Trans-HHS Workshop:
Diet, DNA Methylation Processes and Health

Folic Acid Supplementation and Prevention of Birth Defects[1,2]

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ABSTRACT Based on animal studies, epidemiologic studies and intervention trials, maternal folic acid is known to be protective for neural tube defects (NTD), primarily spina bifida and anencephalus. To reduce the risk of NTD, the U.S. Food and Drug Administration mandated that all enriched cereal grain products be fortified with folic acid as of January 1998. Recent data demonstrate that this public health action is associated with increased folate blood levels among U.S. women of childbearing age and that the national rate of spina bifida has decreased by 20%. Rates of anencephaly appear not to have declined. Epidemiologic data on use of folate and folate antagonists have also implicated folic acid in prevention of other birth defects such as facial clefts and cardiac and limb defects. Dietary folic acid is likely to be inadequate for maximal protection against NTD. Because about half of pregnancies in the U.S. are unplanned, according to the March of Dimes, birth defect prevention includes a recommended daily dose of 400 μg synthetic folic acid for women of childbearing age. Uniform compliance is estimated to decrease the incidence of NTD by up to 70%. This could reduce the overall incidence from 2 to 0.6 per 1000 pregnancies and prevent disease in ~2000 babies per year in the U.S. Four thousand micrograms of folic acid per day is recommended for women with previous pregnancies affected by NTD. J. Nutr. 132: 2356S–2360S, 2002.

KEY WORDS: • *folate* • *folic acid* • *neural tube defects* • *birth defects* • *spina bifida* • *ancencephalus*

Birth defects are the leading cause of infant mortality and have been so for the past 25 y, causing 22% of all infant deaths. Approximately 3–4% of all live births are affected by a birth defect; the etiologies of most of them are unknown (1,2). The relationship between serious birth defects and their prevention by folic acid is well established. Much of the birth defect data focus on the well-substantiated relationship between folic acid and prevention of neural tube defects (NTD),[4] and this emphasis is reflected in this review.

The neural tube is the embryonic structure that develops into the brain and spinal cord. This structure, which starts out as a tiny ribbon of tissue, normally folds inward to form a closed tube by the 28th day after conception. NTDs occur when the embryonic neural tube fails to completely close during development. NTDs are malformations of the developing brain and spine, most commonly spina bifida and anencephaly. Spina bifida ("open spine") is a defect of the spine that can causes paralysis and hydrocephalus. Children with the severe form of spina bifida have some degree of leg paralysis and impaired bladder and bowel control. Anencephaly is a fatal condition in which a baby is born with a severely underdeveloped brain and skull. Absence of the majority of the brain and surrounding tissue results in death before or shortly after birth. Anencephaly is responsible for about 30% of NTDs (2).

There are an estimated 4000 pregnancies affected each year by an NTD, with some fetal demise through spontaneous or induced losses. In the past few years, birth defect surveillance through the efforts of the U.S. Centers for Disease Control and Prevention (CDC) has recorded 2500 live births per year in the U.S. with NTDs (~1 in 1600 births) (2). Most recently, this number has decreased to 2000 per year (3), as will be described below. Seven percent of infant deaths from birth defects are a result of NTDs.

Because NTDs occur early in fetal development, prevention would be most effective at the earliest phase of pregnancy, often before women know they are pregnant. Hence, the best public health interventions must target all fertile women: millions of women who are of childbearing age.

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[4] Abbreviations used: CDC, U.S. Centers for Disease Control and Prevention; DHFR, dihydrofolate reductase; FR, folate receptor; MRC, Medical Research Council; MTHFR, methylenetetrahydrofolate reductase; NHANES, National Health and Nutrition Examination Survey; NTD, neural tube defects; RBC, red blood cell.

