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# Folic Acid Supplementation for the Prevention of Neural Tube Defects: An Update of the Evidence for the U.S. Preventive Services Task Force

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## ABSTRACT

**Background:** Neural tube defects (NTDs) are among the most common birth defects in the United States.

**Purpose:** To update the evidence on folic acid supplementation in women of childbearing age for the prevention of neural tube defects in their offspring.

**Data Sources:** MEDLINE and Cochrane Library searches (from January 1995 through November 2007), recent systematic reviews, reference lists of retrieved articles, and expert suggestions.

**Study Selection:** English language studies were selected to answer the following two questions: Does folic acid supplementation in women of childbearing age reduce the risk of a pregnancy affected by a neural tube defect? Does folic acid supplementation in women of childbearing age increase the risk of any harmful outcomes for either the woman or the infant? The following study types were selected: for potential benefits of folic acid—randomized, controlled trials (RCTs), case-control studies, cohort studies, systematic reviews and meta-analyses; for potential harms of folic acid—RCTs, case-control studies, systematic reviews, meta-analyses, and large observational studies.

**Data Extraction:** All studies were reviewed, abstracted, and rated for quality using predefined U.S. Preventive Services Task Force criteria.

**Data Synthesis:** Four observational studies reported benefit, in reduction of risk of NTD associated with folic acid-containing supplements. Differences in study type and methods prevent the calculation of a summary of the reduction in risk. The one included study on harms reported that the association of twinning with folic acid intake disappeared after adjusting for in vitro fertilization and for underreporting of folic acid intake.

**Limitations:** There is limited evidence on dose. We found no evidence on the potential harm of masking vitamin B12 deficiency in women of childbearing age. Our search focused on NTDs and therefore does not provide a comprehensive review of the effects of folic acid on all possible outcomes.

**Conclusions:** New observational evidence supports previous RCT evidence that folic acid–containing supplements reduce the risk of NTD-affected pregnancies. The association of folic acid use with twin gestation may be confounded by fertility interventions including ovulation stimulation and in vitro fertilization.

## INTRODUCTION

Neural tube defects (NTDs) are among the most common birth defects in the United States.(1) Estimates of disease burden are difficult to determine because affected pregnancies are sometimes spontaneously or electively aborted and are underreported on birth certificates.(2) The Centers for Disease Control and Prevention (CDC) estimates that the rates in 2005 for two of the most common NTDs, spina bifida and anencephaly, were 17.96 per 100 000 live births and 11.11 per 100 000 live births, respectively.(3)

NTDs cover a range of congenital malformations affecting the brain and spinal cord. This spectrum of congenital defects has been defined as:

> "Anencephaly (the total or partial absence of the cranial vault, the covering skin and the brain tissue), spina bifida (non-closure of the spine resulting in herniation or exposure of the spinal cord, the meninges or both; in some cases together with hydrocephalus), encephalocoele (herniation of the meninges and brain tissue outside the cranium, covered by normal or atrophic skin)."(6)

The embryological events leading to NTDs occur early in pregnancy. These events begin at approximately the twenty-first day after conception, and neural tube closing occurs by approximately 28 days after conception.(5)

Although neural tube defects (NTDs) span a range of disorders and possible etiologies, a number of risk factors for the disorders have been identified. Two well-established risk factors are having a history of a previous fetus or child with NTD and/or a first-, second-, or third-degree relative with NTD.(7, 8) There are ethnic and racial variations in the prevalence: Hispanics and non-Hispanic whites have higher rates of NTDs than African-Americans and Asians.(9) In a large population-based study in California, the prevalence in Hispanic women was found to be 1.12 per 1000 women screened (95% CI 1.04-1.21), the prevalence in Caucasian women was 0.96 per 1000 (95% CI 0.89-1.04), in African-American women 0.75 per 1000 (95% CI 0.59-0.91) and in Asian women 0.75 per 1000 (95% CI 0.60-0.90).(10) There are likely environmental or diet-related differences that contribute to these differences in prevalence of NTDs; however, the prevalence of mutations in certain enzymes may also differ among these population groups. Two enzymes that have been linked to increased risk for certain NTDs are methylenetetrahydrofolate reductase (MTHFR) and methionine synthase (MTRR).(11)

Maternal medical conditions such as diabetes and obesity have been associated with an increased risk of NTDs. Women with epilepsy who take certain anti-epileptic medications, such as valproic acid or carbamazepine, are also at increased risk. The risk of an open NTD in a woman taking these medications in the first trimester of pregnancy is reportedly 1-2%. Other medications associated with NTDs include vitamin A and Warfarin.(11)

The effectiveness of folate in preventing NTDs has been reported in multiple studies.(6) The mechanism of action has not been established. However, a main function for folate is in one-carbon transfers, which are important in methylation reactions and in purine and pyrimidine synthesis. Folate is necessary for the regulation of DNA synthesis and function and therefore affects important events in embryogenesis that may lead to NTDs.

There are three main approaches to achieving adequate levels of folate in women who are capable of becoming pregnant in the United States: ensuring a healthy diet that includes foods fortified with folic acid; providing folic acid supplements; and providing a combination of supplements and a folic acid-rich diet. Despite the US recommendations and efforts in the United States to increase folic acid intake, in 1997 intake of supplements by women had not achieved desired levels: only about 30% of women reported taking a daily supplement containing folic acid.(12) Overall, 26% of women reported taking at least 400 micrograms of folic acid per day via supplements within the previous month and 34.3% of women of reproductive age reported consuming over 400 micrograms per day via a combination of fortified foods and supplements. Recently, the 2007 March of Dimes Gallup survey used a random-digit dialed telephone interview in women of childbearing age. Of this population, 40% reported taking folic acid daily, as compared to 32% in 2003, 40% in 2004, and 33% in 2005. When stratified by age, 47% women from 25-34 years of age reported taking a daily supplement with folic acid as compared to 30% of women aged 18-24.(13)

In 1998, the U.S. Food and Drug Administration (FDA) mandated fortification of all enriched grain products at the level of 0.14 mg/100 grams of grain.(1) In the United States, fortification was expected to add approximately 100 μg of folic acid per day to the average American diet and increase the percentage of women of childbearing age consuming at least 400 μg/day from 29% to 50%.(1) In reality, the first expectation was met but the second was not: the proportion of women of childbearing age who consume more than 400 μg/day of folate varies by race/ethnicity from 23% to 33%.(1) According to more recent data from the 2001-2002 National Health and Nutrition Examination Survey (NHANES 2001-2002), limited to non-pregnant female participants between 15 and 49 years of age, the adjusted mean daily consumption of folic acid via fortified foods was estimated at 128 micrograms and was found to be 18% lower in non-Hispanic black women (109 micrograms/day) than in non-Hispanic white women (133 micrograms/day). Consumption of more than 400 micrograms/day from fortified foods was reported by only 8% of women.(14) There were differences by race/ethnicity in intake: 40.5% of non-Hispanic white, 19% of non-Hispanic black women, and 21.0% of Hispanic women reported consuming over 400 micrograms per day from all sources.

Despite less than ideal population intake, the prevalence of NTDs reported on birth certificates in the United States decreased from 37.8 per 100 000 live births before fortification to 30.5 per 100 000 live births conceived after mandatory folic acid fortification, representing a 19% decline.(15) More recently, the CDC reported that 23 population-based surveillance systems showed an approximately 26% decline in NTD prevalence rates in the United States before and after mandated fortification.(16)

The last U.S. Preventive Services Task Force (USPSTF) recommendation on the use of folic acid in women of childbearing age was made in 1996. At that time the USPSTF recommended that all women planning a pregnancy or capable of conception take a supplement containing folic acid. They found insufficient evidence to recommend for or against counseling women to increase their dietary folate consumption as an alternative to taking a folic acid supplement.

