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The Maternal Diet Can Prevent Neural Tube Defects

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THE MATERNAL DIET CAN PREVENT NEURAL TUBE DEFECTS

by

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Abstract

Maternal diet and nutritional status have a direct impact on pregnancy course and outcome. Nutrition is critical to the development of the human embryo and fetus. Each year in the United States, approximately 4000 pregnancies are affected by neural tube defects (NTD). The most important finding in recent years has been the relationship between maternal folic acid status and NTDs. Other studies have found an association between zinc deficiency and an increased risk for having an NTD-affected pregnancy. Furthermore, some of the latest research has linked maternal obesity with the development of NTDs. The cumulative data from several studies have clearly indicated enormous savings in direct medical costs associated with the prevention of NTDs. Improving the nutritional status of women of childbearing age can help to prevent NTDs, resulting in substantial savings.

Of all periods in the life cycle, pregnancy is one of the most critical. When a woman becomes pregnant, all the experiences of her past join with those of the present to lay the foundation of a new life whose potential, in turn, will influence the welfare of generations to come. At no other time does the well being of one individual so directly depend on the well being of another as it does during pregnancy (1). Between the moment of conception and the moment of birth, innumerable events determine the course and outcome of fetal development and, ultimately, the health of the newborn infant. Many of these events are beyond a woman's control, but a woman's nutrition throughout the teen and adult years, including pregnancy is within her control. Nutrition is critical to maternal health, prenatal development, and the development of the child long after birth (2). During pregnancy, malnutrition exerts its effects by acting on critical periods in the development of the embryo and fetus. A woman's nutrient needs during pregnancy are higher than at any other time in her adult life and are greater for certain nutrients than for others (2). The role of specific nutrients and nutrition in general, to promote optimal outcomes of pregnancy for both infants and mothers is under study (3). One area currently being studied is the role the maternal diet plays in altering the risk for the development of neural tube defects (4).

Each year in the United States, approximately 4000 pregnancies are affected by neural tube defects. The term neural tube defect (NTD) applies to any malformation of the embryonic brain and/or spinal cord. The embryonic neural tube is formed 20 to 28 days after fertilization, which is generally before the pregnancy is confirmed (5). The various forms of NTD are characterized by incomplete development of the central nervous system and its closely related structures. They may range in severity from

anencephaly, to incomplete formation of the spinal cord, cranial bones, vertebral arches, meninges, and overlying skin (6). Anencephaly, the absence of the cerebral hemispheres, is incompatible with life. The absent brain is sometimes replaced by malformed cystic neural tissue, which may be exposed or covered with skin. Varying portions of the brainstem and spinal cord may be missing or malformed. It usually results in death in utero or during the first few days after birth (7). Another NTD is an encephalocele, which is a protrusion of nervous tissue and meninges through a skull defect, associated with incomplete closure of the cranial vault. Most encephaloceles can be removed without leaving a major functional disability (7).

Spina bifida is a defective closure of the vertebral column. It is one of the most serious NTD associated with prolonged life. Its severity varies from the occult type to a completely open spine (rachischisis) with severe neurologic disability and death. In spina bifida cystica, the protruding sac can contain meninges (meningocele), spinal cord (myelocele), or both (myelomeningocele) (7). Spina bifida is most common in the lumbar, low thoracic, or sacral region, and usually extends for 3 to 6 vertebral segments. When the spinal cord or lumbosacral nerve roots are involved in the spina bifida, varying degrees of paralysis occur below the involved level. The paralysis usually affects bladder and rectal functions. The neurogenic bladder causes an incomplete bladder emptying and a high incidence of urinary tract infection, which may, in turn, lead to renal disease (8).

Neural tube defects are the most common of severe birth defects. In 1992, more than 4,600 babies with a primary diagnosis of NTD were discharged from U.S. hospitals, representing $141 million in hospital charges (9). One leading manifestation of NTD, spina bifida, costs as much as $200 million annually in direct medical costs. The lifetime

economic cost to society per person with spina bifida is about $258,000 (9). Studies have shown the B vitamin, folic acid, to reduce the occurrence of spina bifida and anencephaly by at least 50% when consumed daily before conception and during early pregnancy (5,9,10,11). Based on the 1995 manufacture's suggested retail prices for multivitamin supplements with folate ($25 per person), the total annual cost of supplementation for all pregnant women is approximately $162 million. Thus, the enormous lifetime cost of NTD offsets the cost of supplements (9).

