes), 2 cases of spina bifida with multiple defects (one infant had ventricular septal defect with pleural effusion, the other had multiple defects and a short umbilicus), 1 meningocele with asymmetry of the skull, 1 case of iniencephaly with rachischisis, 3 cases of encephalocele, 15 cases of anencephaly, and 1 case of anencephaly with encephalocele and rachischisis.

A woman was considered an MV user if she reported taking an MV supplement at least once a week. Eighty-seven percent of the users took MVs 7 days per week and 13% took MVs between 1 and 6 days per week.

The distribution of MV use in the entire population was as follows: 2927 women (12.9%) did not use MVs at any time, 230 (1.0%) used MVs only before conception, 12 297 (54.0%) used MVs in the first trimester only, and 7261 (31.9%) used MVs both before and after conception. In the analyses those few women who used MVs before conception only were included in the nonuser category.

Table 1 presents the crude prevalence of NTD outcomes comparing women who took MVs in the first trimester of pregnancy alone, as well as those who took MVs both before and after conception, with those who did not use MVs at any time after conception. Among the 12 297 women who took MVs in the first trimester only, there were 29 (2.4 per 1000) who had NTD outcomes. The PR estimate for users in the first trimester only compared with nonusers was 0.68 (95% CI, 0.34 to 1.35). Among the 7261 women who used MVs before and after conception, 9 had an NTD outcome. The PR estimate for women who used MVs before and after conception was 0.36 (95% CI, 0.15 to 0.83).

Table 2 provides the results of an analysis of 19 558 women with first-trimester MV use divided into two categories: those who used MVs during weeks 1 through 6 (11 675 [60%]) and those who started using MVs during or after the seventh week (7883 [40%]). The prevalence of NTDs among women who used MVs in weeks 1 through 6 was 13 per 11 675 (1.1 per 1000; crude PR, 0.32; 95% CI, 0.15 to 0.68 compared with nonusers); among those who started using

Table 1.—Prevalence of Neural Tube Defects Comparing Women Who Did Not Use Multivitamins During Pregnancy With Women Who Used Multivitamins Only in the First Trimester and Women Who Used Multivitamins Both Before and After Conception

| | None* | 1st Trimester Only | Before Conception and 1st Trimester | Total† |
|---|---|---|---|---|
| No. of cases | 11 | 29 | 9 | 49 |
| Total | 3157 | 12297 | 7261 | 22715 |
| Prevalence per 1000 | 3.5 | 2.4 | 1.2 | 2.2 |
| Prevalence ratio estimate | 1.00 | 0.68 | 0.36 | ... |
| 95% confidence interval | ... | 0.34-1.35 | 0.15-0.83 | ... |

*Reference category (includes 230 women who took multivitamins only before conception).
†There were 61 women for whom time of multivitamin use was unknown.

Table 2.—Prevalence of Neural Tube Defects Comparing Women Who Did Not Use Multivitamins After Conception With Women Who Used Multivitamins in First 6 Weeks* of Pregnancy and Women Who Started Multivitamin Use Only After Week 6*

| | Week of Pregnancy Multivitamins Were Used | | | |
|---|---|---|---|---|
| | None | 1-6 | 7+ Only | Total† |
| No. of cases | 11 | 13 | 25 | 49 |
| Total | 3157 | 11675 | 7883 | 22715 |
| Prevalence per 1000 | 3.5 | 1.1 | 3.2 | 2.2 |
| Prevalence ratio estimate | 1.00 | 0.32 | 0.91 | ... |
| 95% confidence interval | ... | 0.15-0.68 | 0.45-1.80 | ... |

*As reported in response to the question, "Did you take a multivitamin in the first 3 months of pregnancy?" If yes, "What week of pregnancy did you start the multivitamin?" (in practice based on date of last menstrual period).
†There were 61 women for whom time of multivitamin use was not known.

MVs in week 7 or later, the prevalence was 25 per 7883 (3.2 per 1000; crude PR, 0.91; 95% CI, 0.45 to 1.80 compared with nonusers).

Multivitamin use was further divided into two groups: women who used MVs that contained folic acid during weeks 1 through 6 and those whose MVs did not contain folic acid. For women who took MVs containing folic acid, the prevalence was 10 per 10 713 (0.9 per 1000; PR, 0.27; 95% CI, 0.12 to 0.59 compared with nonusers of MVs). The prevalence was 3 per 926 (3.2 per 1000; PR, 0.93; 95% CI, 0.26 to 3.3) for those who took an MV that did not contain folic acid (Table 3).

Table 4 compares the experience of the 10 713 women who used folic acid–containing MVs in weeks 1 through 6 with that of the 11 937 women who did not. The PR estimate for users compared with nonusers was 0.29 (95% CI, 0.15 to 0.55).