The purpose of this review is to update the evidence on folic acid supplementation in women of childbearing age. The USPSTF decided to focus its new review on folic acid supplementation; therefore, this review does not include a review of the evidence on fortification, counseling to increase dietary intake, or on screening for neural tube defects. Because this current review is an update, it includes only new literature published since 1995. The analytic framework developed for this review following USPSTF methods is shown in Figure 1. The USPSTF developed 2 key questions (KQs) from the analytic framework to guide its consideration of the evidence on folic acid supplementation. The key questions are:

> KQ1: Does folic acid supplementation in women of childbearing age reduce the risk of a pregnancy affected by a neural tube defect?
>
> KQ2: Does folic acid supplementation in women of childbearing age increase the risk of any harmful outcomes for either the woman or the infant?

In addition to these key questions, which define the scope of this systematic review, the USPSTF requested that information be gathered to answer 2 additional questions to provide context for their recommendation. The 2 contextual questions are:

1. What is the current dietary intake of folic acid (average level of folate) in women of childbearing age?

2. What is the most effective dose of folic acid supplementation?

The results of these two contextual questions are discussed in the Introduction and Discussion sections of this report.

## METHODS

### Data Sources and Searches

We performed a systematic search for English language articles published between January 1, 1995, and November 30, 2007, through a MEDLINE search using the terms "neural tube defects," "folic acid," "pregnancy," "twinning," and "twins." Additional studies were identified through a search of the Cochrane database, through discussions with experts, and by hand-searching of reference lists from included studies and major review articles and studies.

## Study Selection

Two reviewers independently reviewed the titles and abstracts and selected articles for inclusion based on predetermined inclusion and exclusion criteria. In general, studies were selected for benefits if they were RCTs, case-control studies, cohort studies or systematic reviews; and for harms if they were RCTs, case-control studies, cohort studies, systematic reviews or large observational studies; reported overall effect on reduction of neural tube defects or effect on harms in association with folic-acid containing supplements; and provided new evidence that was not in the 1996 USPSTF report. Studies were excluded if they included no new evidence since the 1996 review; were subanalyses of data without overall effect on NTD or harms; did not report effect of supplements separate from dietary effects; were letters, editorials, or non-systematic reviews; were performed in special or high-risk populations; or were performed in a country or population with widespread malnutrition or otherwise not generalizable to the United States. More details on search terms and inclusion/exclusion criteria are described in Appendix 1. Disagreements about inclusion of an article were discussed and selected based on consensus; if necessary a third reviewer was used for disagreements.

## Data Extraction and Quality Assessment

For all citations that met initial eligibility criteria, the full articles were reviewed, abstracted, and quality-rated independently by two reviewers. Studies were ultimately included if they were rated fair or good based on USPSTF criteria. Consensus about article abstraction data and quality was achieved through discussion by the two reviewers; disagreements were resolved by the involvement of a third reviewer. Data on the following items were extracted from the included studies: methods; exposure assessment; case ascertainment; selection of participants; dose of folic acid; sample size; size of effect on NTDs, other congenital abnormalities, and twinning; and information on confounders. Quality-rating of articles for all KQs was performed using standard USPSTF methodology on internal and external validity. We evaluated the quality of RCTs and cohort studies on the following items: initial assembly of comparable groups, maintenance of comparable groups, important differential loss to follow-up or overall high loss to follow-up, measurements (equality, reliability, and validity of outcome measurements), clear definition of the interventions, and appropriateness of outcomes. We evaluated systematic reviews and meta-analyses on the following items: comprehensiveness of sources considered, search strategy, standard appraisal of included studies, validity of conclusions, recency, and relevance. More complete criteria and definitions for USPSTF quality ratings are listed in the Appendix 2.

## Data Synthesis and Analysis

Data from studies included for KQ1 and KQ2 were synthesized qualitatively in tabular and narrative format. Synthesized evidence was organized by key question.

## Role of the Funding Source

The general work of the USPSTF is supported by the Agency for Healthcare Research and Quality. This specific review did not receive separate funding.

## RESULTS

Our search for evidence from PubMed, Cochrane library, reference lists, and experts returned 810 articles. Details on reasons for exclusions are in Figure 2. The most common reason for exclusion was incorrect study type; most were letters, editorials, or non-systematic reviews. Many were also excluded because the studies were not on folic acid supplementation; most of these were on folic acid fortification of foodstuffs. There were several additional common reasons for exclusion: the study was in a setting that was not thought to be generalizable to the United States; the study was a subanalysis of previously included data; or the study did not report overall outcomes as benefits in the reduction of NTD or harms associated with folic acid. Four studies for the key question on benefits and one study for the key question on harms ultimately met inclusion criteria and were of appropriate methodological quality. Studies that initially met inclusion criteria, and that were abstracted and quality-rated but ultimately excluded are discussed in Table 1 (KQ1-benefits) and Table 2 (KQ2 - harms).

### KQ1: Does folic acid supplementation in women of childbearing age reduce the risk of a pregnancy affected by a neural tube defect?

#### Summary of Study Results

Observational studies on the benefits of folic acid supplementation provide generally consistent evidence that folic acid supplementation in the periconceptional period reduces the risk of neural tube defects in offspring. This evidence was provided by three fair- or good-quality cohort, case-control, and meta-analytic studies that found statistically significant benefit; one small, fair-quality case-control study reported benefit that was not statistically significant. In addition to NTDs, the cohort and meta-analysis found reductions in cardiovascular congenital abnormalities associated with folic acid-containing multivitamins.

#### Study Characteristics

The search for literature to answer this question returned four articles that met the inclusion/exclusion criteria, were published within the search timeframe, and were of appropriate methodological quality. Detailed study characteristics and outcomes are available in Table 3.

A 2004 cohort study by Czeizel and colleagues followed 3056 women considering pregnancy who were recruited from the Hungarian Periconceptional Service. Women in the supplemented group were given multivitamins containing 0.8 mg of folic acid one month prior to planned conception. Women were recruited into the cohort as unsupplemented controls and matched to the supplemented group by age, socio-economic status, and employment status if they arrived for their first prenatal visit between 8 and 12

weeks gestation and had not taken folic acid–containing supplements in the periconceptional period. The study design had some methodological problems that led to a fair rating. There was potential for self selection bias as evidenced by the higher maternal rate of personal, family, offspring congenital abnormalities and the higher rate of prior fetal and infant loss in the supplemented group. Measurement of exposure to folic acid was performed earlier in the pregnancy in the supplemented group than in the unsupplemented group.

Two case-control studies were found in the literature search. These studies explored the association between exposure to folic acid supplementation in the periconceptional period and NTD in women residing in two geographic areas—California counties and the state of South Carolina. Both studies ascertained cases in a comprehensive way through review of records from hospitals and genetic clinics, and controls were randomly selected from hospitals in proportion to their contribution to all births in the respective populations. The first case-control study by Shaw and colleagues in 1995 was rated good because of accurate ascertainment of cases, selection of cases and controls without obvious biases, response rates of 88% in both cases and controls, exposure measurement applied equally to cases and controls, and exploration of reporting bias by asking mothers whether they believed folic acid had protective, causal, or no effect on birth defects. The second case-control study, by Thompson and colleagues in 2003, had accurate ascertainment of cases and selection of cases and controls without obvious biases, but had a small sample size, differential measurement assessments, and differential response rates in the cases and controls. These methodological problems led to a fair rating.

