

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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Background: Neural tube defects (NTDs) are among the most common birth defects in the United States. In 1996, the U.S. Preventive Services Task Force (USPSTF) recommended that all women planning a pregnancy or capable of conception take a supplement containing folic acid to reduce the risk for NTDs.

Purpose: To search for new evidence published since 1996 on the benefits and harms of folic acid supplementation for women of childbearing age to prevent neural tube defects in offspring, to inform an updated USPSTF recommendation.

Data Sources: MEDLINE and Cochrane Central Register of Controlled Trials searches from January 1995 through December 2008, recent systematic reviews, reference lists of retrieved articles, and expert suggestions.

Study Selection: English-language randomized, controlled trials; cohort studies; case-control studies; systematic reviews; and meta-analyses were selected if they provided information on the benefits and harms of folic acid supplementation in women of childbearing age to reduce NTDs in offspring.

Data Extraction: All studies were reviewed, abstracted, and rated for quality by using predefined USPSTF criteria.

Data Synthesis: Four observational studies reported benefit of reduction of risk for NTDs 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 adjustment for in vitro fertilization and underreporting of folic acid intake.

Limitations: The evidence on dose was limited. No evidence was found on the potential harm of masking vitamin B₁₂ deficiency in women of childbearing age. The search focused on the association of NTDs with supplementation only and therefore does not provide a comprehensive review of the effects of folic acid on all possible outcomes or of the effects of dietary intake of folic acid.

Conclusion: New observational evidence supports previous evidence from a randomized, controlled trial that folic acid-containing supplements reduce the risk for NTD-affected pregnancies. The association of folic acid use with twin gestation may be confounded by fertility interventions.

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Neural tube defects (NTDs) are among the most common birth defects in the United States (1). It is difficult to estimate disease burden because affected pregnancies are sometimes spontaneously or electively aborted and are underreported on birth certificates (2). The Centers for Disease Control and Prevention estimate that the rates in 2005 for 2 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).

The U.S. Preventive Services Task Force (USPSTF) last issued a recommendation on the use of folic acid in women of childbearing age in 1996. At that time, it recommended that all women planning a pregnancy or capable of conception take a supplement that contained 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 update does not include a review of the evidence on fortification, counseling to increase dietary intake, or screening for neural tube defects. We include only literature published since 1995 because it is an update of the previous USPSTF review. **Figure 1** shows the analytic framework developed for this review, which follows USPSTF methods. The USPSTF developed 2 key questions (KQs) from the analytic framework to guide its consideration of the evidence on folic acid supplementation:

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

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

METHODS

Data Sources and Searches

We performed a systematic search in MEDLINE for English-language articles published between January 1995 and December 2008 by using the terms *neural tube defects*,

See also:

Print

Related article. 626

Summary for Patients. I-50

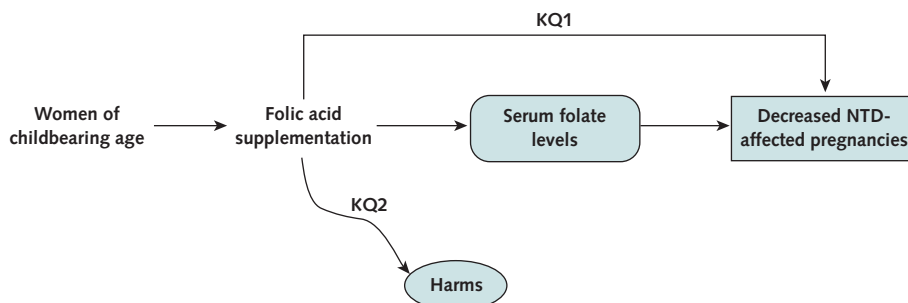
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Appendix

Appendix Tables

Conversion of graphics into slides

Figure 1. Analytic framework for the U.S. Preventive Services Task Force review on folic acid supplementation for the prevention of NTDs.



KQ = key question; NTD = neural tube defect.

folic acid, pregnancy, twinning, and twins. We identified additional studies by searching the Cochrane Central Register of Controlled Trials, having discussions with experts, and hand-searching reference lists from included studies and major review articles and studies.