Folate plays a crucial role in the development of the central nervous system during the early weeks of gestation. The neural tube forms from the 17^{th} to 19^{th} day after conception and closes by the 20^{th} to the 28^{th} day (11). For fighting NTD, folic acid needs to be available in adequate amounts during the first part of pregnancy (12). Because at least 50% of pregnancies are unplanned, the daily intake of adequate folic acid by all women of childbearing age is important (13).

Folate has an essential role in the transport of single-carbon units needed for DNA synthesis, cell division, and tissue growth. Folate also plays a role in DNA methylation, an important factor in genetic expression and maintenance of chromosome structure (10). The major form of folate in serum is 5-methyl-tetrahydrofolate. This is an important transport form of folate and is the principal methyl donor for methionine synthase, a B_{12}-requiring enzyme. Methionine, an essential amino acid, loses its methyl group by reactions involving S-adenosylmethionine. The resulting homocysteine can be remethylated in the presence of adequate amounts of 5-methyl-tetrahydrofolate and vitamin B_{12} to regenerate methionine, thus conserving the supply of this essential nutrient. Failure of regeneration may result in the accumulation of potentially toxic

homocysteine levels (5). During the period of neurulation, a time of rapid cell proliferation and complex developmental processes, flaws in these folate-mediated one-carbon transfers could be detrimental to normal development and closure of the neural tube (13).

Recent information may help to explain folic acid's role in the prevention of NTDs. First, in recent studies, homocysteine has been found to be elevated, in both the plasma and amniotic fluid, in women with NTD-affected pregnancies as compared with control subjects (4,5,6). Second, it appears that a frank deficiency of folic acid is not required in women to have an NTD-affected pregnancy (5,6). However, there does appear to be a trend toward lower folic acid levels in women with NTD-affected pregnancies as compared with control patients. This information suggests that there may be a defect or block in a metabolic step or steps involving homocysteine metabolism, causing an elevated homocysteine level. Higher homocysteine levels could indicate inadequate remethylation to form methionine (5,6). One explanation may be that, at the critical period of neural tube formation, the genetic information is sensitive to the metabolic consequences of a lower than normal folate status. Perhaps, high homocysteine, low methionine, or some other signal results in the incomplete closure of the tube (5). At this time, the exact mechanism by which folate affects NTD is not understood, but the benefits of folate for women of childbearing age are clear (1,2,3,4,5,6,9,10,11,12,13).

Data from several studies have shown that NTD risk can be significantly lowered when women take a folic acid-containing multivitamin supplement daily during the periconceptional period (1,2,3,4,5,6,9,10,11,12,13). A summary of four intervention

studies and four observational studies of folic acid and neural tube defects can be found in Appendix A and B, respectively (6). All of the four intervention studies indicated a reduction in risk for NTD-affected pregnancy in folic acid supplemented women with a prior NTD-affected pregnancy. The women in the intervention studies were given supplements ranging from 0.36-5 mg of folic acid, taken from one month before conception through the first trimester. The reduction in risk ranged from 60% to a complete protective effect in the women supplemented with 5 mg of folic acid (6). Three of the four observational studies showed a lowered risk of NTDs for women who had not had a prior NTD-affected pregnancy and who consumed 0.4-0.8 mg of folic acid daily from multivitamin supplements (6). The study that reported the absence of a relationship between the periconceptional use of vitamins and the occurrence of NTD lacked specific information on dietary intake as well as biochemical indicators of vitamin status. It is possible that women included in the NTD group had risk factors that could not be overcome by multivitamin supplementation (e.g. older age) and that women from less economically advantaged backgrounds may not have been equally represented in this study (5,6).