The fourth study was a meta-analysis of studies on pre- and periconceptional multivitamin use and congenital malformations. This fair-quality meta-analysis was recent and relevant and searched several resources for evidence; however, it did not include expert consultations and did not report a standard appraisal of study methodology. In addition, the applicability of this meta-analysis to the current review is limited because it excluded studies on folate-only supplementation and included several studies that were excluded by us: studies that were published prior to 1995, that were performed in special populations or that did not report NTD as a separate outcome.

**Study Results**

The Czeizel cohort study reported that 1 NTD and 9 NTDs occurred in the supplemented and unsupplemented women, respectively, for an adjusted odds ratio (aOR) of 0.11 (95% CI, 0.01-0.91); the odds ratio (OR) was adjusted for birth order, chronic maternal disorders, and history of previous fetal death or congenital abnormality. The meta-analysis also found a protective effect of folic acid–containing multivitamins in NTDs with an OR of 0.67 (95% CI, 0.58-0.77) in case-control studies and an OR of 0.52 (0.39-0.69) in RCTs and cohort studies. Both the Czeizel study and the meta-analysis found a statistically significant association between folic acid supplementation and a reduction in cardiovascular congenital abnormalities. In addition, there was a significant effect of folic acid–containing multivitamin use on congenital limb defects in the meta-analysis. No

consistent effect of folic acid–containing multivitamins, either on orofacial clefts or on urinary tract congenital abnormalities, was seen in the Czeizel study or the meta-analysis.

The Shaw 1995 case-control study reported an OR of 0.65 (95% CI, 0.45-0.94) for use of folic acid–containing supplements in the 3 months before conception, and an OR of 0.60 (95% CI, 0.46-0.79) for supplement use in the 3 months after conception. The 2003 study by Thompson and colleagues reported an OR of 0.55 (0.25-1.22) for regular use, and an OR of 0.92 (0.55-1.55) for some use of folic acid–containing supplements, but neither of these findings was statistically significant.

Several differences in these case-control studies may explain differences in results. The 2003 Thompson study was smaller and adjusted for dietary folate intake. Additionally, the exposure timeframes were different: the Shaw study measured exposure in 2 time frames, 3 months before and 3 months after conception, while the Thompson study combined these same 6 months of periconception time into one measure of exposure.

### KQ2: Does folic acid supplementation in women of childbearing age increase the risk of any harmful outcomes for either the woman or the infant?

#### Summary of Study Results

We found one study of fair quality on the harms of twinning that suggests that the association of folic acid supplementation with twinning may be the result of confounding by infertility treatment and by differential reporting of folic acid use. We found no clear, consistent evidence that preconceptional folic acid use results in increased rate of twinning. We did not find any studies on other previously suggested potential harms such as masking of vitamin B12 deficiency.

#### Study Characteristics

We identified one study meeting the inclusion and quality criteria that addressed whether folic acid supplementation in women of childbearing age increases the risk of harmful outcomes for either the woman or the infant.(17) Details of this study are in Table 4. This retrospective cohort study examined the association between risk of twinning in 176 042 women who gave birth in Norway between December 1998 and December 2001 and their history of multivitamin or folic acid supplementation before or during pregnancy. Assessment of exposure was by birth attendant at the time of delivery, thus introducing potential problems with both measurement validity and differential recall. Overall, six percent of women in this study reported folic acid supplementation preconceptionally; however 24% of women who became pregnant through in vitro fertilization (IVF) reported supplementation. Given the concern for underreporting of folic acid use (calculated to be about 45% when the authors linked the pregnancies in this analysis to another large cohort study with more accurate assessment of folic acid exposure) and potential confounding by IVF, the authors included adjustment for these factors.

This study was rated fair, given its use of reasonable, albeit not the best, methods for exposure assessments, and its consideration of and accounting for most confounders, including IVF. Recall by mothers was likely imperfect, given that exposure was assessed at delivery, and there may have been differential recall of exposure by mothers with or without twin pregnancies. The exact dose, timing, and duration of the interventions were not clear, but all important outcomes were assessed.

### Study Results

After adjusting for age and parity, the authors reported an OR of 1.59 (95% CI 1.41-1.78) for twin delivery after preconceptional folic acid supplementation. In a subgroup analysis of women who did not report IVF, the risk of twinning was lower and non-significant (OR 1.13, 95% CI 0.97-1.33), as expected given the increase in multiple gestation associated with IVF and other assisted reproductive technologies. The odds of having twins of unlike sex, an outcome used as a proxy for dizygotic twinning, were increased in women taking folate, (OR 1.43, 95% CI 1.12-1.83). The authors then adjusted for both a 45% underreporting of supplementation as well as an estimated 12.7% of unidentified IVF pregnancies. When the likely underreporting for folic acid use and IVF were accounted for, the OR for twin delivery after preconceptional supplementation fell to 1.02, and was no longer statistically significantly greater than the risk for women who did not take folic acid (95% CI, 0.85-1.24).

## DISCUSSION

### Dose of Folic Acid Supplementation

There are considerable difficulties in determining the most effective dose, form, and timing of folic acid supplementation for prevention of first NTDs. RCTs provide the best opportunity to make these determinations, but there has only been a single RCT assessing women without a history of a previously affected child.(18) In this study, women who were treated periconceptionally with 0.8 milligrams per day had a statistically significantly lower risk of NTDs, but there was no opportunity to study other doses in the setting of this RCT. Observational studies have also attempted to answer these questions about dosage, but are plagued by difficulties of accurate exposure assessment (dose, form, and timing); heterogeneity with respect to whether studies accounted for supplements, fortified foods, and dietary intake of naturally-occurring folate; and variability in bioavailability of various sources.

One of the studies cited in the 1996 USPSTF report presented findings that could serve as an estimate of the minimal dose effective at reducing NTD risk.(19) This study derived relative risks according to daily folic acid dose among 18 case mothers and 322 control mothers, and found that patients taking 0.4 mg/day from one month before to one month after the last menstrual period had a 70% reduction in risk of NTD (RR 0.3, 95% CI 0.1,-0.6), while those taking less than 0.4 mg (but still taking some supplementation) had a non-statistically significant reduced risk of 50% (RR 0.5, 95% CI 0.2-1.5); the latter group consisted of only 53 women however, making definitive conclusions difficult.(19)

Due to the difficulty of assessing doses in observational studies, other studies have attempted to answer this question by relating the risk of NTDs instead to serum or red blood cell folate levels. In a case-control study in Dublin, Ireland, that examined maternal red-cell and plasma folate levels and risk of NTD, the risk of NTD decreased from 6.6 per 1000 births when red-cell folate levels were below 150 nanograms/mL to 0.8 per 1000 births at a level of greater than 400 nanograms/mL.(20) Most importantly, NTD risk was found to be inversely associated with red-cell folate levels in a continuous relationship. In another study by Daly (21), data demonstrated that women living in a country without mandatory fortification who take 200 micrograms of additional folic acid per day achieve red cell folate levels that exceed 400 nanograms/mL, the level shown in the previous study to be associated with decreased NTD risk. The authors estimated that adding 400 micrograms, 200 micrograms, and 100 micrograms daily could reduce NTD incidence by 47%, 41% and 22% respectively.(21)

More recently, Wald and colleagues (24) used published data on "dose" (folic acid intake) and "response" (serum folate concentrations and risk of NTD) to develop a model with which to predict responses to specific increases in folic acid intake. They used data on the effects of folic acid supplementation on serum folate from 13 published studies and then attempted to relate doses to outcomes of NTDs based on serum folate levels from the Daly (20) study mentioned above. At an increased level of 0.2 mg/day, the % risk reduction of NTDs was calculated to be 36% for a woman with a background serum folate of 2.5 ng/mL, 23% with baseline folate of 5.0 ng/mL (typical western diet, according to authors), and 13% with a background of 10 ng/mL. The authors of this study assert that maximal benefit can be derived by taking an additional 5.0 mg per day (in the form of a supplement), which would reduce risk of NTDs by 85% in women with a background folate intake of 5.0 ng/mL. However, the authors discount concerns about adverse effects of high levels of folate supplementation, despite a lack of studies investigating harms of such doses.