Study Selection

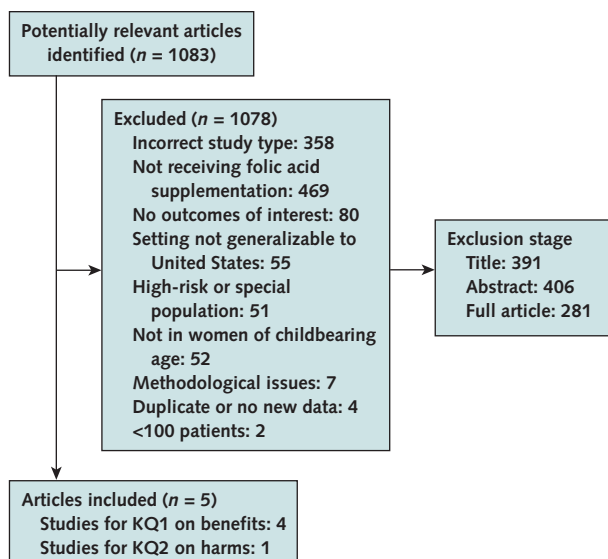
Two reviewers independently reviewed the titles and abstracts and selected articles for inclusion on the basis of predetermined inclusion and exclusion criteria. In general, we selected randomized, controlled trials (RCTs); case-control studies; cohort studies; and systematic reviews that reported an overall effect on reduction of NTDs or an effect on harms associated with folic acid-containing supplements and provided new evidence that was not in the 1996 USPSTF report. We excluded studies that did not include new evidence since the 1996 review; did not report outcome data on NTDs or harms associated with folic acid supplementation; did not report on the effect of supplements separate from dietary effects; were letters, editorials, or nonsystematic reviews; were performed in special or high-risk populations; or were performed in a country or population with widespread malnutrition or that was otherwise not generalizable to the United States. The **Appendix** (available at www.annals.org) provides more details on search terms and inclusion and exclusion criteria. We discussed and settled disagreements about inclusion of an article by consensus; if necessary, we involved a third reviewer for disagreements.

Data Extraction and Quality Assessment

For all citations that met initial eligibility criteria, 2 reviewers reviewed, abstracted, and independently quality-rated the full articles. We ultimately included studies that were rated fair or good on the basis of USPSTF criteria. We achieved consensus about article abstraction data and quality through discussion by the 2 reviewers and resolved disagreements by involving a third reviewer. We extracted data from included studies on the following items: 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. We used standard USPSTF methodology on internal and external validity to quality-rate the articles for all KQs. We evaluated the quality of RCTs and cohort studies on 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 interventions, and appropriateness of outcomes. We evaluated systematic reviews and meta-analyses on comprehensiveness of sources considered, search strategy, standard appraisal of included studies, validity of conclusions, recency, and relevance. **Appendix Table 1** (available at www.annals.org) lists more

Figure 2. Study flow diagram.



KQ = key question.