In general, studies have shown that the level of folic acid supplementation most often associated with NTD prevention is 0.4-0.8 mg per day (4,5,6,9,10,11,12,13). Butterworth (5) reviewed several studies that have shown women who consume adequate folate from natural sources or from foods fortified with folic acid, have a reduced risk of having a NTD-affected pregnancy. However, the risk reductions were not as great or as consistent as that seen in folate-supplemented women. A 1997 survey of 2,001 women aged 18-45 found that 66% of the women surveyed had heard about the importance of

folic acid, but only 30% took a multivitamin containing folic acid (10). This indicates that more effort is needed to educate women of childbearing age that folic acid may lower the risk of having a baby with a neural tube abnormality (10).

In 1992, the Public Health Service recommended that all women of childbearing age who are capable of becoming pregnant consume 0.4 mg of folic acid daily (10). In August of 1991, the Center for Disease Control issued a separate guideline for women who have had a prior pregnancy affected by NTDs and who are planning to start a new pregnancy. Since the risk of a subsequent child having a NTD is high, the guideline called for the consumption of a 4.0 mg daily dose of folic acid, from at least one month before conception through the first three months of pregnancy (6). One of the most rigorously conducted studies on folate supplementation was a randomized controlled trial sponsored by the British Medical Research Council. The study showed that high-dose folic acid supplements (4.0 mg per day) used by women who had a prior NTD-affected pregnancy reduced the risk of having a subsequent NTD-affected pregnancy by 70% (6).

Several forms of folate are found naturally in food; the majority of these are tetrahydro derivatives bearing multiple glutamic acid units in gamma peptide linkage. This configuration requires cleavage by an enzyme known as conjugase to bring about optimal bioavailability and intestinal absorption (5). Inhibitory compounds known as conjugase inhibitors affect availability of folate from food. The conjugase inhibitors prevent the digestion of folate, which is necessary for folate absorption (15). As much as 50 to 95% of food folate may be destroyed during household preparation, food processing, and storage (14). The synthetic form of folate used in vitamin supplements does not require the conjugase digestion to promote intestinal absorption (5).

In March of 1996, the Food and Drug Administration issued regulations requiring that folic acid be added to enriched cereal grain products, such as flours, cereals, corn meals, pasta, and rice, by January of 1998. Although improved dietary intake of folic acid has been suggested as the optimal approach to increase folic acid intake, it is unlikely that necessary population-wide dietary changes can be easily accomplished. Dietary folate is absorbed only 50% as effectively as supplemental folic acid, so at least 0.8 mg of dietary folate would be needed on a daily basis to obtain the suggested 0.4 mg daily intake (13). The average consumption of dietary folate by women of childbearing age in the United States has been estimated to be about 0.2 mg per day (13). Since the average consumption among women in the United States represents a fourfold deficit in meeting the suggested amount, at this time supplemental folic acid appears necessary to provide protection against NTDs (13).

Folic acid supplement pills containing 0.4 mg of folic acid are readily available, as are multivitamin preparations containing folic acid. The larger doses (4.0 mg) of folic acid may require a prescription. Folic acid is a water soluble vitamin, so any excess consumed is usually excreted in the urine. The effects of higher doses are not well known, although they include complicating the diagnosis of vitamin B_{12} deficiency in certain people. Irreversible neurologic damage may occur if B_{12} is not diagnosed and treated. Therefore, women should be careful to keep their total daily folate consumption at less than 1 mg per day, unless advised otherwise by their physician (6). It is important to caution against unreasonably high expectations of folic acid. Supplementation is expected to avert some, but not all, NTDs (6,9). Thus, it is apparent that there may be other factors involved in the development of NTDs.

It has been postulated that dietary zinc deficiency may be teratogenic, and may be a contributing factor in the development of NTDs (16). Zinc is known to play a critical role as a cofactor for numerous enzyme functions, including protein synthesis, nucleic acid metabolism, and gene expression (17). Studies have shown that in the presence of high concentrations of folate, zinc and folate form a complex in the intestinal lumen. This complex then inhibits zinc absorption (15). Furthermore, the hydrolysis of dietary folates requires a zinc-dependent enzyme in order to be metabolized and absorbed. Folic acid has been shown to impair zinc absorption when the dietary zinc intake is low, but not when zinc intake is high (18).