Table 5 presents the results stratified by knowledge of the amniocentesis result prior to interview. For subjects who did not know the amniocentesis result prior to interview, the PR estimate comparing women who received folic acid in weeks 1 to 6 with those who did not was 0.1 (95% CI, 0.02 to 0.44). For women who knew their amniocentesis result prior to interview, the PR estimate was 0.5 (95% CI, 0.22 to 1.30).

Folic acid intake was calculated from the diet portion of the questionnaire for the 11 944 women who did not receive folic acid from an MV (Table 6). The prevalence of NTDs among those with a daily folic acid intake of less than 100 μg was 4 per 551 (7.3 per 1000). The prevalence among those with a daily folic acid intake of 100 μg or more was 35 per 11 393 (3.1 per 1000). The PR for women consuming 100 μg or more of folic acid daily was 0.42 (95% CI, 0.15 to 1.13) compared with women with a daily folic acid intake of less than 100 μg. Among women who did not use supplements, dietary intake of preformed vitamin A was not related to the risk of NTDs.

The use of folic acid–containing MVs was compared with nonuse in women with and without a family history of an NTD. Among women with a family history of an NTD, the prevalence of NTDs was 1 per 287 (3.5 per 1000) in women who took folic acid–containing MVs in weeks 1 to 6 and 4 per 307 (13.0 per 1000) among women who did not. Among those with no family history of an NTD, the prevalence of NTDs was 9 per 10 380 (0.9 per 1000) for women who received folic acid and 35 per 11 654 (3.0 per 1000) among women who did not. The PR estimates for folic acid use compared with nonuse were 0.27 and 0.29, respectively, for women with and without a family history of an NTD.

In a multivariate logistic regression analysis, the PR estimate for NTDs comparing women who received MVs containing folic acid in weeks 1 through 6 with those who did not was 0.30 (95% CI, 0.15 to 0.63) after controlling for number of previous pregnancies, family history of an NTD, maternal age, maternal education, current smoking, and dietary folic acid intake (Table 7).

After analysis of the results relating to folic acid, we reviewed the results relative to the relation of vitamin A intake and NTDs. We found that most MVs that contain folic acid also contain vitamin A and vice versa. Therefore, there was insufficient information to clearly separate the effects of these two nutrients. In a random sample of 150 MV users, 145 women used MVs containing vitamin A in the form of retinol. The doses ranged from 1250 IU to 10 000 IU, with 57% of the women getting 8000 IU of vitamin A in their preparations, 20% with 5000 IU, 16% with 4000 IU, 3% each getting 1250 or 10 000 IU, and less than 1% getting 6000 IU. There were only 659 women who received

Table 3.—Prevalence of Neural Tube Defects According to Intake of Folic Acid–Containing Multivitamins and Their Time of Use*

| | | Weeks 1-6† | | Weeks 7+ Only‡ | |
|---|---|---|---|---|---|
| | None | Folic Acid + | Folic Acid − | Folic Acid + | Folic Acid − |
| No. of cases | 11 | 10 | 3 | 25 | 0 |
| Total | 3157 | 10713 | 926 | 7795 | 66 |
| Prevalence per 1000 | 3.5 | 0.9 | 3.2 | 3.2 | ... |
| Prevalence ratio estimate | 1.00 | 0.27 | 0.93 | 0.92 | ... |
| 95% confidence interval | ... | 0.12-0.59 | 0.26-3.3 | 0.45-1.87 | ... |

*As reported in response to the question, "Did you take a multivitamin in the first 3 months of pregnancy?" If yes, "What week of pregnancy did you start the multivitamin?" (in practice based on date of last menstrual period).
†Among women who took multivitamins in weeks 1 to 6, folate content was not known in 36 instances.
‡Among women who took multivitamins in weeks 7+, folate content was not known in 22 instances.

Table 4.—Prevalence of Neural Tube Defects Comparing Women Who Did Not Use Multivitamins Containing Folic Acid in First 6 Weeks of Pregnancy With Those Who Did*

| | Folic Acid − | Folic Acid + | Total |
|---|---|---|---|
| No. of cases | 39 | 10 | 49 |
| Total | 11944 | 10713 | 22657† |
| Prevalence per 1000 | 3.3 | 0.9 | 2.2 |
| Prevalence ratio estimate | 1.00 | 0.29 | ... |
| 95% confidence interval | ... | 0.15-0.55 | ... |

*As reported in response to the question,"Did you take a multivitamin in the first 3 months of pregnancy?" If yes, "What week of pregnancy did you start the multivitamin?" (in practice based on date of last menstrual period).
†Folate content of multivitamin was unknown in 58 people.