Table. Characteristics and Results of Studies Included for Key Questions 1 and 2

Study, Year (Reference)	Design and Methods	Participants	Interventions
Czeizel et al, 2004 (4)	Cohort	Hungary, 1 May 1993 to 30 April 1996. Supplementation group: 3056 offspring of women considering pregnancy, recruited from Hungarian Periconceptual Service. Originally created for the RCT of folate and NTD. Exclusions: unable to receive multivitamins (7), declined multivitamins (186), had induced abortion (15), had ectopic pregnancy (15), had early miscarriage (488), did not take supplement (147), outcomes could not be clarified (54), had late abortion (4), were lost to follow-up (9). Nonsupplementation group: 3056 women who had not received supplements, recruited in their 8th–12th gestational week from regional antenatal care clinics. Matched to supplementation group by age, quality of schools, employment status, and residence.	Multivitamin tablets, containing 0.8 mg of folic acid, 1 month before planned conception and supplied every third month for up to 12 months. Nonsupplementation group received routine care.
Goh et al, 2006 (7)	Meta-analysis. Searched up to July 2005 in MEDLINE, PubMed, EMBASE, Toxline, HealthSTAR, and Cochrane Central Register of Controlled Trials in all languages, using the search terms <i>multivitamin</i> , <i>pregnancy</i> , and <i>malformation</i> . Reviewed reference lists of all collected articles for potential studies. Two reviewers assessed articles for possible inclusion.	41 studies eligible on the basis of inclusion criteria: 27 case-control studies, 4 RCTs, and 10 cohort studies. Inclusion criteria: RCT, case-control, or cohort study; reported pre- and periconceptual multivitamin intake; had a control group; and reported raw data of rates of congenital malformation outcomes. Exclusion criteria: studies on specific vitamins; exposure to known teratogens; or review article, letter, or abstract.	Multivitamin use before or in first trimester of pregnancy.
Shaw et al, 1995 (5)	Case-control study	Pregnant women and their offspring in California counties (except Los Angeles, Riverside, and Ventura counties). NTD cases: 538 diagnosed prenatally and electively aborted from February 1989–January 1991 or born from June 1989–May 1991, ascertained from all hospitals and genetic clinics. Control participants: 539 births without major structural malformations from June 1989–May 1991, selected in proportion to hospital's contribution to total births. Exclusions: not English- or Spanish-speaking (29 case patients, 32 control participants), previous NTD (11 case patients and 1 control participant), and interview data not available (75 case patients, 72 control participants), of 665 case patients and 644 control participants who were originally eligible. Case-patient mothers were more likely to be Hispanic and <25 years of age and have completed fewer years of school.	Folic acid from supplements or multivitamins in the 3 months before and the 3 months after conception. Amount of folic acid was estimated from personal interview on type, brand, and frequency of use.
Thompson et al, 2003 (6)	Case-control study that explored multivitamin folic acid use, dietary folate intake, and risk for NTDs	Pregnant women residing in South Carolina who gave birth between October 1992 and September 1997. Investigators obtained 170 cases by monitoring amniocentesis programs, perinatal centers, all medical practitioners providing care to pregnant women, medical records from hospitals with delivery or newborn units, and vital records. Investigators randomly selected 269 control participants from hospital, in proportion to the hospital's estimated contribution to the total population of infants born, concurrently with case patients. Case patients and control participants were similar with respect to age, education, gravidity, month that prenatal care began, previous NTD, body mass index, smoking, alcohol use, drug use, chronic conditions, and multivitamin use. A higher proportion of case patients were white (79.3% vs. 69.1%) and reported having been exposed to environmental tobacco smoke (51.4% vs. 26.1%).	Average daily folic acid supplement intake in periconceptual period (3 months before and 3 months after conception) was assessed by maternal interview conducted within 2 weeks of discharge (live baby born to control participant or baby with NTD) or 4 weeks of termination; 77% of women with NTD-affected pregnancies and 86% of control participants were interviewed within 6 months of delivery (means not given).

Table—Continued

Outcomes	Results	Notes
NTDs, abnormalities of urinary tract, cardiovascular, and orofacial clefts	<p>NTDs: 1 NTD in supplementation group and 9 NTDs in nonsupplementation group; adjusted OR, 0.11 (CI, 0.01–0.91)</p> <p>Urinary tract congenital abnormalities: adjusted OR, 0.71 (CI, 0.33–1.50)</p> <p>Cardiovascular congenital abnormalities: adjusted OR, 0.60 (CI, 0.38–0.96)</p> <p>Orofacial clefts: adjusted OR, 1.63 (CI, 0.31–28.8)</p> <p>All ORs adjusted for birth order, chronic maternal disorder, and history of previous fetal death or congenital abnormalities</p>	Fair quality: adherence was assessed for supplementation group by personal interview at 4 separate visits, “tick-off” form for basal body temperature before conception, and counting unused tablets; measurement of exposure to supplements differed in the 2 groups; potential self-selection; no adjustment for important potential confounders.
Congenital malformations, including NTDs, associated with multivitamin use before and in first trimester of pregnancy	<p>NTD: OR in case–control studies, 0.67 (CI, 0.58–0.77); OR in RCTs and cohorts studies, 0.52 (CI, 0.39–0.69)</p> <p>Cleft palate: OR in case–control studies, 0.76 (CI, 0.62–0.93); OR in RCTs and cohort studies, 0.42 (CI, 0.06–2.84)</p> <p>Urinary tract anomalies: OR in case–control studies, 0.48 (CI, 0.30–0.76); OR in RCTs and cohort studies, 0.68 (CI, 0.35–1.31)</p> <p>Cardiovascular defects: OR in case–control studies, 0.78 (CI, 0.67–0.92); OR in RCTs and cohort studies, 0.61 (CI, 0.40–0.92)</p> <p>Limb defects: OR in case–control studies, 0.48 (CI, 0.30–0.76); OR in RCTs and cohort studies, 0.57 (CI, 0.38–0.85)</p> <p>Congenital hydrocephalus: OR in case–control studies, 0.37 (CI, 0.24–0.56); OR in RCTs and cohort studies, 1.54 (CI, 0.53–4.50)</p>	Fair quality: searched multiple databases and reference lists but did not include experts, no standard appraisal of included studies explicitly stated, studies on folate-only supplementation were excluded, included several studies published before 1995 or performed in special populations.
Risk for NTDs associated with folic acid–containing supplement	<p>NTD risk and any use of folic acid in 3 months before conception: OR, 0.65 (CI, 0.45–0.94)</p> <p>Any use of folic acid in 3 months after conception: OR, 0.60 (CI, 0.46–0.79)</p>	Good quality: accurate ascertainment of cases; selection of case patients and control participants seems nonbiased, with exclusion criteria applied equally to both; response rates >80%; exposure measurement applied equally to each group; attention to appropriate covariates and confounding variables.
Risk for NTDs associated with folic acid–containing supplement	<p>Regular use (3 times/week): 16 case patients and 43 control participants; adjusted OR, 0.55 (CI, 0.25–1.22)</p> <p>Some use: 123 case patients and 188 control participants; adjusted OR, 0.92 (CI, 0.55–1.55)</p> <p>No use: 40 case patients and 57 control participants (reference)</p> <p>Adjusted for age, race, body mass index, environmental tobacco smoke exposure, and dietary folate</p>	Fair quality: potential for selection bias (25 of 71 women with NTD pregnancies chose not to participate, but participation rate in the NTD group was similar to that of the control group); measurement of exposure assessed by interview at different times for case patients with terminated pregnancies versus control participants or patients who had babies with NTDs; small sample size; few received multivitamins regularly in 6 months periconceptionally.