Other investigators have also taken into account both folic acid supplementation and dietary folate. In one study, dietary and supplement information from 23 228 women in the northeastern United States was collected in the early second trimester of pregnancy (25). Accounting for differing bioavailability of folate from varying sources, the authors converted all sources of folate to dietary folate–equivalent units and estimated the prevalence of NTDs according to individual and total sources of folate. They identified a dose-response relationship between increasing folate equivalents per day and decreasing prevalence of NTDs (p-value for linear trend of 0.016). Thompson and colleagues (26) also attempted to relate the risk of NTDs to total intake. In a case-control study of 487 women, they demonstrated decreased risk of NTDs at the highest quartiles of total folate intake (0.880-3.125 mg/day) (OR 0.35, 95% CI 0.17-0.72), but no significant dose-response relationship with supplement dose alone.

**Benefits of Folic Acid Supplementation**

New evidence from observational studies provides weight to previous evidence from controlled trials that folic acid supplementation provides benefit in reduction of risk from NTD-affected pregnancies. We found four studies of the benefits of supplementation, of fair or good quality, published since the previous 1996 USPSTF report. Odds ratios for reductions in NTDs associated with periconceptional folic acid supplementation ranged from 0.11 to 0.65 in cohort and case-control studies. A meta-analysis reported an OR for NTDs inversely associated with multivitamin use of 0.67 in case-control studies and 0.52 in RCTs and cohort studies.

A study that was excluded from our review because it was performed in a population not generalizable to the United States deserves discussion. This cohort study evaluated the pregnancy outcomes of 130 142 women in 3 provinces in China who were asked during their premarital medical examination to take a 0.4 mg daily folic acid supplement.(27) Periconceptional use of a folic acid supplement was associated with an approximately 40-80% reduction in risk of NTD-affected pregnancies; the reduction was greater in a region with higher pre-study rates of NTDs. While the direct applicability of these specific rate reductions to the United States population is limited by the differences in the two countries' nutritional levels, these results nevertheless lend additional strength to the evidence on benefit.

**Harms of Folic Acid Supplementation**

The only RCT included in the 1996 USPSTF report on the prevention of first occurrence NTDs noted an increase in the risk of twinning among multivitamin users (28). These findings were not statistically significant when the data were re-analyzed and twin deliveries were considered as the outcome instead of twin births (29). In our current review, we attempted to identify all studies published since 1996 that examined twinning as an outcome. The one fair-quality study that was included found no association between preconceptional folic acid use and twinning; this study differed from previous studies because it accounted for both the high rate of underreporting of folic acid use (seen in many populations and studies) and the use of IVF. Another study, excluded based on population, that found no association with twinning was the prospective study from China discussed above, in which exposure assessment was likely fairly accurate and IVF and ovulation induction were not prevalent confounding factors (39).

One additional study that warrants discussion here was excluded from the systematic review because it was of a study type that did not meet inclusion criteria (31). The authors of this study used Australian pregnancy and birth and morbidity and mortality data as well as pooled estimates of relative risks for NTDs and twinning from previous trials to model the impact of periconceptional folate on NTDs and twinning in a hypothetical cohort of 100 000 pregnancies. They concluded that, although full supplementation could result in 119 fewer NTDs (95% CI, -74-141), it would result in approximately 1144 (95% CI, -200-3174) more twin births after 20 weeks' gestation. The estimated RR of twinning was from studies that did not address potential confounders such as IVF or ovulation induction. Although this study offers a unique perspective, its applicability to a United States population is limited.

Other potential concerns about folic acid supplementation include masking of vitamin B12 deficiency. Our current review yielded no evidence to support or refute this possible harm. However, given the low prevalence of vitamin B12 depletion in young women, it is unlikely that folic acid supplementation in women of childbearing age would result in a significant number of cases of neurological sequelae due to masking of vitamin B12 deficiency. In a study using data from NHANES and the Hispanic Health and Nutrition Examination Survey, the CDC National Center for Health Statistics reported in 1998 that under 1% of the total population between 4 and 50 years of age had serum vitamin B12 less than 100 pg/mL, the level below which vitamin B12 deficiency is likely.(32) Furthermore, in an ecologic study comparing patients in pre- and post- folic acid fortification periods, there was no evidence of an increase in low vitamin B12 levels without anemia.(33) Finally, folic acid supplementation is often given in the form of a multivitamin or prenatal vitamin that includes supplementation with B12, reducing the likelihood of masking of B12 deficiency in this population.

## Limitations

This review looked specifically for studies on NTDs and therefore does not include a comprehensive picture of how folic acid–containing supplements may prevent other congenital abnormalities. We did not review the evidence on counseling to increase dietary intake of folic acid. We reviewed the overall effect of folic acid on NTDs and did not comprehensively review the evidence on how the effect may differ among ethnic groups and among groups with genetic differences that may affect the metabolism of folic acid.

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**Figure 1.** Analytic Framework for the USPSTF Review on Folate Supplementation for the Prevention of Neural Tube Defects

