

# Folate and neural tube defects<sup>1-3</sup>

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## ABSTRACT

A protective effect of folate against the development of neural tube defects (NTDs), specifically, anencephaly and spina bifida, is now well recognized, having been established by a chain of clinical research studies over the past half century. This article summarizes the more important of these studies, which have led to the current situation in which all women capable of becoming pregnant are urged to ingest folic acid regularly. The recommended intakes are 4 mg/d for those at high risk (by virtue of a previous NTD pregnancy outcome) and 0.4 mg/d for all others. However, a reduction in NTD births did not follow promulgation of these recommendations, and so folic acid fortification was mandated in the United States and some other countries. Although some controversy remains about the adequacy of fortification levels, the process was followed by significant improvement in folate indexes and a reduction of 25–30% in NTD frequency (about one-half of the proportion of cases assumed to be responsive to folate). The folate-NTD relation represents the only instance in which a congenital malformation can be prevented simply and consistently. Nevertheless, several research gaps remain: identification of the mechanism by which the defect occurs and how folate ameliorates it; characterization of the relative efficacy of food folate, folic acid added to foods, and folic acid by itself; delineation of the dose-response relations of folate and NTD prevention; and more precise quantification of the dose needed to prevent recurrences. *Am J Clin Nutr* 2007;85(suppl):285S–8S.

**KEY WORDS** Folate, folic acid, neural tube defect, spina bifida, anencephaly, congenital malformations, birth defects

## INTRODUCTION

A relation between folate and neural tube defects (NTDs), first suggested a little >50 y ago (1), has become recognized as a result of a large number of clinical investigations. Research in this area has received a great deal of attention for several reasons. In the first place, the relative importance of congenital malformations in general has increased as other major causes of fetal and infant morbidity and mortality (eg, preterm birth, infections) have come under control. Furthermore, NTDs are common (the most common malformations of the central nervous system and probably second only to cardiac defects among major congenital anomalies) and they represent a major public health problem by virtue of their mortality, morbidity, social cost, and human suffering. Finally, and perhaps most importantly, it now being clear that maternal folic acid supplementation prevents a substantial proportion of NTDs, we have identified for the first time a situation in which a congenital defect is clearly amenable to preventive measures.

## DEFINITIONS AND EPIDEMIOLOGIC CONSIDERATIONS

*Folate* is a generic term for a water-soluble, B-complex vitamin that serves as an essential coenzyme in single-carbon transfers in the metabolism of nucleic and amino acids and thus fills an important function in purine and pyrimidine metabolism. It occurs in certain natural foods as polyglutamate, a form less absorbed than free folate. *Folic acid* (a monoglutamic acid) is the oxidized and most active form of the vitamin; found rarely in food, it is the form used in vitamin preparations and food fortification. The distinction between food folate and folic acid is important because of differing bioavailability (ie, food folate is only about half as available as folic acid consumed on an empty stomach).

Isolated (ie, nonsyndromic) NTDs are congenital malformations that result from failure of the neural tube to close during embryogenesis. Normally, the primordial central nervous system begins as a plate of cells early in embryonic life, which folds on itself to form a tube. Closure of the tube occurs in the interval of days 21–28 postconception. Failure of closure results in NTDs, of which there are 2 main forms depending on whether the cranial or caudal end of the neural tube is involved. The most important cranial defect is *anencephaly*, in which the cerebral cortex and overlying bony calvarium fail to develop. The principal caudal defect is *spina bifida* (including meningocele and meningomyelocele), a midline defect in the lumbosacral region in which the spinal cord is dysplastic and the overlying spinal column is absent. Anencephaly, which accounts for about one-third of cases, is invariably lethal, with death either before or shortly after birth. Spina bifida (about two-thirds of NTDs) causes paraplegia, with paralysis of the lower extremities and impaired bladder and bowel function, but it is not usually fatal unless accompanied by other conditions.