The requirement for zinc increases during pregnancy. The total incremental zinc need for pregnancy in humans is about 15 mg. However, women do not appear to increase their zinc intakes during pregnancy. The Recommended Dietary Allowance (RDA) for non-pregnant women is 12 mg of zinc per day (14). The average daily intake remains constant at near 10 mg. Endogenous fecal zinc losses have not been measured in pregnant women, but urinary zinc losses increase during pregnancy. By late pregnancy, the concentration of circulating zinc is about 15 to 35% lower in pregnant women than in nonpregnant women (18). This decline occurs as early as the first gestational month, which is when the final closure of the neural tube takes place.

If women do not increase their zinc intakes, adequate delivery of zinc to the developing fetus must be achieved by adjustments in zinc utilization (18). Studies have failed to show an improvement in zinc absorption in human pregnancy. Unfortunately, it seems that a zinc-deficient diet does not effectively move zinc from maternal bones. This storage pool appears somewhat unavailable so that dietary deficiency can quickly have an

impact on the mineral balance of the maternal organism as well as the development of the fetus (1). If a women's zinc intake is low, and she is consuming the recommended amount of folate, the folate may be impairing zinc absorption, leading to a zinc deficiency. It is also important to consider the effect of a zinc deficiency on the metabolism and absorption of folate, which requires a zinc dependent enzyme. If in fact a zinc deficiency causes NTDs, the zinc-folate nutrient interactions may help to explain some of the NTD-affected pregnancies seen in women who consume adequate folate.

In experimental animals, maternal zinc deficiency has consistently produced defects of the central nervous system (1,2,18,19,20,21,22). Zinc deficiency has been specifically implicated in the development of anencephaly and spina bifida (18). Animal studies have shown that even short periods of maternal zinc deficiency have deleterious implications for the fetus (16,20). This transient deficiency may be totally undetectable with respect to maternal health. The pregnant rat is unable to mobilize sufficient zinc from her own tissues to supply the needs of normal fetal development during periods of inadequate dietary zinc (22).

The relationship between zinc nutriture and human birth defects is less clear. Few studies with humans have been conducted on the development of NTDs in infants of zinc deficient mothers. Epidemiologic data seem to support a relationship between zinc deficiency and central nervous system malformation in humans (22). There are reports of women from Sweden, Turkey, and Ireland who gave birth to offspring with congenital malformations. The mother's of these NTD-affected infants had significantly lower plasma zinc concentrations at delivery than mothers who had had normal offspring (15,20,21). A study by Hurd et al (21) found an association between zinc deficiency,

spina bifida, and anencephaly from studying the international incidence of NTD-affected pregnancies. The results showed that significant zinc deficiency has been found in Egypt, Turkey, and Iran where high rates of central nervous system anomalies are seen (22). Another study done by Hambridge et al (16) reported the pregnancy outcomes of patients with acrodermatitis enteropathica, the most severe zinc deficiency state recognized in man. The infants of these mothers were born with NTDs, in which zinc deficiency may have been a contributing factor.

Although the potential value of zinc supplementation has been examined, the data don't seem to be as sufficient as the data from studies on folate supplementation to require preconceptional zinc supplementation in an effort to reduced NTDs. However, since the zinc intake of women of childbearing age appears to be marginal, and because of the zinc-folate interactions, it seems appropriate to include guidelines for optimizing zinc intake in prenatal nutrition counseling (1).

Besides deficiencies in folate and zinc contributing to NTDs, recent reports have shown that overnutrition, as evidenced by maternal obesity, may also cause congenital defects, particularly of the central nervous system (23,24,25). An association linking obesity to NTDs is of considerable public health importance because the prevalence of overweight women is high and continues to increase in the United States, and because NTDs are one of the most common and severe congenital malformations (23). Latest estimates from the United States indicate a national average prevalence of obesity of 33% in women, and rates close to 50% in certain ethnic and regional subgroups (25). Furthermore, as many as 10% of women in the United States are obese periconceptionally (24).