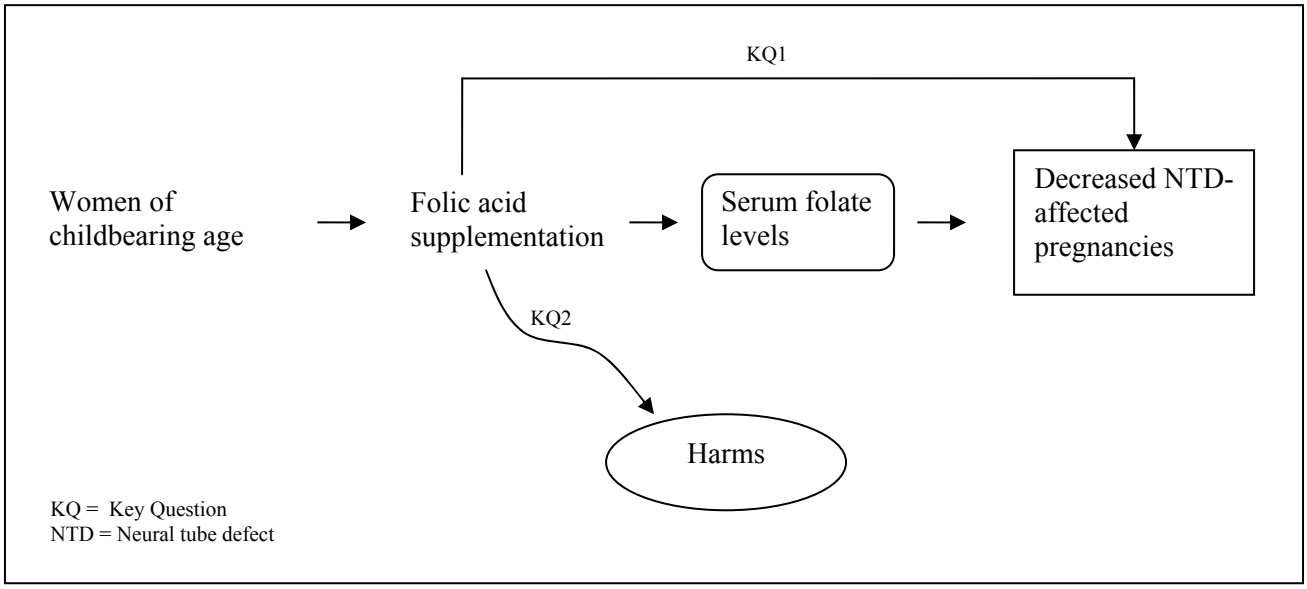

**Figure 2.** Search results and article flow.

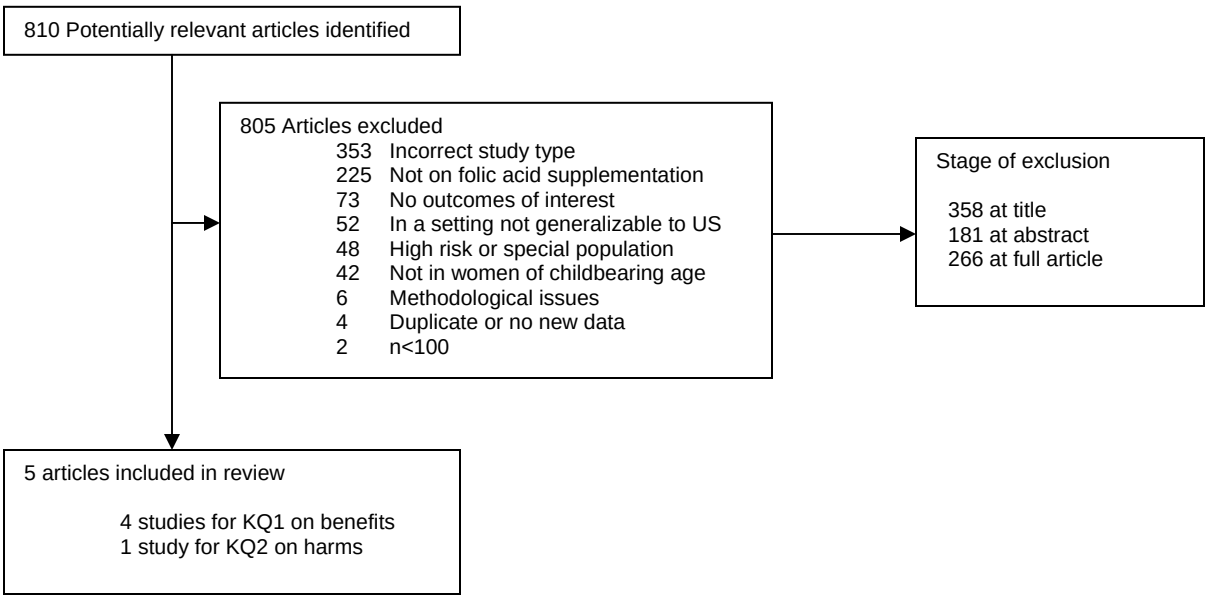

**Table 1.** Studies excluded after abstraction and quality rating - key question on benefits

| Study | Methods | Notes/Reason for Exclusion |
|---|---|---|
| Czeizel, 1996 (34) | Case-control study of participants of the Hungarian Case Control Surveillance of Congenital Abnormalities from 1980 to 1991, exploring the relationship of folic acid with congenital anomalies | Retrospective exposure assessment poses potential recall bias. |
| | Cases: 17 300 mothers of infants with congenital anomalies<br>Population controls: 30 663 mothers of infants with no congenital abnormalities matched to cases by sex, birth week, residence<br>Patient controls: 607 mothers of infants with Down Syndrome | Differential measurement of exposure causes potential measurement bias.<br><br>Lower response rate in controls |
| | Exposure is folic acid supplementation:<br>Dosing included folic acid (3 mg tablets, 1-3 times per day) or multivitamin (folic acid dose not reported)<br>Timing included preconception, 1st month of pregnancy, 2-3rd month of pregnancy, 4-9th month of pregnancy, and unknown | No adjustment for smoking |
| Locksmith, 1998 (35) | Review of studies examining the use of supplemental folic acid for prevention of NTDs | Study type not included in review (not a systematic review) |
| Kallen, 2002 (36) | Cohort study of 5331 infants registered in the Swedish Medical Birth Registry between 1995 and 2001 whose mothers had reported use of folic acid in early pregnancy, examining the relationship of folic acid and congenital malformations | Used involuntary childlessness as proxy for infertility |
| | Exposure is folic acid in early pregnancy (doses ranging from 0 to 5 mg of folic acid).<br><br>Outcome is congenital malformations including NTDs.<br><br>Subgroup analyses: women with subfertility problems and use of antiepileptic drugs | Exposure assessed by questionnaire at gestational week 10-12: drugs taken "since she became pregnant"<br><br>No information about dose, timing |
| Lumley, 2001 (37) | Systematic review of randomized and quasi-randomized studies published until April 2001 relating to whether NTDs can be reduced by increased consumption of multivitamins or folate before pregnancy or in first two months of pregnancy | Studies included were not recent (many published prior to 1995 and included in USPSTF previous evidence report). |
| Medveczky, 2004 (38) | Case control study of participants in the Hungarian Case Control Surveillance of Congenital Abnormalities from 1980 to 1996, to explore the association between socioeconomic status, periconceptional folic acid/multivitamin supplementation and NTDs in Hungary | No information on overall effect of folic acid on NTDs |
| | Cases: 1202 mothers of infants or fetuses with NTDs<br>Population controls: 38 151mothers of infants without congenital anomalies matched for sex, week of birth and district of residence<br>Patient controls: 22 475 mothers of infants with congenital anomalies other than NTDs | |
| | Exposure is periconceptional or pregnancy folic acid use and employment status classification | |
| Moore, 2003 (25) | Prospective cohort study of 23 228 women predominantly from northeastern U.S. who were in early second | This was a study of dose-response re-examining |

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| | trimester and had either serum alpha-fetoprotein screening test or amniocentesis to examine the effect of folic acid dose during early pregnancy<br><br>Exposure: folate from food, supplements, or fortified grains (converted to dietary folate−equivalents (DFEs):<br>13 431 women had intake of 0 DFEs/day<br>2489 women had intake of 1-399 DFEs/d<br>1812 women had intake of 400-799 DFEs/d<br>5,494 women had intake of ≥800 DFEs/d<br><br>Outcome: infant with NTD | data from study reviewed in 1996 USPSTF report; no new information about overall benefits of folic acid supplementation |
| Shaw, 2002 (39) | Case-control study of live births and fetal deaths (at >20 weeks) from January 1987 to December 1989 in most California counties, to evaluate possible interactions of periconceptional vitamins with selected factors on congenital anomalies<br><br>Cases: mothers of infants or fetuses with congenital anomalies (265 with NTDs)<br>Controls: 734 mothers of infants without any major anomalies, randomly selected in same geographic area and time period<br><br>Exposure: preconceptional vitamins in addition to smoking, fever, alcohol, race/ethnicity, education, BMI | No information on overall effect of folic acid on NTDs |
| Shaw, 1998 California (40) | Case-control study of live births and fetal deaths (at >20 weeks) and fetuses with NTDs that were electively terminated from 1987-1991 who were included in two previous studies by the California Birth Defects Monitoring Program to examine potential interaction between infant MTHFR C677T polymorphism and maternal use of vitamin supplements with folic acid<br><br>Cases: mothers of infants or fetuses with NTDs<br>Controls: mothers of infants without any major anomalies<br><br>Exposure: Periconceptional or pregnancy use of folic acid and presence of MTHFR C677T polymorphism | No information on overall effect of folic acid on NTDs |
| Shaw, 2001 (41) | Case-control study of fetuses, live births, and fetuses with NTDs that were electively terminated from 1989 to 1991 in most California counties to examine the potential relationship between weight gain during pregnancy and risk of NTDs<br><br>Cases: mothers of infants or fetuses with NTDs<br>Controls: mothers of infants without any major anomalies<br><br>Exposure: Periconceptional or pregnancy use of folic acid and weight gain during pregnancy | No information on overall effect of folic acid on NTDs |
| Shaw, 1996 (42) | Case-control study of fetuses, live births and fetuses with NTDs that were electively terminated from 1989 to 1991 in most California counties, to investigate the potential association between maternal obesity, folic acid supplementation, and NTD risk<br><br>Cases: mothers of infants or fetuses with NTDs<br>Controls: mothers of infants without any major anomalies<br><br>Exposure: Periconceptional or pregnancy use of folic acid and prepregnancy BMI | No information on overall effect of folic acid on NTDs |
| Suarez, 2000 (43) | Case-control study of infants and fetuses in 14 Texas counties along the U.S.-Mexico border between 1995-1999, to examine the relationship between folic acid intake and NTDs | Study performed in high-risk population |