DISCUSSION

25-y history of NTD and folate deficiency

A number of large-population studies associating maternal folate deficiency with NTDs serve as the scientific basis for communication of prevention. Folic acid deficiency was suspected as contributing to NTDs as far back as the 1970s, but the conclusive proof was not demonstrated by large intervention studies until the mid-1980s and early 1990s. In 1976 Smithells et al. (4) published an early paper suggesting that deficiencies in folic acid and/or other micronutrients may predispose developing fetuses to NTDs. In a subsequent paper in 1980, Smithells et al. (5) reported on possible prevention of NTDs by periconceptional vitamin supplementation. In the 1990s, multiple intervention trials demonstrated a substantial reduction in the incidence of NTDs with preconception folic acid treatment. These studies include a British Medical Research Council (MRC) study in which women with a previous history of a pregnancy affected by an NTD were treated with large doses of folic acid (6). This intervention resulted in a 72% reduction in risk of a second NTD-affected pregnancy. Prevention of similar magnitude was reported in a European intervention trial of previously unaffected women (7). Eight of nine studies published from 1981 to 1992 demonstrated a 50–70% reduction in NTDs with preconceptional folic acid intake (reviewed in Refs. 8–10). Perhaps, ironically, all of this progress has occurred even as the biochemical basis of NTDs is still not known, although the relationship to healthy DNA replication and the importance of decreasing homocysteine is under consideration.

In a 1999 study, the CDC reported its results of a randomized control trial of folic acid in China in two distinct regional populations (11). Women took 400 μg of folic acid daily from the time of their premarital examination until the end of their first trimester of pregnancy. Among the fetuses or infants (at least 20 wk gestational age) of women who did not take any folic acid, the rates of NTDs were 4.8 per 1000 pregnancies in the northern region and 1.0 per 1000 in the southern region. Among the fetuses or infants of the women with periconceptional use of folic acid, the rates were 1.0 per 1000 in the northern region and 0.6 per 1000 in the southern region. Regional differences in NTD rates, both with and without folic acid treatment, are likely attributable to dietary and genetic differences. The authors note: "The greatest reduction in risk occurred among the fetuses or infants of a subgroup of women in the northern region with periconceptional use who took folic acid pills >80% of the time" (11).

Mechanisms of NTD and folic acid prevention

Increased serum or red blood cell (RBC) folate concentrations are associated with decreased risk of NTDs. Serum homocysteine levels are inversely proportional to folate levels, and homocysteine levels increase with folate depletion. Hence one of the big mysteries is whether birth defects, including NTDs, are due to low folate or high homocysteine levels, to both, or to other downstream effects. Inborn errors of folate and homocysteine metabolism may be involved in the etiology of NTDs. Although some cases of NTDs are induced by hyperhomocysteinemia resulting from genetic polymorphism of a thermolabile enzyme, the cause of most NTDs is unknown (7,12). Several studies have demonstrated that mutations in the methylenetetrahydrofolate reductase (MTHFR; EC 1.5.1.20) gene represent genetic risk factors for NTDs (13). There are additional folate-related genes that contribute to NTD pathogenesis, including mutant forms of folate receptors (FR) such as FRα. Genetic associations between molecular variations of the FRα gene and NTDs have been documented, suggesting that this gene may be a risk factor for human NTD (12).

Several animal models are being used to investigate the biochemical and molecular basis of NTDs (reviewed in Ref. 14). Animal models have raised the possibility of an important role for the folate binding proteins in NTDs (15). Neural tube closure and proper cardiac development in a chick embryo model (16) were impaired in a dose-dependent manner by direct treatment with homocysteine. These results support the hypothesis that homocysteine itself causes dysmorphogenesis of the neural tube, suggesting that the effects of folate may be, at least in part, mediated through homocysteine.

Why is the neural tube so sensitive to maternal folate or homocysteine? As described below, other developing organ systems can also be seriously affected, but probably to somewhat lesser extents. One possible mechanism relates to the important role of folate for one carbon metabolism in nucleic acid and amino acid biosynthesis in rapidly dividing cells. Another theory implicates impaired cellular remodeling (apoptosis) of the neural tube in folate-associated NTDs (14).