Occurrence rates for NTDs show marked variability according to geographic area, socioeconomic status, and ethnic background. The highest rates, at least in the Western world, are found in Ireland and Scotland, where the frequency approaches 10 per 1000 births. US figures overall are much lower, although considerable geographic variation exists, with levels highest in the Southeast. Moreover, NTD births have steadily declined over the past 30 or 40 y, in part because of prenatal diagnosis and selective

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**TABLE 1**

Case-control occurrence studies of maternal folic acid (FA) ingestion and neural tube defects (NTDs) in offspring<sup>1</sup>

| Author and reference        | Intervention        | NTD reduction |
|-----------------------------|---------------------|---------------|
|                             |                     | %             |
| Mulinare et al, 1988 (2)    | MV + FA 0.8 mg      | -60           |
| Bower and Stanley, 1989 (3) | FF + MV + FA 0.8 mg | -75           |
| Mills et al, 1989 (4)       | FF + MV + FA 0.8 mg | Nil           |
| Werler et al, 1993 (5)      | MV + FA 0.4 mg      | -40           |
| Shaw et al, 1995 (6)        | FA                  | -35           |

<sup>1</sup> MV, multivitamins; FF, food folate.

abortion, which confounds the interpretation of results of putative preventive measures. Nevertheless, estimates are that  $\geq 2500$  infants with NTDs are born annually in the United States and that  $\approx 30\,000$  Americans are living with spina bifida.

NTDs are believed to reflect a combination of genetic predisposition and environmental influences. The wide geographic variations in incidence has suggested to many the importance of environmental factors and, among those factors, nutrition has been a particularly popular subject for both speculation and research. Most of the attention has focused on folic acid and this article will summarize the substantial body of clinical investigation since Hibbard first proposed the relation in 1964 (1).

### RETROSPECTIVE AND OTHER NONRANDOMIZED STUDIES

Several investigators have examined the relation between maternal folic acid ingestion and NTDs in offspring through the use of a case-control design, a retrospective method in which cases (in this instance NTD infants) are compared with controls (infants without NTDs born to otherwise identical women) for the exposure of interest (ie, folic acid supplements in early gestation). The case-control design offers a major advantage in providing risk estimates quickly and efficiently but has several disadvantages, chiefly its reliance on recall that may well differ between cases and controls.

The results of 5 case-control studies of the relation between maternal folic acid supplementation and NTDs in offspring reported between 1988 and 1995 are summarized in **Table 1**. All were occurrence studies (ie, they involved women without previous NTD-affected pregnancies) and most involved folic acid supplements begun before conception and continued through the first several weeks of gestation; in some instances, dietary folate intake was also evaluated. Mulinare et al (2), in a study in Atlanta, GA, found multiple vitamins containing up to 0.8 mg folic acid associated with a risk reduction of 60% in NTDs. Bower and Stanley (3), who evaluated spina bifida cases in Western Australia, reported high dietary folate intake and multivitamin supplement use associated with a 75% risk reduction. By contrast, Mills et al (4) found no protective effect of dietary folate and multivitamins plus 0.8 mg folic acid in a population-based case-control study in California and Illinois. However, Werler et al (5), in Boston, Philadelphia, and Toronto, and Shaw et al (6), in California, observed reductions of 40% and 35%, respectively, with multivitamins containing folic acid begun before conception. Thus, 4 of the 5 retrospective studies found statistically

significant reductions in risk in the range of 35–75%; the reason one study (4) found no effect is unclear.

One prospective (but nonrandomized) cohort study has been described. Milunsky et al (7) assessed dietary folate plus a folic acid-containing multivitamin begun  $\geq 1$  mo before conception and continued through the first trimester. There were 10 NTD pregnancies among 10 713 women who took folic acid compared with 39 among 11 944 women who took multivitamins without folic acid, a relative risk of 0.28 ( $P < 0.05$ ), meaning a risk reduction of 72%.

### CONTROLLED TRIALS

Several prospective trials, some randomized and some not, have been reported. All but one represented recurrence trials in that they involved women who had previously experienced NTD pregnancies. This design was presumably chosen because of the well-known increased risk of recurrence (ranging from 2% to 10%), meaning that statistical significance might be reached with smaller study populations. This is an understandable choice but, because 90% or more of NTD cases involve women who have not had an affected pregnancy previously, one that limits the relevance of the findings.