Cases: 148 mothers of infants or fetuses with NTDs
Controls: 158 mothers of infants without any congenital abnormalities

Exposures are preconceptional supplement use (any use in 5.4% of cases and 3.2% of controls and daily use in 2% of cases and 2.5% of controls) and estimated dietary folate

**Table 2**. Studies excluded after abstraction and quality rating - key question on harms.

| Study | Methods | Notes/Reason for Exclusion |
|---|---|---|
| Lumley, 2001 (31) | Modeling study based on relative risks for NTDS and twins after folic acid supplementation (in a hypothetical cohort of 100,000 women)<br><br>Sources of data from registries in Victoria and Western Australia<br><br>Hypothetical exposure: adequate folic acid supplementation in 100,000 women<br><br>Outcomes: absolute difference in overall NTDs and twin gestations, perinatal and postnatal deaths | Study type not included in review |
| Ericson, 2001 (44) | Retrospective cohort study of 442 906 deliveries in Sweden between 1995 and 1999<br><br>Exposure: folic acid (n=2569) or multivitamin (n=1971) use since pregnancy reported at 10 weeks of gestation<br><br>Outcome: twin gestation identified at delivery<br><br>Subset analyses: women not reporting unwanted childlessness; unlike sex twin pairs | Potential confounding by patients undergoing IVF or ovulation stimulation; subgroup analysis on women without "period of involuntary childlessness", but authors reported known underreporting of infertility history (40% of women who underwent IVF or ovulation stimulation did not report involuntary childlessness.)<br><br>Measurement validity issues: exposure measured at 10 weeks; reported folic acid use was 0.6% in this study based on Birth Registry, as compared to 8% in concurrent study.<br><br>No information on doses or timing of initiation of folic acid<br><br>Potential differential recall based on knowledge of twin gestation by 8-10 weeks |
| Czeizel, 2004 (45) | Case-control study of 38 151 subjects from the Hungarian Case Control Surveillance of Congenital Abnormalities (HCCSCA) study between 1980 and 1996 who did not have any congenital abnormalities (the control group from the previous study)<br><br>Cases: 395 twins<br>Controls: 27 756 singleton pregnancies<br><br>Exposure: folic acid use in pregnancy reported in prenatal log books and in questionnaire completed after delivery<br><br>Including: no supplement, folic acid alone (dose range from 3-9 mg per day), multivitamin (folic acid dose range 0.1-1 mg per day), or folic acid and multivitamin | No adjustment for possible confounders: IVF, ovulation induction, smoking<br><br>No information on doses or timing of initiation of folic acid<br><br>Potential differential recall based on knowledge of twin gestation early in pregnancy or twin delivery |
| Kallen, 2004 (46) | Retrospective cohort study of 576 873 women registered in the Swedish Medical Birth Registry between 1995 and 2001, examining the relationship of folic acid and dizygotic twinning<br><br>Exposure: folic acid use before conception or before first appointment (usually between 8-10 weeks) (n=6953)<br><br>Outcome: unlike-sexed twin gestation identified at delivery | Incomplete information on doses (women likely took either 400 micrograms or 5 mg) or whether prenatal vitamins with folic acid were included in analysis<br><br>No information on timing of initiation or duration of exposure<br><br>Initial comparability of groups unknown<br><br>Potential differential recall based on knowledge of twin gestation by 8-10 weeks |

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| Subset analysis: non-Swedish (by nationality or birth) women not reporting unwanted childlessness, use of ovulation induction or use of gestagens, | Residual confounding possible if incomplete reporting of fertility treatments.<br><br>Unclear how many women were included in the final analysis. |

**Table 3.** Characteristics and results of studies included for key question 1: Folic acid supplementation and NTD reduction

| Study | Methods | Participants | Interventions | Outcomes | Results (95% CI) | Notes |
|---|---|---|---|---|---|---|
| Czeizel, 2004 (47) | Cohort study of pregnant women who received folic acid– containing MVI prior to conception and women who did not take any supplements periconceptionally.<br><br>USPSTF Level: II-2 | Hungary, May 1, 1993 to April 30, 1996<br><br>Supplemented group – 3981 women considering pregnancy recruited from Hungarian Periconceptional Service (HPS) originally created for the RCT of folate and NTD.<br><br>Exclusions: 7 Unable to use MVI, 186 didn't want to use MVI, 15 had induced abortion, 15 ectopic, 488 had miscarriage prior to 14 weeks, 147 didn't take MVI. Outcomes couldn't be clarified in 54 (1.7%)<br><br>Unsupplemented group – 3069 women recruited from regional antenatal care clinics between 8th and 12th gestational week who had not taken supplements. Outcomes couldn't be clarified in 47 (1.5%). 15% used supplements. Matched to supplemented group by age, quality of schools, employment status, and residence.<br><br>Supplemented group more highly educated, higher employment status, less likely to smoke (8% vs 18%). Supplemented group more likely to have prior fetal and infant deaths and more likely to have family history of congenital anomalies. | Multivitamin tablets containing 0.8 mg of folic acid 1 month prior to planned conception and supplied every 3rd month for up to 12 months.<br><br>Compliance for supplemented group was assessed by personal interview at 4 separate visits, "tick-off" form for basal body temperature prior to conception, and counting unused tablets.<br><br>Women were considered "fully supplemented" if they missed no more than one day for 28 days prior to conception and/or the 3rd missed menstruation. "Partially supplemented" women missed 2 or more tablets.<br><br>Unsupplemented group received routine care. | Presence of NTD, abnormalities of urinary tract, cardiovascular, limbs, pyloric stenosis, and orofacial clefts. | <u>NTD</u><br>1 NTD in supplemented and 9 NTDs in unsupplemented group.<br><br>aOR = 0.11<br>(0.01-0.91)<br><br><u>Urinary tract CAs</u><br>aOR = 0.71<br>(0.33-1.50)<br><br><u>Cardiovascular CAs</u><br>aOR = 0.60<br>(0.38-0.96)<br><br>Orofacial clefts<br>aOR = 1.63<br>(0.31-28.8)<br><br>Adjusted ORs not calculated for pyloric stenosis or limb deficiencies.<br><br>All ORs adjusted for birth order, chronic maternal disorder, history of previous fetal death or CAs. | Fair quality<br><br>Measurement of exposure to supplements was different in the two groups.<br><br>Women with personal, family, or offspring history of congenital abnormalities or fetal/infant loss likely self-selected into supplemented group.<br><br>Collected information on SES but did not adjust for these and other potential confounders. |
| Goh, 2006 (48) | Meta-Analysis<br><br>Searched up to July 2005 in Medline, PubMed, EMBASE, Toxline, HealthSTAR, and Cochrane in all languages. Search terms: | Initial search returned 92 articles. 41 studies eligible based on inclusion criteria: 27 case control studies, 4 RCTs, and 10 cohort studies.<br><br>Inclusion criteria: RCTs, case-control, or cohort study; reported pre- and periconceptional multivitamin intake; | Multivitamin use before or in first trimester of pregnancy | Risk of congenital malformations, including NTD, associated with multivitamin use before and in first trimester of pregnancy | <u>NTD</u><br>"Consistent protective effect"<br><br>OR 0.67 (0.58-0.77) in CCS<br><br>OR 0.52 (0.39-0.69) in | Fair quality<br><br>Searched multiple databases and reference lists but did not include experts. |