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Table—Continued

Study, Year (Reference)	Design and Methods	Participants	Interventions
Vollset et al, 2005 (8)	Retrospective cohort	All women giving birth in Norway between December 1998 and December 2001 ($n = 176\ 042$). Information on IVF obtained from Norway registry and by contacting fertility clinics in Denmark and Sweden.	Folic acid tablets containing either 0.2 mg or 0.4 mg; multivitamins containing 0–0.2 mg of folic acid per tablet. Folic acid use assessed by birth attendant, who checked off use of multivitamin or folic acid before or during pregnancy. Preconceptional folate use in 6% (24% among IVF pregnancies).

IVF = in vitro fertilization; NTD = neural tube defect; OR = odds ratio; RCT = randomized, controlled trial.

complete criteria and definitions for USPSTF quality ratings.

Data Synthesis and Analysis

We qualitatively synthesized data from studies included for KQ1 and KQ2 in tabular and narrative format. We organized synthesized evidence 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

We identified 1083 articles, of which 4 met our inclusion criteria for benefits and 1 for harms. **Figure 2** details the reasons for exclusions. **Appendix Tables 2 and 3** (available at www.annals.org) discuss studies that initially met inclusion criteria and were abstracted and quality-rated but were ultimately excluded for KQ1 (benefits) and KQ2 (harms), respectively.

Key Question 1

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

Our literature search to answer this question returned 4 articles that met the inclusion criteria, were published within the search time frame, and were of appropriate methodological quality. The **Table** lists detailed study characteristics and outcomes. Observational studies on the benefits of folic acid supplementation provide generally consistent evidence that folic acid supplementation in the periconceptional period reduces the risk for neural tube defects in offspring. This evidence was provided by 3 fair- or good-quality cohort, case-control, and meta-analytic studies that found statistically significant benefit; a 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.

The first study, by Czeizel and colleagues (4), recruited a cohort of 6112 women from the Hungarian Preconception Service. Women in the supplementation group received 0.8 mg of folic acid per day beginning 1 month before planned conception. Women who presented at 8 to 12 weeks of gestation with no periconception folic acid supplementation served as control participants and were matched 1-to-1 by age, socioeconomic status, and employment status with 3056 women who received supplements. We rated this study fair quality because women in the supplementation group were more likely than control participants to have congenital abnormalities or a history of congenital abnormalities among family or offspring and because exposure to folic acid supplementation was assessed earlier in the supplementation group than in the control group. One NTD occurred in the supplementation group and 9 in the control group, of 3056 women in each group. Although this difference was statistically significant after adjustment for birth order, chronic maternal disorders, and history of previous fetal death or congenital abnormality, our confidence in the statistical estimates is reduced, given the small number of events. Of note, this study also reported that women who received supplements had infants with significantly fewer cardiovascular congenital abnormalities than did control participants.