Studies have shown a two-fold increase in the risk for NTD-affected pregnancies among obese women compared with women who were of average weight prior to pregnancy (23,24,25). The increased risk was seen in women whose prepregnancy body mass index (BMI) exceeded 29 kg/m^2 compared with women of an average BMI. Obesity in these reports was defined using the Institute of Medicine's definition of obesity as a BMI greater than 29 kg/m^2 (23,24).

Werler et al (23), gathered data from a case-control surveillance program of birth defects. The subjects filled out a questionnaire, which included detailed inquiries about vitamin supplementation, dietary intake of more than 100 specific food items, prepregnant weight, and maternal height. The results showed a positive association between increasing prepregnant weight and NTD risk, regardless of folate intake. There was a threefold increase in risk estimated for the heaviest women, independent of whether folate intake was below or above the recommended level of 0.4 mg. In normal-weight women, those consuming high levels of folate had a relative risk of NTDs in line with findings concerning the protective effects of folic acid. However, in women weighing more than 70 kg, folate had no significant protective effect (23). The finding that folate seems to lose it's protective effect in obese women is in line with another clinical trial, where the protective effect of zinc supplementation in reducing low birth weight infants was achieved only in nonobese women (25).

A population-based case-control study by Shaw et al (24) also showed an increased risk in neural tube defect-affected pregnancies among obese women. Subjects in this study were interviewed to collect information on each woman concerning medical, reproductive, and family history, as well as aspects of her employment, hobbies, vitamin

intake, and activities associated with various lifestyle factors. Potential maternal covariates considered included age, race/ethnicity, education, gravidity, alcohol use, diabetes, any use of a vitamin containing folic acid in the period 3 months before and after conception, and dietary intake of folate, zinc, and fat (24). The increased risk was not attributable to maternal nonuse of a vitamin containing folic acid, diabetes, use of diet pills, lower dietary folate or zinc intake, or an NTD-pregnancy history. Adjustments for maternal age, education, gravidity, use of vitamins, and use of alcohol did not change the odds ratio.

Compared to women with a prepregnancy BMI of 19-27 kg/m^2, women whose BMI was 28 kg/m^2 or more were at an increased risk for having an NTD-affected pregnancy, and women whose BMI was 38 kg/m^2 or more had the largest risk. In both studies (23,24), the risk for anencephaly was similar between women with a BMI greater than 29 kg/m^2 and women with a BMI of 29 kg/m^2 or less. The risk for spina bifida and other NTDs was substantially higher for those women with a BMI greater than 29 kg/m^2. Shaw et al (24) observed an increased NTD risk for shorter obese women.

The mechanism by which increased weight may affect NTD risk is not clear, but these data suggest it may involve something other than folate and zinc intake. Women who are overweight may have compromised nutrient intakes and therefore may be at an increased risk for delivering an infant with a congenital anomaly. The association between obesity and an increased risk of NTD in the studies by Werler (23) and Shaw (24) was not readily explained by any known social, dietary, or medical confounders and appeared to be independent of folate and zinc intake, suggesting that it might arise from a

specific pathophysiologic disturbance of obesity. The many metabolic and endocrine changes associated with obesity provide numerous potential candidates (25).

Obesity affects metabolic factors that may, in turn, have a direct effect on neural tube development. Many alterations in metabolism have been observed in the person who is obese. These may be discussed in terms of the effects on the adipose cell in particular and effects on metabolism in general (8). As the adipocyte enlarges, it adapts metabolically, with progressive inhibition of lipogenesis and inhibition of pentose shunt enzymes. As fat cells become larger, they also become insulin-resistant. Among other consequences, they have a decreased number of receptor sites on their cell membranes (8). A variety of biochemical abnormalities may be present in the obese. Some of these abnormalities include an abnormal glucose tolerance test, increased fasting levels of plasma glucose and plasma insulin, and increased insulin response to a glucose load and other cues for insulin release. Other biochemical abnormalities in the obese include hypertriglyceridemia, elevated fasting levels of free fatty acid, and elevated plasma ketones (8). Perhaps the changes caused by obesity affect the delivery of nutrients needed for closure of the neural tube during gestation. The efficiency of placental nutrient transfer is a determinant of fetal well-being. Obesity may have a deleterious effect on the utilization and transfer of nutrients by reducing the surface area of the villi, promoting insufficient vascularization, or by changing the hydrostatic pressure in the intervillous space. All of these changes could potentially limit the supply of nutrients available to the fetus, inhibit normal growth, and consequently contribute to the development of neural tube defects (1).