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| | "multivitamin," "pregnancy," and "malformation;"<br><br>Reviewed reference lists of all collected articles for potential studies.<br><br>Two reviewers assessed articles for possible inclusion. | contained a control group; reported raw data of rates of outcomes.<br><br>Exclusion criteria: studies on specific vitamins, exposures to known teratogens; review article, letters, abstracts. | | | RCTs/cohorts<br><br><u>Cleft palate</u><br>OR 0.76 (0.62-0.93) in CCS<br>OR 0.42 (0.06-2.84) in RCTs/cohorts<br><br><u>Urinary tract anomalies</u><br>OR 0.48 (0.30-0.76) in CCS<br>OR 0.68 (0.35-1.31) in RCTs/cohorts<br><br><u>Cardiovascular defects</u><br>OR 0.78 (0.67-0.92) in CCS<br>OR 0.61 (0.40-0.92) in RCTs/cohorts<br><br><u>Limb defects</u><br>OR 0.48 (0.30-0.76) in CCS<br>OR 0.57 (0.38-0.85) in RCTs/cohorts<br><br><u>Congenital hydrocephalus</u><br>OR 0.37 (0.24-0.56) in CCS<br>OR 1.54 (0.53-4.50) in RCTs/cohorts | No standard appraisal of included studies explicitly stated.<br><br>Studies on folate-only supplementation were excluded.<br><br>Included several studies that were excluded by us because of publication prior to 1995, because the studies were performed in special populations or did not report NTD as a specific outcomes. This limits applicability to the current review. |
| Shaw, 1995 (49) | Case Control Study exploring exposure to folic acid supplementation in cases of NTD and controls without a birth defect<br><br>USPSTF Level: II-2 | Pregnant women and their offspring in California counties (except Los Angeles, Riverside, and Ventura)<br><br><u>NTD cases</u>: 665 ascertained; diagnosed prenatally and elective abortions during Feb 1989–Jan 1991 and NTD births: June 1989–May 1991; Ascertained from all hospitals and genetic clinics.<br><br><u>Controls</u>: – births without major structural malformations: June 1989–May 1991; selected in proportion to hospital's contribution to total births | Folic acid from supplements or multivitamins in the 3 months before and the 3 months after conception.<br><br>Amount of folic acid estimated from personal interview on type, brand, and frequency of use. Prenatal vitamins assumed to have 0.8 mg; multivitamins assumed to have 0.4 mg; if no information on frequency or type of supplement, folic | Risk of NTD associated with folic acid–containing supplement | NTD risk and use of folic acid in 3 months before conception:<br>OR = 0.65 (0.45-0.94)<br><br>Use of folic acid in 3 months after conception:<br>OR = 0.60 (0.46-0.79) | Good quality<br><br>Accurate ascertainment of cases; selection of cases/controls appears nonbiased with exclusion criteria applied equally to both; response rates >80%; exposure measurement applied equally to each group; |

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| | | Exclusions:<br>Not English or Spanish speaking (29 cases, 32 controls); prior NTD (11 cases and 1 control).<br><br>Interviews conducted in 88.0% of cases, mothers at an average of 4.9 months after actual/estimated (for terminations) date of delivery and 88.2% of control mothers at an average of 4.6 months after date of delivery.<br><br>6.9% of cases refused to be interviewed and<br>5% could not be located; 6.2% of controls refused to be interviewed and 5% could not be located. Non-participants similar to study participants in race/ethnicity.<br><br>Cases mothers more likely to be Hispanic, less than 25 years old and completed fewer years of school. | acid intake considered to be zero. | | | attention to appropriate covariates and confounding variables.<br><br>Reporting bias explored:<br>Women who thought "no effect" of vitamins on birth defects had larger reduction in risk (OR = 0.51, 0.31-0.85) for use 3 months prior to conception.<br>Women who thought vitamins were "protective" = OR = 0.89 (0.46-1.7).<br>Women who thought vitamins "causal" to birth defects = OR = 0.40 (0.04-7.6). |
| Thompson, 2003 (26) | Case Control Study exploring multivitamin folic acid use, dietary folate intake, and risk of NTDs.<br><br>USPSTF Level: II-2 | Pregnant women residing in South Carolina who delivered in October 1992 through September 1997.<br><br>Cases obtained through monitoring of amniocentesis programs, perinatal centers, all medical practitioners providing care to pregnant women, medical records from hospitals with delivery/newborn units and vital records. CDC surveillance team verified completeness of case ascertainment in years one and four of the study.<br><br>Controls randomly selected from hospital in proportion to the hospital's estimated contribution to the total population of infants born. Selected concurrently with cases. | Average daily folic acid supplement intake in periconceptional period (3 months before and 3 months after conception)<br><br>By maternal interview conducted within 2 weeks of discharge (liveborn control or NTD baby) and 4 weeks of termination.<br>77% of women with NTD affected pregnancies and 86% of controls interviewed within 6 months of delivery (means not given)<br><br>Assumed Prenatal MVI = 0.8 mg | Risk of NTD associated with folic acid–containing supplement | Regular use: 16 cases and 43 controls, a OR 0.55 (0.25-1.22)<br>Some use: 123 cases and 188 controls, aOR = 0.92 (0.55-1.55)<br>No use: 40 cases and 57 controls, reference<br>Adjusted for age, race, BMI, ETS exposure and dietary folate | Fair quality<br><br>Potential for selection bias:<br>25/71 women with NTD pregnancies chose not to participate, but participation rate similar for NTD patients as compared to controls.<br><br>Measurement by interview of exposure assessed at different times for cases |

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| | First occurrence of singleton isolated NTD = 312<br>3 excluded because taking anticonvulsant medication.<br>2 women gave birth to twins excluded.<br>1 mother with NTD excluded.185 agreed to participate (72.3%)<br><br>Controls = 398 eligible.<br>289 (72.6%) agreed to participate.<br>1 excluded because taking anticonvulsant medication.<br><br>Cases and controls similar with respect to age, education, gravidity, month began PNC, previous NTD, BMI, smoking, ETOH, drug use, chronic conditions, MVI use.<br>Higher proportion of cases were white (79.3% vs. 69.1%) and reported having been exposed to ETS (51.4% vs. 26.1%). | Regular Use = 0.4 – 0.8 mg or more at least 3 times per week in periconceptional period.<br>Some intake = less than 3 times per week or in partial months.<br>No use = none at any time in periconceptional period. | terminated versus controls/NTD babies creating potential for differential measurement bias.<br><br>Sample size was small. Few took MVI regularly in 6 months periconceptionally.<br><br>6 month periconceptional period is not very precise timeframe for critical period of NTD. |

Abbreviations: aOR = adjusted odds ration; CCS = case control studies; CA = congenital abnormalities; CI = confidence interval; ETS = environmental tobacco smoke; MVI = multivitamins; NTD = neural tube defects; RCT = randomized controlled trials