We found 2 case-control studies 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 2 areas—most California counties and South Carolina. The **Table** details the study design. We rated the 1995 case-control study by Shaw and colleagues (5) good quality because it accurately ascertained cases, selected case-patients and control participants without obvious biases, had response rates of 88% among both case patients and control participants,

Table—Continued

Outcomes	Results	Notes
Risk for twin gestation after preconceptional folate use	OR, 1.59 (CI, 1.41–1.78) OR in subset of women who did not report IVF, 1.13 (CI, 0.97–1.33) after adjustment for the underreporting (12.7% unidentified IVF; 45% unidentified folate use) OR for twin delivery, 1.02 (CI, 0.85–1.24), adjusted for age and parity	Fair quality: exposure measured at delivery, possible recall and potential differential recall problems for twin gestations compared with singletons, authors modeled for underreporting of folate use and unidentified IVF pregnancies.

applied exposure measurement equally to case-patients and control participants, and explored reporting bias. We rated the 2003 case-control study by Thompson and colleagues (6) fair quality because it had a small sample size, differential measurement assessments, and differential response rates among case patients and control participants. Shaw and colleagues (5) reported an odds ratio (OR) of 0.65 (95% CI, 0.45 to 0.94) for any reported use of folic acid-containing supplements in the 3 months before conception and an OR of 0.60 (CI, 0.46 to 0.79) for supplement use in the 3 months after conception. Thompson and colleagues (6) reported an OR of 0.55 (CI, 0.25 to 1.22) for regular use (at least 3 times/wk) and an OR of 0.92 (CI, 0.55 to 1.55) for some use of folic acid-containing supplements, but neither of these findings was statistically significant. In both studies, it was difficult to accurately assess the dose and frequency of supplement intake because of the reliance on self-reporting and variability in supplement composition. Several differences in these case-control studies may explain differences in results. Thompson and colleagues' study (6) was smaller and adjusted for dietary folate intake. In addition, the exposure timeframes were different: Shaw and colleagues (5) measured exposure in 2 time frames, 3 months before and 3 months after conception, whereas Thompson and colleagues (6) combined these same 6 months of periconceptional time into 1 measure of exposure.

The fourth study was a meta-analysis of studies on pre- and periconceptional multivitamin use and congenital malformations. We rated this meta-analysis fair quality because it did not include consultation with expert informants to identify additional potential evidence not identified in the literature search and did not report a standard appraisal of study methodology (7). This meta-analysis may not fully meet strict systematic review inclusion criteria because it excluded studies on folate-only supplementation and included several studies that we excluded. We nevertheless include it here because it was published since the previous USPSTF review and the USPSTF may decide that it provides useful information as it deliberates on rec-

ommendations. The meta-analysis found that folic acid-containing multivitamins had a protective effect against NTDs, with an OR of 0.67 (CI, 0.58 to 0.77) in case-control studies and an OR of 0.52 (CI, 0.39 to 0.69) in RCTs and cohort studies. In addition, it found a significant effect of folic acid-containing multivitamin use on congenital limb defects. The meta-analysis found no consistent effect of folic acid-containing multivitamins on either orofacial clefts or urinary tract congenital abnormalities.

Key Question 2

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

We found no studies that demonstrated an association of folic acid supplementation with twin pregnancy or masking of B₁₂ deficiency. Of note, 1 fair-quality study suggested that confounding by infertility treatment explains previously reported associations of folic acid and twin pregnancy.

The retrospective cohort study (8) examined the association between risk for 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. The Table provides details of this study. Twenty-four percent 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 investigators 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 investigators adjusted for these factors. We rated this study fair quality because it used reasonable, albeit not the best, methods for exposure assessments; recall by mothers was probably imperfect, given that exposure was assessed at delivery; mothers with or without twin pregnancies may have had differential recall of exposure; and the exact dose, timing, and duration of the interven-

tions were not clearly described. After adjusting for age and parity, the investigators reported an OR of 1.59 (CI, 1.41 to 1.78) for twin delivery after preconceptional folic acid supplementation. In a subgroup analysis of women who did not report IVF, the risk for twinning was lower and nonsignificant (OR, 1.13 [CI, 0.97 to 1.33]). The investigators 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 decreased to 1.02 and was no longer significantly greater than the risk among women who did not take folic acid (CI, 0.85 to 1.24).