Ethnic and genetic variations in NTD risk

Population-based research has revealed the heterogeneous frequency of defects in folic acid metabolism, although the key factors remain poorly characterized. The most striking report is the 40% increase in incidence of NTDs in offspring of Hispanic descent compared to Caucasians and African Americans (**Table 1**) (17,18). One component of ethnic variations in susceptibilities to NTDs may be dietary factors. For example, Hispanic women may take fewer vitamin supplements and some may eat fewer folate-fortified wheat grains in favor of generally nonfortified products such as tortillas made from corn. It is likely that differences in the incidence of NTDs within U.S. and other populations may also reflect differing genetic susceptibilities. These differences may be due, at least in part, to hitherto unrecognized polymorphisms in genes for proteins of folate metabolism involved in absorption, activation or use or differences in effects from/on homocysteine or other pathways and mediators.

Folate and non-NTD birth defects

While the relationship between folate and NTD is well established, folate deficiency may also be related to other serious birth defects. A randomized control trial of periconception folic acid–containing multivitamin supplementation demonstrated a reduced occurrence of urinary tract and cardiovascular congenital abnormalities and congenital limb deficiencies (7). The occurrence of orofacial cleftings, cleft lip and cleft palate, may also be reduced by a high dose of folic acid. This preventive effect may be the result of other mechanisms of action. One investigator has suggested that cleftings may be related to the compensation of impaired mitosis caused by a folate deficiency (7). A large-population CDC study demonstrating protection against NTDs by folic acid (11) also analyzed other birth defects. The preliminary data suggest that periconceptional folic acid protects against all major defects as a group, atrial and ventricular septal defects, limb deformities, omphalocele and cleft lip and/or cleft palate.

To further explore the relationship between folate and non-NTD birth defects, a large epidemiologic database of women taking medications during pregnancy was examined for the risks of non-NTD birth defects such as oral clefts and

TABLE 1

Relative risk for spina bifida by race/ethnicity, U.S. 1983–1990[1]

Race/ethnicity	Adjusted relative risk (95% CI)
White	1.00
Black	0.80 (0.72–0.88)
Hispanic	1.41 (1.26–1.58)
Asian/Pacific Islander	0.51 (0.38–0.70)
Native American	1.13 (0.74–1.74)

[1] The rates for Native Americans are based on small numbers and may reflect statistical artifacts. Data are based on 16 state-based birth defects surveillance systems.
Source: CDC, Neural Tube Defect Surveillance, 2001.
Prepared by The March of Dimes Perinatal Data Center, 1999.

cardiovascular and urinary tract defects in babies born to mothers taking two general classes of folate antagonists (19,20). The analysis revealed two important findings: 1) a 2- to 3.5-fold increased risk of these birth defects following first trimester treatment with folate antagonists, with the timing of the exposure during pregnancy strengthening the argument for causality; and 2) in women taking prenatal multivitamins containing folic acid, minimization of risk from drugs that act by inhibiting dihydrofolate reductase (DHFR; EC 1.5.1.3) but not from anticonvulsant drugs. This latter group presumably exert their antifolate activity through some other mechanism(s). The former group of medications includes relatively commonly prescribed medications trimethoprim, triamterene, methotrexate, aminopterin and sulfasalazine. The anticonvulsants studied were carbamazepine, phenytoin, phenobarbital and primidone.

The striking protective effects of folic acid on birth defects for mothers taking DHFR inhibitors help confirm the importance of folic acid, and its deficiencies, in the development of non-NTDs as well as NTDs (19,20). Equally striking was the finding that folic acid had no appreciable protective effect in mothers taking antiepileptics. These data suggest that antiepileptics interfere at other levels of folate metabolism. Perhaps larger doses or a longer duration of folic acid treatment prior to conception would reduce the risk of birth defects in their offspring. Of course, drug dosing may be affected by folic acid, so drug levels would need to be studied in experimental and clinical settings. The mothers in the study with affected babies may have had increased susceptibilities to folic acid deficiencies that were unmasked by drug provocation. Clearly, folic acid supplementation must be given to all women of childbearing age taking antifolate medications. Moreover, the risks of birth defects must be weighed for women needing to take anticonvulsants. Modified pharmacologic approaches to prevention may be effective, such as use of other forms or higher doses of folic acid.