Over the years, many hypotheses have evolved to explain why some people become fat while others remain lean. A defect in thermogenesis has been proposed as a factor in excessive weight gain. In people of normal weight, adaptive thermogenesis usually occurs. In this process, there is an increase in metabolic rate stimulated by eating, which serves the purpose of burning off excess energy in the form of heat (26). A defect in the thermic effect of food may alter the process of digestion, absorption, and metabolism of nutrients, making them less available for utilization in the growth of the fetus. This may be one possible explanation as to why zinc and folate intakes seem to lose their protective effects in obese people. In a study by Fernandez-Lopez et al (27) on obese rats, obesity did not seem to have a significant effect on the ability to absorb and retain the minerals studied, but obesity was thought to effect the ability to use the substrates. If obesity affects the human body's ability to use nutrients, this could help to explain why obese women are at an increased risk for having NTD-affected pregnancies, since the nutrients would be unavailable for use by the embryo and fetus.

The relation between heavy prepregnant weight and NTD risk appears to be clear, but the underlying mechanism is not (23,24,25). Therefore, nutrition, metabolism, and obesity must be studied further to better explain their roles in the development of the neural tube. It seems that prevention of excess weight gain in women of childbearing age would be an effective intervention to decrease the risk of having an NTD-affected pregnancy.

Human studies and studies on experimental animals have clearly shown that maternal diet and the nutritional status of women have a direct impact on the pregnancy course and outcome. Improving the overall nutrition status of women can help to reduce

the incidence and prevalence of NTDs. Efforts should specifically be directed toward increasing the awareness of the benefits of folic acid among women of childbearing age and preventing excess weight gain in adolescents and young women. Although the potential value of zinc supplementation has been examined in several populations, the data are judged to be insufficient at the present time to mandate the institution of preconceptional zinc supplementation practices. However, since the zinc intake of women is marginal, and because zinc is needed for the absorption and metabolism of folate, prenatal nutrition counseling might appropriately include guidelines for optimizing zinc intake, which may help to prevent NTDs (1). Strategies to prevent NTDs through achieving optimal nutritional status in women of childbearing age, are urgently needed and deserve considerable funding, since the economic benefits in reducing the burden of NTDs would be substantial.

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Appendix A
Intervention Studies of Folic Acid and Neural Tube Defects

Study	Design	Subjects	Exposure	Results	Comments
Laurence, et al, 1981 (1)	Randomized controlled trial in Wales	Pregnant women with prior NTD-affected pregnancy Supplemented mothers took 4 mg of folic acid daily. Unsupplemented mothers took a placebo.	Supplemented women were given 4 mg of folic acid or placebo daily at least 1 month before conception through 1s^{t} trimester.	2 NTD-pregnancies among 60 supplemented women. 4 NTD-pregnancies among 51 placebo-treated women. Relative risk=0.40, not statistically significant.	60% reduction in risk.
UK MRC study, 1991 (2)	Randomized controlled multicenter trial in UK and Hungary	Pregnant women with prior NTD-affected pregnancy: Supplemented mothers took 4 mg of folic acid daily. Unsupplemented mothers took a placebo.	Women given 4 mg of folic acid or placebo daily at least 1 month before conception through the 1st trimester.	6 NTD-pregnancies among 593 supplemented women. 21 NTD-pregnancies among 602 unsupplemented women. Relative risk=0.28, $p<0.05$	72% reduction in risk.
Smithells, et al, 1983 (3)	Nonrandomized controlled multicenter trial in UK	Pregnant women with prior NTD-affected pregnancy: Supplemented mothers took 0.36 mg folic acid + multivitamins daily. Unsupplemented mothers took nothing.	Women given 0.36 mg of folic acid + multivitamins or reported no use from 1 month before conception through the 1st trimester.	3 NTD-pregnancies among 454 supplemented women. 24 NTD-pregnancies among 519 unsupplemented women. Relative risk=0.14, $p<0.05$	86% reduction in risk.
Vergel, et al, 1990 (4)	Nonrandomized controlled trial in Cuba	Pregnant women with prior NTD-affected pregnancy: Supplemented mothers took 5 mg folic acid daily. Unsupplemented mothers took nothing.	Women given 5 mg of folic acid or reported no use from 1 month before conception through the 1st trimester.	0 NTD-pregnancies among 81 supplemented women. 4 NTD-pregnancies among 114 untreated women. Indeterminant protective effects, not statistically significant.	Complete protective effect.