DISCUSSION

New evidence from observational studies provides weight to previous evidence from controlled trials that folic acid supplementation provides benefit in reduction of risk for NTD-affected pregnancies. We found 4 fair- or good-quality studies of the benefits of supplementation 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; however, some of these studies had small samples, which limits our confidence in the statistical estimates. A meta-analysis reported ORs 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 we 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 (9). Periconceptional use of a folic acid supplement was associated with an approximately 40% to 80% reduction in risk for NTD-affected pregnancies; the reduction was greater in a region with higher prestudy rates of NTDs. Although the direct applicability of these specific rate reductions to the U.S. population is limited by the differences in the 2 countries' nutritional levels, these results nevertheless lend additional strength to the evidence on benefit.

The only RCT included in the 1996 USPSTF report on the prevention of first-occurrence NTDs noted an increase in the risk for twinning among multivitamin users (10). These findings were not statistically significant when the data were reanalyzed and twin deliveries were considered as the outcome instead of twin births (11). In our review, we attempted to identify all studies published since 1996 that examined twinning as an outcome. The 1 fair-quality study that we 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. The previously discussed prospective study from China, which we excluded because of population, found no association with twinning; exposure assessment was probably fairly accurate and IVF and ovulation induction were not prevalent confounding factors (9).

Another potential concern about folic acid supplementation is masking of vitamin B₁₂ deficiency. We found 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 neurologic sequelae due to masking of vitamin B₁₂ deficiency. In a study that used data from the National Health and Nutrition Examination Survey and the Hispanic Health and Nutrition Examination Survey (12), the Centers for Disease Control and Prevention National Center for Health Statistics reported in 1998 that less than 1% of the total population between 4 and 50 years of age had a serum vitamin B₁₂ level less than 100 pg/mL, the level below which vitamin B₁₂ deficiency may occur. An ecologic study (13) that compared patients before and after folic acid fortification periods found no evidence of an increase in low vitamin B₁₂ levels without anemia. Finally, folic acid supplementation is often given in the form of a multivitamin or prenatal vitamin that includes supplementation with vitamin B₁₂, which reduces the likelihood that vitamin B₁₂ deficiency would be masked in this population.

Gaps in the Evidence

Determining the most effective dose, form, and timing of folic acid supplementation to prevent first NTDs presents considerable difficulties. Randomized, controlled trials provide the best opportunity to make these determinations, but only 1 RCT (10) assessed women without a history of a previously affected child. In this study, women who were treated periconceptionally with 0.8 mg/d had a significantly lower risk for NTDs, but this RCT offered no opportunity to study other dosages. Observational studies have also attempted to answer these questions about dosage, but are plagued by difficulties with 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.

Limitations of the literature make it difficult to determine the combined effect of supplementation and dietary intake of folic acid on population rates of NTDs. Epidemiologic studies suggest that dietary intake varies by race or ethnicity. In addition, intake of dietary folic acid may be decreasing because of the recent popularity of low-carbohydrate diets, which eschew food products that are commonly fortified with folic acid.

Limitations of This Review

We looked specifically for studies on NTDs and therefore did 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 or among groups with genetic differences that may affect the metabolism of folic acid.

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Requests for Single Reprints: Reprints are available from the Agency for Healthcare Research and Quality Web site (www.preventiveservices.ahrq.gov).

Current author addresses are available at www.annals.org.

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APPENDIX: 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. Study not on folic acid supplementation.
2. Incorrect study type.
3. Setting not generalizable to U.S. population.
4. Not in women of childbearing age.
5. No outcomes of interest.
6. High-risk or special population, such as women who had a previous NTD-affected pregnancy.
7. Fewer than 100 participants.
8. Duplicate study.

Appendix Table 1. U.S. Preventive Services Task Force Hierarchy of Research Design and Quality Rating Criteria*

Hierarchy of research design

- I: Properly conducted 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
- 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 equal to or greater than 80%, 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% or attention to some but not all important confounding variables
- Poor: Major selection or diagnostic work-up biases, response rates less than 50%, 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, crossovers, adherence, and 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 (>100) and broad spectrum of patients
- Fair: Evaluates relevant available screening tests, uses reasonable although not the 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 use of an inappropriate reference standard, screening test improperly administered, biased ascertainment of reference standard, or very small sample size or very narrowly 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 (>100) and broad spectrum of patients with and without disease
- Fair: Evaluates relevant available screening test, uses reasonable although not the best standard, interprets reference standard independently of screening test, moderate sample size (50–100 subjects) and a “medium” spectrum of patients
- Poor: Has fatal flaw, such as use of an inappropriate reference standard, screening test improperly administered, biased ascertainment of reference standard, or very small sample size or very narrowly selected spectrum of patients

RCT = randomized, controlled trial.