Table 5.—Use of Multivitamins Containing Folic Acid in First 6 Weeks of Pregnancy* in Relation to the Prevalence of Neural Tube Defects According to Knowledge of Amniocentesis Results at the Time of Interview†

| | Folic Acid − | Folic Acid + | Total |
|---|---|---|---|
| **Results Unknown to Patient** | | | |
| No. of cases | 11 | 1 | 12 |
| Population | 3073 | 3453 | 6526 |
| Prevalence ratio estimate | 1.00 | 0.10 | ... |
| 95% Confidence interval | ... | 0.02-0.40 | ... |
| **Results Known to Patient** | | | |
| No. of cases | 11 | 8 | 19 |
| Population | 213 | 289 | 502 |
| Prevalence ratio estimate | 1.00 | 0.54 | ... |
| 95% Confidence interval | ... | 0.22-1.30 | ... |

*As reported in response to the question, "Did you take a multivitamin in the first 3 months of pregnancy?" If yes, "What week of pregnancy did you start the multivitamin?" (in practice, based on date of last menstrual period).
†The remaining women did not have amniocentesis.

Table 6.—Prevalence of Neural Tube Defects According to Reported Folic Acid Intake From Diet Alone Among Women With No Folic Acid Intake From Multivitamins in First 6 Weeks of Pregnancy*

| | Estimated Folic Acid (μg/d) in Diet† | | |
|---|---|---|---|
| | <100 | 100+ | Total |
| No. of cases | 4 | 35 | 39 |
| Total | 555 | 11389 | 11944 |
| revalence per 1000 | 7.3 | 3.1 | ... |
| Prevalence ratio estimate | 1.00 | 0.42 | ... |
| 95% confidence interval | ... | 0.16-1.15 | ... |

*As reported in response to the question, "Did you take a multivitamin in the first 3 months of pregnancy?" If yes, "What week of pregnancy did you start the multivitamin?" (in practice based on date of last menstrual period).
†Those for whom information on multivitamin use was incomplete were excluded.

Table 7.—Multivariate Logistic Regression Analysis of Risk of Neural Tube Defect Comparing Women Who Did Not Use Multivitamins Containing Folic Acid in First 6 Weeks of Pregnancy With Those Who Did*

| | Prevalence Ratio | 95% Confidence Interval |
|---|---|---|
| Use of multivitamins containing folic acid (weeks 1-6) | | |
| Not† | 1.0 | ... |
| Yes | 0.3 | 0.15-0.63 |
| No. of previous pregnancies | | |
| 0† | 1.0 | ... |
| 1-2 | 1.4 | 0.64-3.0 |
| 3+ | 1.6 | 0.56-4.5 |
| History of neural tube defect | | |
| Not† | 1.0 | ... |
| Yes | 5.9 | 2.33-14.8 |
| Maternal age, y | | |
| <20† | 1.0 | ... |
| 20-29 | 1.3 | 0.16-11.55 |
| 30-39 | 0.7 | 0.35-1.30 |
| ≥40 | 0.8 | 0.10-6.58 |
| High school education or more | | |
| Not† | 1.0 | ... |
| Yes | 0.8 | 0.43-1.62 |
| Smoking | | |
| Not† | 1.0 | ... |
| Yes | 0.91 | 0.42-1.20 |
| Dietary folic acid intake, μg | | |
| <100† | 1.0 | ... |
| ≥100 | 0.70 | 0.22-2.22 |

*As reported in response to the question, "Did you take a multivitamin in the first 3 months of pregnancy?" If yes, "What week of pregnancy did you start the multivitamin?" (in practice based on date of last menstrual period).
†Reference category.

MVs in weeks 1 through 6 that contained vitamin A without folic acid. Among these women, 1 (1.5 per 1000) had an NTD outcome. There were 141 women who received an MV that contained folic acid but not vitamin A. None of these women had an NTD outcome. Similarly, there were few women unexposed to MVs in weeks 1 through 6 who reported a diet low in folic acid but high in vitamin A or vice versa. Therefore, there was insufficient information to evaluate the independent effects of these two nutrients.

We also reviewed the results relative to vitamins C, D, and E and zinc. Zinc intake did not appear to be associated with the risk of an NTD. There were insufficient data to evaluate the independent effects of vitamins C, D, and E.

Fewer than 2% of women reported use of supplemental folic acid or vitamin A alone. None of these women had an NTD outcome.