**Table 4.** Characteristics and results of studies included for key question 2: Folic acid supplementation and harms

| Study | Methods | Participants | Interventions | Outcomes | Results (95% CI) | Notes |
|---|---|---|---|---|---|---|
| Vollset, 2005 (17) | Retrospective cohort study exploring association of twin gestation with folate use before or during pregnancy. | Norway, December 1998 to December 2001.<br><br>All women giving birth in Norway between (n= 176,042). | Folic acid tablets contain either 0.2 mg or 0.4 mg.<br><br>Multivitamins contain 0-0.2 mg folic acid per tablet.<br><br>Folic acid use assessed by birth attendant who checks off use of multivitamin or folic acid before or during pregnancy .<br><br>Preconception folate use in 6% (24% among IVF pregnancies.) | Risk of twin gestation after preconceptional folate use | OR: 1.59 (1.41-1.78)<br><br>OR in subset of women who did not report IVF: 1.13 (0.97-1.33)<br><br>OR for unlike sex pairs (proxy for dizygotic): 1.43 (1.12-1.83)<br><br>When modeling the underreporting (12.7% unidentified IVF; 45% unidentified folate use):<br>OR for twin delivery: 1.02 (0.85-1.24)<br><br>In unlike sex pairs:<br>1.26 (0.91-1.73)<br><br>Adjusted for age and parity | Fair quality<br><br>Exposure measured at delivery; may be problems with recall and potential differential recall for twin gestations as compared to singletons; authors did model for underreporting of folate use and unidentified IVF pregnancies. |

**Appendix 1:** PubMed search terms and exclusion criteria

PubMed search terms and limits:
("neural tube defects"[MeSH Terms] OR "spina bifida"[All Fields] OR "neural tube damage"[All Fields] OR "neural tube defect"[All Fields] OR "neural tube defects"[All Fields] OR "neural tube disorders"[All Fields] AND (("1995/01/01"[PDAT] : "2007/11/30"[PDAT]) AND English[lang])) AND (("folic acid"[MeSH Terms] OR folic acid[Text Word]) AND (("1995/01/01"[PDAT] : "2007/11/30"[PDAT]) AND English[lang])) AND (("pregnancy"[MeSH Terms] OR pregnancy[Text Word]) AND (("1995/01/01"[PDAT] : "2007/11/30"[PDAT]) AND English[lang])) AND

Exclusion Criteria for Folic Acid in Pregnancy Review

1. A study not on folic acid supplementation
2. Incorrect study type
3. In a setting not generalizable to US
4. Not in women of child bearing age
5. No outcomes of interest
6. High risk or special population (ex. Prior NTD)
7. n < 100
8. Duplicate study

## Appendix 2. USPSTF HIERARCHY OF RESEARCH DESIGN AND QUALITY RATING CRITERIA[1,2]

### HIERARCHY OF RESEARCH DESIGN

I Properly conducted randomized controlled trial (RCT)
II-1: Well-designed controlled trial without randomization
II-2: Well-designed cohort or case-control analytic study
II-3: Multiple time series with or without the intervention; dramatic results from uncontrolled experiments
III: Opinions of respected authorities, based on clinical experience; descriptive studies or case reports; reports of expert committees

### DESIGN-SPECIFIC CRITERIA AND QUALITY CATEGORY DEFINITIONS

#### Systematic Reviews

**Criteria:**

- Comprehensiveness of sources considered/search strategy used
- Standard appraisal of included studies
- Validity of conclusions
- Recency and relevance are especially important for systematic reviews

**Definition of ratings from above criteria:**

Good: Recent, relevant review with comprehensive sources and search strategies; explicit and relevant selection criteria; standard appraisal of included studies; and valid conclusions.

Fair: Recent, relevant review that is not clearly biased but lacks comprehensive sources and search strategies.

Poor: Outdated, irrelevant, or biased review without systematic search for studies, explicit selection criteria, or standard appraisal of studies.

#### Case-Control Studies

**Criteria:**

- Accurate ascertainment of cases
- Nonbiased selection of cases/controls with exclusion criteria applied equally to both
- Response rate
- Diagnostic testing procedures applied equally to each group
- Measurement of exposure accurate and applied equally to each group
- Measurement of exposure accurate and applied equally to each group
- Appropriate attention to potential confounding variables

**Definition of ratings based on criteria above:**

Good: Appropriate ascertainment of cases and nonbiased selection of case and control participants; exclusion criteria applied equally to cases and controls; response rate equally to or greater than 80 percent; diagnostic procedures and measurements accurate and applied equally to cases and controls; and appropriate attention to confounding variables.

Fair: Recent, relevant, without major apparent selection or diagnostic work-up bias but with response rates less than 80 percent or attention to some but not all important confounding variables.

Poor: Major section or diagnostic work-up biases, response rates less than 50 percent, or inattention to confounding variables.

**Randomized Controlled Trials and Cohort Studies**

**Criteria:**

- Initial assembly of comparable groups
  - -for RCTs: adequate randomization, including first concealment and whether potential confounders were distributed equally among groups
  - -for cohort studies: consideration of potential confounders with either restriction or measurement for adjustment in the analysis; consideration of inception cohorts
- Maintenance of comparable groups (includes attrition, cross-overs, adherence, contamination)
- Important differential loss to follow-up or overall high loss to follow-up
- Measurements: equal, reliable, and valid (includes masking of outcome assessment)
- Clear definition of the interventions
- All important outcomes considered

**Definition of ratings based on above criteria:**

Good: Evaluates relevant available screening tests; uses a credible reference standard; interprets reference standard independently of screening test; reliability of test assessed; has few or handles indeterminate results in a reasonable manner; includes large number (more than 100 broad-spectrum of patients.

Fair: Evaluates relevant available screening tests; uses reasonable although not best standard; interprets reference standard independent of screening test; moderate sample size (50 to 100 subjects) and a "medium" spectrum of patients.

Poor: Has fatal flaw such as: Uses inappropriate reference standard; screening test improperly administered; biased ascertainment of reference standard; very small sample size or very narrow selected spectrum of patients.

**Diagnostic Accuracy Studies**

**Criteria:**

- Screening test relevant, available for primary care, adequately described
- Study uses a credible reference standard, performed regardless of test results
- Reference standard interpreted independently of screening test
- Handles indeterminate result in a reasonable manner
- Spectrum of patients included in study
- Sample size
- Administration of reliable screening test

**Definition of ratings based on above criteria:**

Good: Evaluates relevant available screening test; uses a credible reference standard; interprets reference standard independently of screening test; reliability of test assessed; has few or handles indeterminate results in a reasonable manner;

includes large number (more than 100) broad-spectrum patients with and without disease.

Fair: Evaluates relevant available screening test; uses reasonable although not best standard; interprets reference standard independent of screening test; moderate sample size (50-100 subjects) and a "medium" spectrum of patients.

Poor: Has fatal flaw such as: Uses inappropriate reference standard; screening test improperly administered; biased ascertainment of reference standard; very small sample size or very narrow selected spectrum patients.

**Appendix 2: Reference List**


1. Harris R, Atkins D, Berg AO, Best D, Eden KB, Feightner JW et al. *US Preventive Services Task Force Procedure Manual*. Rockville, MD: Agency for Healthcare Research and Quality, 2001.

2. Harris RP, Helfand M, Woolf SH, Lohr KN, Mulrow CD, Teutsch SM et al. Current methods of the US Preventive Services Task Force: a review of the process. *Am J Prev Med* 2001; 20(3 Suppl):21-35.

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