The first of the investigations considered here was a controlled but nonrandomized trial involving several institutions in Britain, reported by Smithells et al (8) in 1983. Nearly 1000 women with previous NTD pregnancies were enrolled and the supplement was 0.36 mg folic acid plus multivitamins, begun  $\geq 2$  mo before conception and continued through the first trimester. Three NTD pregnancies occurred among 454 supplemented women compared with 24 among 519 unsupplemented women, a relative risk of 0.14 ( $P < 0.05$ ). Although strongly suggestive, this report was not regarded as definitive because assignment to treatment groups was not random.

The definitive recurrence trial was the multiinstitutional study sponsored by the Medical Research Council (MRC; United Kingdom) and reported in an article published in 1991 (9). The design involved a masked, randomized comparison of folic acid (4 mg) and placebo, each begun  $\geq 1$  mo before conception and continued through the first trimester, in a large number of women who had previously had NTD-affected pregnancies. There were 6 NTD cases among 593 supplemented women compared with 21 among 692 placebo control women, a risk reduction of 72%; among women known to have initiated folic acid before conception, risk was reduced by 83%. The dose chosen for this study, 4 mg, was based on an earlier randomized recurrence study (10) that found a substantial risk reduction but one that did not reach statistical significance because of small sample size. The MRC investigators reasoned that they should test this dose because if no protection resulted with a lower dose it could always be argued that the dose was inadequate, whereas if there were no protection at 4 mg, there would be no basis to test a higher dose.

Other investigations yielded results consistent with those of the MRC study. As already noted, a 1981 randomized trial in Wales (10) found 2 NTDs among 60 supplemented women compared with 4 among 51 unsupplemented women, a difference that reached statistical significance only after the treated group was adjusted for noncompliance as determined by blood analysis. Another randomized trial, this one in Ireland (11), assessed recurrence with a folic acid supplement at a much lower dose (0.36 mg); there were no NTDs among 172 supplemented women and

1 in 89 unsupplemented women, again a nonsignificant difference with small sample size. A controlled but nonrandomized trial in Cuba (12) yielded consistent findings; there were no NTDs among 81 supplemented women (5 mg folic acid) and 4 in 114 unsupplemented women, a nonsignificant difference.

As noted earlier, all clinical trials but one assessed recurrence, whereas primary occurrence accounts for the most NTD cases and is therefore the more important public health problem. The only reported trial aimed at occurrence was reported by Czeizel and Dudas in 1992 (13). The subjects were Hungarian women planning pregnancy who were randomly assigned to a supplement of 0.8 mg folic acid plus multivitamins or a supplement of trace elements. No NTD cases occurred in 2104 supplemented women compared with 6 cases among 2052 unsupplemented women, a statistically significant difference ( $P = 0.029$ ).

### RECOMMENDATIONS AND POLICY DEVELOPMENT

The studies summarized above, especially the definitive randomized trials addressing recurrence and occurrence, quickly led to strong recommendations. Shortly after publication of the MRC study of recurrence in 1991 (9), the Centers for Disease Control and Prevention (CDC) advised women at high risk by virtue of a previous NTD pregnancy to plan subsequent pregnancies so that they could ingest folic acid at a level of 4 mg daily, beginning before conception and continuing through the first trimester of pregnancy (14). In 1992, with publication of the report of occurrence prevention by Czeizel and Dudas (13), CDC came out with the recommendation that all reproductive-aged women should consume 0.4 mg of folic acid in addition to a folate-rich diet (15).