Table comes from:
Recommendations for the Use of Folic Acid to Reduce the Number of Cases of Spina Bifida and Other Neural Tube Defects. *MMWR-Morbidity and Mortality Weekly Report.* 1992;41(RR-14):1-7.

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Appendix B
Observational Studies of Folic Acid and Neural Tube Defects

Study	Design	Subjects	Exposure	Results	Comments
Mulinare, et al, 1988 (1)	Case/control in metropolitan Atlanta	NTD case babies and normal control babies. Pregnant women without a prior NTD-affected pregnancy.	Multivitamin supplement containing 0-0.8 mg of folic acid at least 1 month before conception through the 1st trimester.	24 supplemented and 157 unsupplemented NTD case-women. 405 supplemented and 1,075 unsupplemented controls. Odds ratio=0.40, $p<0.05$	60% reduction in risk.
Bower and Stanley, 1989 (2)	Case/control in Western Australia	Spina bifida case babies and normal control babies. Pregnant women without a prior NTD-affected pregnancy.	Dietary folate and multivitamin supplement at least 1 month before conception through the 1st trimester.	77 NTD cases; 154 control mothers in study. The highest folate quartile was compared with the lowest. An increasing protective effect was observed from the lowest to the highest quartile. Odds ratio=0.25, $p<0.05$.	75% reduction in risk.
Mills, et al, 1989 (3)	Case/control in California and Illinois	NTD case babies and normal control babies. Pregnant women without a prior NTD-affected pregnancy.	Multivitamin + folate supplement containing up to 0.8 mg of folic acid + diet at least 1 month before conception through the 1st trimester.	89 supplemented and 214 unsupplemented NTD case-women. 90 supplemented and 196 unsupplemented women controls. Odds ratio=0.91, not statistically significant.	No protective effect.
Milunsky, et al, 1989 (4)	Prospective cohort in New England	NTD case babies and normal control babies. Pregnant women without a prior NTD-affected pregnancy.	Multivitamin + folate supplement containing 0.1-1.0 mg of folic acid + diet at least 1 month before conception through the 1st trimester.	10 NTD-pregnancies among 10,713 women who took multivitamin + folate. 49 NTD-pregnancies among 11,944 women who took multivitamins without folate. Relative risk=0.28, $p<0.05$.	72% reduction in risk.

Table comes from:
Recommendations for the Use of Folic Acid to Reduce the Number of Cases of Spina Bifida and Other Neural Tube Defects. *MMWR-Morbidity and Mortality Weekly Report.* 1992;41(RR-14):1-7.

Studies summarized above:

1. Mulinare J, Cordero JF, Erikson JD, Berry RJ. Periconceptional use of multivitamins and the occurrence of neural tube defects. *JAMA*. 1988;260:3141-5.
2. Bower C, Stanley FJ. Dietary folate as a risk factor for neural-tube defects: evidence from a case-control study in Western Australia. *Med J Aust.* 1989;150:613-9.
3. Mills JL, Rhoads GG, Simpson JL, Cunningham GC, Conley MR, Lassman MR, Walden ME, Deppo OR, Hoffman HJ. The absence of a relation between the periconceptional use of vitamins and neural-tube defects. *N Engl J Med.* 1989;321:430-5.
4. Milunsky A, Jick H, Jick SS, Bruell CI, MacLaughlin DS, Rothman KJ, Willett W. Multivitamin/folic acid supplementation in early pregnancy reduces the prevalence of neural tube defects. *JAMA*. 1989;262:1634.