* See references 29 and 30.

Appendix Table 2. Studies Excluded After Abstraction and Quality Rating for Key Question 1 (Benefits)

Study, Year (Reference)	Design and Methods	Notes and Reason for Exclusion
Czeizel et al, 1996 (14)	Case-control study of participants in the Hungarian Case-Control Surveillance of Congenital Abnormalities from 1980 to 1991, which explored the relationship of folic acid with congenital anomalies. Case patients: 17 300 mothers of infants with congenital anomalies. Control population: 30 663 mothers of infants with no congenital abnormalities, matched to case patients by sex, birth week, and residence. Control participants: 607 mothers of infants with the Down syndrome. Exposure: folic acid supplementation. Dosing included folic acid (3-mg tablets, 1–3 times per day) or multivitamin (folic acid dose not reported). Timing included preconception, first month of pregnancy, second–third month of pregnancy, fourth–ninth month of pregnancy, and unknown.	Retrospective exposure assessment poses potential recall bias; differential measurement of exposure causes potential measurement bias; lower response rate in control participants; no adjustment for smoking.
Locksmith and Duff, 1998 (15)	Review of studies that examined the use of supplemental folic acid for prevention of NTDs.	Study type not included in review (not a systematic review).
Källén and Olausson, 2002 (16)	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, which examined the relationship of folic acid and congenital malformations. Exposure: folic acid in early pregnancy (doses ranging from 0–5 mg of folic acid). Outcome: congenital malformations, including NTDs. Subgroup analyses: women who had subfertility problems or received antiepileptic drugs.	Used involuntary childlessness as proxy for infertility. Exposure assessed by questionnaire at 10–12 gestational weeks: drugs taken “since she became pregnant.” No information about dose or timing.
Lumley et al, 2001 (17)	Systematic review of randomized and quasi-randomized studies published before April 2001 that related to whether NTDs can be reduced by increased consumption of multivitamins or folate before pregnancy or in first 2 months of pregnancy.	Studies included were not recent (many were published before 1995 and included in the previous USPSTF evidence report).
Medveczky and Puhó, 2004 (18)	Case-control study of participants in the Hungarian Case-Control Surveillance of Congenital Abnormalities from 1980 to 1996, to explore the association among socioeconomic status, periconceptual folic acid or multivitamin supplementation, and NTDs in Hungary. Case patients: 1202 mothers of infants or fetuses with NTDs. Control population: 38 151 mothers of infants without congenital anomalies, matched for sex, week of birth, and district of residence. Control participants: 22 475 mothers of infants with congenital anomalies other than NTDs. Exposure: periconceptual or pregnancy folic acid use and employment status classification.	No information on overall effect of folic acid on NTDs.
Moore et al, 2003 (19)	Prospective cohort study of 23 228 women, predominantly from the northeastern United States, who were in the early second trimester of pregnancy and had either a serum α -fetoprotein screening test or amniocentesis to examine the effect of folic acid dose during early pregnancy. Exposure: folate from food, supplements, or fortified grains. Outcome: infant with NTD.	This was a study of dose-response that reexamined data from a study reviewed in the 1996 USPSTF report; no new information about overall benefits of folic acid supplementation.
Shaw et al, 2002 (20)	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 periconceptual vitamins with selected factors on congenital anomalies. Case patients: mothers of infants or fetuses with congenital anomalies (265 with NTDs). Control participants: 734 mothers of infants without any major anomalies, randomly selected from the same geographic area and time. Exposure: preconceptional vitamins, in addition to smoking, fever, alcohol consumption, race or ethnicity, education, and body mass index.	No information on overall effect of folic acid on NTDs.
Shaw et al, 1998 (21)	Case-control study of live births and fetal deaths (at >20 weeks) and fetuses with NTDs that were electively terminated from 1987 to 1991 and were included in 2 previous studies by the California Birth Defects Monitoring Program to examine potential interaction between infant <i>MTHFR</i> C677T polymorphism and maternal use of vitamin supplements with folic acid. Case patients: mothers of infants or fetuses with NTDs. Control participants: mothers of infants without any major anomalies. Exposure: periconceptual or pregnancy use of folic acid and presence of <i>MTHFR</i> C677T polymorphism.	No information on overall effect of folic acid on NTDs.
Shaw et al, 2001 (22)	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. Cases: mothers of infants or fetuses with NTDs. Controls: mothers of infants without any major anomalies. Exposure: periconceptual or pregnancy use of folic acid and weight gain during pregnancy.	No information on overall effect of folic acid on NTDs.
Shaw et al, 1996 (23)	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 risk for NTDs. Case patients: mothers of infants or fetuses with NTDs. Control participants: mothers of infants without any major anomalies. Exposure: periconceptual or pregnancy use of folic acid and prepregnancy body mass index.	No information on overall effect of folic acid on NTDs.
Suarez et al, 2000 (24)	Case-control study of infants and fetuses in 14 Texas counties along the U.S.–Mexico border between 1995 and 1999, to examine the relationship between folic acid intake and NTDs. Case patients: 148 mothers of infants or fetuses with NTDs. Control participants: 158 mothers of infants without congenital abnormalities. Exposure: preconceptional supplement use (5.4% of case patients and 3.2% of control participants had any use; 2% of case patients and 2.5% of control participants had daily use) and estimated dietary folate.	Study performed in a high-risk population.