## COMMENT

We observed a substantially reduced risk of NTDs among women who took standard doses of MV/folic acid supplements during the first 6 weeks of pregnancy. The timing of the interviews in early pregnancy and the fact that the vast majority of mothers were unaware of pregnancy outcome minimized the possibility that this finding was due to their selective participation or biased recall of supplementation. In addition, detailed information on the precise timing and folic acid content of the MV preparation used (not necessarily available in other studies) allowed for more detailed evaluation of the relation of folic acid to NTDs. Multivariate control of potential confounding by number of previous pregnancies, family history of an NTD, maternal age, maternal education, current smoking, and dietary folic acid intake did not affect the PR estimate for folic acid supplementation during weeks 1 through 6 of pregnancy.

The current results are strikingly similar to those previously reported. In the study of Smithells et al,[10] among women who had a history of a prior NTD birth, the crude PR estimate for NTD comparing women who received MVs containing folic acid with those who did not was 0.18. In the current study, among women with a family history of an NTD, the PR estimate comparing women who received folic acid–containing MVs with women who did not was 0.27. In the study of Mulinare et al,[14] the relative risk estimate for NTD comparing women who used MVs both before and after conception with women who did not use MVs was 0.40. In the current study, the PR estimate for similar categories of women was 0.36. Our results, together with those of other studies, point to the importance of vitamin intake, in particular folic acid, in the prevention of NTDs and indicate that the apparent protective effect of MV supplements is limited to use in the first 6 weeks of pregnancy. Our results also suggest that in the absence of ingestion of MVs containing folic acid during the first 6 weeks of pregnancy, a diet deficient in folic acid may increase the risk of NTDs. Neither the current nor earlier studies could rule out the possibility that vitamin A, C, D, or E, either alone or in combination with folic acid, is protective against the development of NTDs, since a substantial majority of MVs contained all of these vitamins together with folic acid.

In the vast majority of instances, women in this study were interviewed before the outcome of pregnancy was known. Furthermore, since the questions related to recent history, possibilities for recall error were relatively small. Unawareness of the outcome reduces opportunities for biased responses that could distort the results.

Our study population is generally well educated and not statistically representative of the general population, but we have no reason to hypothesize that the higher risk for NTD among our subjects should make their response to folic acid biologically different from that of other women. On the other hand, the findings of this study cannot be statistically generalized to predict the public health effect of folic acid supplementation in a general population.

An additional observation of great in-

terest in this study was the strikingly higher prevalence of NTDs in women with a positive family history who did not take supplements (13.0 per 1000) compared with those with a family history who did (3.5 per 1000). Our finding is consistent with the idea that a genetic predisposition interacts with an essential nutrient deficiency, possibly a deficiency of folic acid.

We believe that the combined data from this and other studies[10,14] provide good evidence that folic acid–containing MVs taken during the first 6 weeks of pregnancy will prevent, by more than 50%, the occurrence of NTDs.

It should be emphasized that the proposed protective effect was present in the use of MVs in the standard prenatal doses. Effects of excessive doses of MVs may be deleterious, and there is no indication that they would be more protective than standard formulas.

## ADDENDUM

Since this study was submitted for publication, an article by Mills et al[20] on the periconceptional use of vitamins and NTDs has been published. In that study the relative risk estimate for having a baby with an NTD comparing women who used MVs early in pregnancy with those who did not was close to 1.0, and the authors concluded that "the periconceptional use of multivitamins or folate-containing supplements by American women does not decrease the risk of having an infant with a neural tube defect."

The important differences between the current study and that of Mills et al relate to the ascertainment and definition of the use of vitamins. In the current follow-up study, a history of MV use was obtained early in pregnancy, near the time of MV use and prior to knowledge of outcome in most cases. By contrast, in their case-control study, Mills et al obtained the history of MV use at least 1 month *after* delivery or after the anomaly was identified. Interviews taking place after pregnancy has been completed are subject to substantial recall error that tends to lead to a null result.[21]

In the current study, the precise week of first use of specific vitamin preparations was elicited. This included prenatal vitamins taken after the woman knew she was pregnant. By contrast, Mills et al obtained a history of MV use within the period 30 days prior to and 45 days after the estimated last menstrual period and explicitly *excluded* vitamins taken after the woman found out she was pregnant. Dr Mills has kindly sent a copy of the questionnaire used in their study. The question on vitamin supplementation reads, "First, during the time period . . . [30 days before estimated last menstrual period to 45 days after estimated last menstrual period] did you take any vitamins, minerals or other food supplements . . . ? These would be vitamins you were taking around the time you got pregnant, *not* the vitamins the doctor suggested you take when you found out you were pregnant." In the current study about 22% of women reported first use of MVs in the first 6 weeks of pregnancy. These women would presumably have been recorded as nonusers in the study of Mills et al.

If there is a protective effect of MV use, misclassification of relevant MV use in the study of Mills et al would have tended to lead to a null result.

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