Folate deficiency is associated with chromosomal breakage in vitro (21). This raises the possibility that at least some of the chromosomal abnormalities and their attendant birth defects may be traced to folate deficiencies. A recent case report speculates that abnormal maternal folate metabolism (a mutation in the MTHFR gene), combined with folate deficiency, may have been predisposing factors for a child born with both Down syndrome and spina bifida (22). The trisomy 21 may have been promoted through folate-dependent mechanisms of chromosomal instability and meiotic nondisjunction. This case may catalyze research on the potential link between disturbed folate metabolism and meiotic (and possibly mitotic) chromosomal errors (21).

Folic acid protection

As described above, a number of studies have shown an association between increased periconceptional intake of folic acid and a reduced incidence of structural developmental anomalies. Increased folic acid intake is associated with significantly fewer NTDs in combination with another major birth defect, particularly orofacial clefts, cardiac and limb defects and omphalocele (7). The combined data, placed on a background of strong biologic plausibility, make it highly likely that periconceptional intake of folic acid can reduce the risk of a variety of major birth defects.

Folic acid from vitamin supplements and fortified foods is more readily absorbed than is folate naturally contained in food. It is estimated that 50% of food folate is absorbed while ~85% of folic acid in fortified foods and 100% of the folic acid in vitamin supplements is absorbed (10). There are two general forms of folic acid used in biological studies: 1) folic acid, a pteroylglutamic acid synthetic, used in supplements and fortified foods; and 2) folates, substituted derivatives of folic acid that appear naturally in foods or that are generated in vivo.

Increasing blood folate levels

The MRC European trial (cited above) concluded in 1991 that "folic acid supplementation starting before pregnancy can now be firmly recommended for all women who have had an affected pregnancy, and public health measures should be taken to ensure that the diet of all women who may bear children contains an adequate amount of folic acid" (6). These data led the U.S. Public Health Service in 1992 to recommend that all fertile women of child-bearing age consume 0.4 mg of folate daily to reduce the risk of NTDs, and women at increased risk—those with previous NTD pregnancies—should consult their doctor before conception (23).

Folate levels can be determined in several ways for individual and population studies. RBC folate levels generally reflect longer-term tissue stores, whereas serum or plasma levels are more indicative of short-term dietary intake (23,24,7). Thus, serum folate concentration may be a valid diagnostic test, particularly if done in conjunction with RBC folate values. Serum studies are technically less demanding and less dependent on sample handling and thus may be at times more appropriate for population field testing.

As of January 1998, the U.S. Food and Drug Administration has required that all enriched flour, rice, pasta, cornmeal and other grain products contain 140 μg of folic acid per 100 g of product. The effects of fortification on folate levels among nonpregnant women of childbearing age (aged 15–44 y) were assessed. The CDC compared serum and RBC folate concentrations for childbearing-aged women who participated in the 1999 National Health and Nutrition Examination Survey (NHANES)—postfortification—to prefortification levels obtained from the third NHANES (NHANES III, 1988–1994). The findings indicate substantial increases in serum and RBC folate concentrations among women of childbearing age. Since NHANES III, mean serum folate concentrations have increased 2.5-fold, from 6.3 to 16.2 μg/mL, and mean RBC concentrations have increased 1.7-fold, from 181 to 315 μg/L (25). Similarly, the roughly bell-shaped distribution of folate levels in this population has shifted overall to higher levels. Findings by other recent studies support the CDC data docu-

menting increased folate levels in women of childbearing age (25).

One of the stated goals of Healthy People 2010 (26) is to increase RBC folate levels in women aged 15–44 to 220 μg/L, so this goal has largely been met. These increased folate levels are largely attributable to the "passive" approach to folate supplementation, namely folic acid fortification, with some effects from folic acid supplementation (3).

Impact of folic acid on risk for NTDs

Since the late 1960s, the reported prevalence of anencephaly and spina bifida in the U.S. has declined. These declines likely represent two factors: better prenatal screening and diagnosis for NTDs and improved maternal oral intake through diet and vitamins. Similar trends have been reported in the United Kingdom (27). A recent published report from the CDC in 2001 shows that as blood serum folate levels have increased over the past few years, the rate of NTDs appears to have declined by 19% (3). To ascertain the true impacts from folic acid, birth certificate surveillance data for these defects may need to include data from pregnancies that are prenatally diagnosed and then lost or terminated (2).