The recommendations were quickly endorsed by the relevant professional organizations, specifically, the American College of Obstetricians and Gynecologists and the American Academy of Pediatrics (16), and the March of Dimes undertook an ambitious program of public education about folate and NTDs. The Food and Nutrition Board of the Institute of Medicine also recommended that all women capable of becoming pregnant should consume 0.4 mg of folate daily from supplements or fortified foods or a combination of the 2 in addition to consuming folate from a varied diet (17). (The distinction between “reproductive-aged women” and “women capable of becoming pregnant” is important because slightly less than one-half of US females aged 15–44 y are at risk of pregnancy; some 22% are permanently sterile, 20% are using a highly effective contraceptive, 5% are already pregnant or recently delivered, and 11% have never had sexual intercourse.) The Food and Nutrition Board noted further that most of the evidence about NTD reduction involved folic acid supplements, and whether food folate (even with the differing bioavailability taken into account) has similar effects remains to be shown.

The proportion of NTDs that can be prevented by periconceptional folic acid has not been established, but the general assumption is that it is probably in the area of 50–60%. The early experience following the recommendations accompanied by extensive lay and professional education campaigns proved disappointing, for there was no apparent effect on the number of NTD pregnancies (18). Even as long as a decade after most countries promulgated recommendations about periconceptional folic acid, the frequency of NTDs recorded in birth defect registries of 13 (mainly Western) European countries showed no discernible

improvement (19). A plausible explanation for this apparent discrepancy involves the fact that closure of the neural tube occurs quite early in embryonic life and therefore supplementation needs to be initiated even before conception to ensure adequate blood and tissue concentrations of the vitamin at the critical time. Given that only about one-half of pregnancies in the United States are planned and that some women are probably not even aware that they are pregnant at the stage at which the neural tube closes, this lack of effect should perhaps not be surprising.

In view of the disappointing results of recommendations and education, after hearings and much deliberation, the Food and Drug Administration decided to mandate fortification of enriched cereal grain products with folic acid at the level of 140  $\mu\text{g}$  per 100 g, an amount chosen to increase folic acid consumption by an average of 0.1 mg. Fortification was optional between March 1996 and December 1997 and became mandatory on 1 January 1998. Subsequently, several studies have reported improvement in blood folate concentrations (18, 20), and NTD rates have declined by 20–30% (21–23). Some have argued, however, that present fortification levels are inadequate and an increase would prevent many more NTD cases (24, 25).

### RESEARCH NEEDS


The most important research need relates to our limited understanding of the mechanism of NTD development and how folate influences this mechanism. The need for mechanistic knowledge is more than academic, for it could well improve results with folic acid supplementation by identifying high-risk subjects or pointing out ways to develop more effective interventions. If, for example, the problem is some genetic aberration in metabolism, the approach would be different from if it was nutritional deficiency.

A second research need, and one related in some ways to the first, is determination of the relative efficacy of food folate, folic acid added to foods, and supplemental folic acid alone. As noted earlier, most of the evidence and all of the definitive evidence involves supplemental folic acid. Thus, the role of the diet and fortified foods remains questionable.

A third research need is accurate delineation of the dose-response relation of folic acid protection against NTDs. The current recommended doses, 4 mg for recurrence and 0.4 mg for occurrence, were chosen arbitrarily and it is certainly possible that better results might accrue with higher doses or that equally good results might result with lower doses. A theoretical model (26), based on reported experience, suggests that the dose of folic acid of 0.4 mg as currently recommended for all women will reduce NTD incidence by only 36%, whereas 5 mg will yield a decline of 85%. However, this requires empirical confirmation. Additionally, better understanding of the dose-response relation would inform the current debate about appropriate levels of food fortification.

Finally, the dose recommended for women who have previously had an NTD pregnancy, 4 mg, needs to be examined. This level was based on the MRC study, which tested only that dose because it had been reported previously to be effective. The problem with the dose is that the largest folic acid tablet manufactured is 1 mg, and thus women must take 4 tablets daily, which may impair compliance. Furthermore, 4 mg is 4 times the



Tolerable Upper Intake Level for adults. Therefore, it is important to know whether recurrent NTDs can be prevented with <4 mg daily. 

During 2004 and 2005, Roy M Pitkin was a member of the scientific advisory board of Herbalife International of America Inc.

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