MTHFR = methylenetetrahydrofolate reductase; NTD = neural tube defect; USPSTF = U.S. Preventive Services Task Force.

Appendix Table 3. Studies Excluded After Abstraction and Quality Rating for Key Question 2 (Harms)

Study, Year (Reference)	Design and Methods	Notes and Reason for Exclusion
Lumley et al, 2001 (25)	Modeling study based on relative risks for NTDs and twins after folic acid supplementation (in a hypothetical cohort of 100 000 women). Data from registries in Victoria and Western Australia. Hypothetical exposure: adequate folic acid supplementation in 100 000 women. Outcomes: absolute difference in overall NTDs and twin gestations and perinatal and postnatal deaths.	Study type not included in review.
Ericson et al, 2001 (26)	Retrospective cohort study of 442 906 deliveries in Sweden between 1995 and 1999. Exposure: folic acid ($n = 2569$) or multivitamin ($n = 1971$) use since pregnancy reported at 10 weeks of gestation. Outcome: twin gestation, identified at delivery. 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 investigators reported known underreporting of infertility history (40% of women who underwent IVF or ovulation stimulation did not report involuntary childlessness). Measurement validity issues: exposure measured at 10 weeks; reported folic acid use in this study was 0.6% on the basis of birth registry data, compared with 8% in concurrent study. No information on doses or timing of initiation of folic acid. Potential differential recall based on knowledge of twin gestation by 8–10 weeks.
Czeizel and Vargha, 2004 (27)	Case-control study of 38 151 participants in the Hungarian Case-Control Surveillance of Congenital Abnormalities study between 1980 and 1996 who did not have any congenital abnormalities (the control group from the previous study). Case patients: 395 twins. Control participants: 27 756 singleton pregnancies. Exposure: folic acid use in pregnancy, reported in prenatal log books and in questionnaire completed after delivery. Included: no supplement, folic acid alone (dosage range, 3–9 mg/d), multivitamin (folic acid dosage range, 0.1–1 mg/d), or folic acid and multivitamin.	No adjustment for possible confounders: IVF, ovulation induction, or smoking. No information on doses or timing of initiation of folic acid. Potential differential recall based on knowledge of twin gestation early in pregnancy or twin delivery.
Källén, 2004 (28)	Retrospective cohort study of 576 873 women registered in the Swedish Medical Birth Registry between 1995 and 2001 that examined the relationship of folic acid and dizygotic twinning. Exposure: folic acid use before conception or before first appointment (usually at 8–10 weeks) ($n = 6953$). Outcome: unlike-sex twin gestation, identified at delivery. Subset analysis: non-Swedish women (by nationality or birth) not reporting unwanted childlessness, use of ovulation induction, or use of gestagens.	Incomplete information on doses (women probably took either 400 μ g or 5 mg) or whether prenatal vitamins with folic acid were included in analysis. No information on timing of initiation or duration of exposure. Initial comparability of groups unknown. Potential differential recall based on knowledge of twin gestation by 8–10 weeks. Residual confounding possible if reporting of fertility treatments was incomplete. Unclear how many women were included in the final analysis.

IVF = in vitro fertilization; NTD = neural tube defects.