The rate of spina bifida has declined significantly between 1995 and 1999 (3,28). The rate of spina bifida in 1999 was 20.8 per 100,000 live births, with 735 reported cases of spina bifida. In contrast, after a decline in the early part of the decade, the anencephalus rate has been stable since 1994. The rate of anencephalus in 1999 was 10.6 per 100,000 live births, with 373 reports of anencephalus in 1999 (28). The Healthy People 2010 Maternal-Child Health objective related to folic acid and NTDs is to reduce the incidence of spina bifida and other NTDs to no more than 3 per 10,000 live births (26). While this goal appears to have been nearly achieved, there are two approaches that provide the prospect of further reductions: to further increase women's folate levels and to discern and treat the plateau rate of anencephaly.

Using data from a case-control study, the risk of an NTD has been found to be inversely proportional to early pregnancy maternal RBC folate levels in a continuous dose-response relationship (9). Whether there is a plateau effect at the highest RBC folate levels (>600 nmol/L) is controversial. The "optimal" blood folate levels in women of childbearing age (how high these levels, both the mean or the distribution) need to be to maximally prevent NTDs and other serious birth defects are unknown. The particular susceptibility to the more severe NTD, anencephaly, is unknown. This defect may correlate with a more severe folate deficiency. Alternatively, other genetic and/or environmental factors may make some fetuses more prone to one or the other defect. These factors may eventually be discerned through animal and genetic epidemiologic research.

Public health messages

In 1998, the Food and Nutrition Board of the Institute of Medicine made a specific recommendation that all women capable of becoming pregnant should take 400 μg of synthetic folic acid per day from fortified foods and supplements, in addition to dietary folate, to ensure adequate amounts are taken (**Table 2**) (23).

Since 1995 the March of Dimes, the CDC and several other organizations of health care professionals have conducted public and professional education campaigns urging women of childbearing age to consume folic acid daily beginning before conception and continuing into the early months of pregnancy. Beginning in 1997, the National Council for Folic Acid (the CDC, the March of Dimes and their partners) embarked on a national campaign to increase consumption of folic acid as a supplement, in a multivitamin and from fortified foods. The March of Dimes, together with the National Council on Folic Acid, is working toward this goal through mass media placements to raise awareness, in particular among the general public and women of childbearing age, about the importance of folic acid for preventing birth defects. Media efforts are comprised of national and local advertising campaigns, publicity and promotion strategies and activities, community action and professional education. This message continues to be that, to ensure adequate maternal folate levels in the 1st mo postconception, women of childbearing age should take 400 μg of synthetic folic acid per day as part of a healthy diet.

TABLE 2

Folic acid recommendations IOM 1998[1]

	Recommended daily folic acid intake
	μg
Men (14 y and older)	400 (any source)
Women (14 y and older)	400 (synthetic + food)
Pregnancy	600 (synthetic + food)
Lactation	500 (any source)
Previous NTD	4000 (synthetic)

[1] Institute of Medicine report, 1997.

Remaining issues

After 25 years of work on birth defect prevention and folate, preventing all of the folate-dependent birth defects—NTDs and others—requires more research on a number of issues. It is our hope that these questions will continue to be raised and attempts will continue to be made to find answers through appropriate research programs involving animal models, epidemiologic studies and ethically sound clinical intervention trials. These complex questions include the following: *1*) What determines the neural tube sensitivity to folate and/or homocysteine? *2*) What are the mechanisms of folic acid protection? *3*) What are the mechanisms for folic acid resistance and how can they be treated? *4*) What are the downstream or different pathways involved in folate and homocysteine metabolism? 5) How can anencephaly be prevented? 6) What are the optimal fortification levels, supplement doses and blood levels for women of childbearing age? *7*) How can these data and concepts translate into population screening for prevention?

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