Evidence Synthesis

Number 230

Folic Acid Supplementation to Prevent Neural Tube Defects: A Limited Systematic Review Update for the U.S. Preventive Services Task Force

Prepared for:
Agency for Healthcare Research and Quality
U.S. Department of Health and Human Services
5600 Fishers Lane
Rockville, MD 20857
www.ahrq.gov

Contract No. 75Q80120D00007, Task Order 01

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AHRQ Publication No. 22-05302-EF-1
August 2023

This report is based on research conducted by the RTI International–University of North Carolina at Chapel Hill Evidence-based Practice Center (EPC) under contract to the Agency for Healthcare Research and Quality (AHRQ), Rockville, MD (Contract No. 75Q80120D00007, Task Order 01). The findings and conclusions in this document are those of the authors, who are responsible for its contents, and do not necessarily represent the views of AHRQ. Therefore, no statement in this report should be construed as an official position of AHRQ or of the U.S. Department of Health and Human Services.

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None of the investigators has any affiliations or financial involvement that conflicts with the material presented in this report.

Acknowledgments

The authors gratefully acknowledge the following individuals for their contributions to this project: Justin Mills, MD, MPH, AHRQ Medical Officer; Tina Fan, MD, MPH, previous Associate Scientific Director and Tracy Wolff, MD, MPH, Scientific Director, AHRQ, U.S. Preventive Services Task Force (USPSTF) Program; current and former members of the USPSTF; external peer reviewers Nancy Rose, MD, University of Utah; Jorge Chavarro, MD, ScD, Harvard University; and Kimberly Gregory, MD, MPH, Cedars-Sinai Medical Center; federal partner reviewers (Centers for Disease Control and Prevention, National Institutes of Health); RTI International–University of North Carolina EPC staff: Christiane Voisin, MSLS, research librarian; Roberta Wines, MPH, and Carol Woodell, BSPH, current and former EPC Program Managers; Nila Sathe, MA, MLIS, quality assurance; Sharon Barrell, MA, editor; and Teyonna Downing, publications specialist.

Suggested Citation

Viswanathan M, Urrutia RP, Hudson K, Middleton JC, Kahwati, LC. Folic Acid Supplementation to Prevent Neural Tube Defects: A Limited Systematic Review Update for the U.S. Preventive Services Task Force. Evidence Synthesis No. 230. AHRQ Publication No. 22-05302-EF-1. Rockville, MD: Agency for Healthcare Research and Quality; 2023.

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Structured Abstract

Purpose: To conduct a limited update of new evidence of the benefits and harms of folic acid supplementation for the prevention of neural tube defects (NTDs) in persons capable of becoming pregnant for the U.S. Preventive Services Task Force (USPSTF) to update its 2017 recommendation.

Data Sources: PubMed/MEDLINE, the Cochrane Library, Embase, and trial registries for publications from July 1, 2015, through July 2, 2021; reference lists of retrieved articles, with surveillance of the literature through February 10, 2023.

Study Selection: Two investigators independently screened studies from the update search using a priori inclusion and exclusion criteria. We included English-language randomized studies and nonrandomized cohort studies with comparisons that focused on the use of folic acid supplementation (by itself or in multivitamin) for the prevention of NTD-affected pregnancies in persons capable of getting pregnant. We also evaluated studies investigating potential harms of folic acid supplementation such as maternal cancer and autism spectrum disorder.

We excluded poor-quality studies, studies not conducted in very highly developed countries, and studies focusing solely on persons on antiseizure medications, persons with a history of NTDs in previous pregnancies, or persons not capable of getting pregnant.

Data Extraction and Analysis: One investigator extracted data and a second checked accuracy. Two reviewers independently rated the methodological quality of the included studies based on predefined criteria.

Results: Twelve observational studies (reported in 13 publications) were eligible for this limited update (N=1,244,072 [from nonoverlapping cohorts]). Of these, three studies (N=990,372) reported on the effect of folic acid supplementation on NTDs. No studies reported on differences by race or ethnicity. For harms, nine studies were eligible; one randomized, controlled trial (N=431) reported on variations in twin delivery, seven observational studies (N=761,125) reported on the incidence of autism spectrum disorder, and one observational study (N=429,004) reported on maternal cancer.

Regarding benefits of folic acid supplementation, two cohort studies and one case-control study in this update reported on the association between folic acid supplementation and NTDs (N=990,372). One cohort study reported a statistically significant reduced risk of NTDs associated with folic acid supplementation taken before pregnancy (adjusted relative risk [aRR]: 0.54 [95% confidence interval {CI}, 0.31 to 0.91]), during pregnancy (aRR, 0.62 [95% CI, 0.39 to 0.97]), and before and during pregnancy (aRR, 0.49 [95% CI, 0.29 to 0.83]), but for only the later of two periods studied (2006 to 2013 and not 1999 to 2005). No other statistically significant benefits were reported overall.

No study reported statistically significant harms (multiple gestation, autism, and maternal cancer) associated with pregnancy-related folic acid exposure.

Limitations: Interventions evaluated by included studies were restricted to folic acid supplementation and did not include interventions such as food fortification, counseling to increase dietary intake, or screening for NTDs. We did not evaluate the association between red blood cell folate concentrations and NTDs. We found limited information on differences in benefits and risks of folic acid supplementation by dose and timing. We found no information about variation in outcomes by duration of use or by race or ethnicity.

Our review was designed to identify evidence that could result in a change in the 2017 USPSTF A recommendation; therefore, it focused only on studies published since 2015 and did not include the previously reviewed evidence. Ethical and logistical issues constrain the conduct of new randomized, controlled trials of folate supplementation versus placebo. All newly available evidence is observational and offers limited ability to control for confounding (including from mandatory food fortification), selection bias, recall bias, and attrition. As a result, included studies have inherent uncertainty regarding case ascertainment (for NTDs and harms) and degree of exposure (dose, timing, and duration) to folic acid supplementation.

Conclusions: New evidence from observational studies provides continued evidence of benefit of folic acid supplementation for preventing NTDs and no evidence of harms related to multiple gestation, autism, or maternal cancer and is consistent with the previously reviewed evidence on this topic. The 2017 USPSTF recommendation supporting folic acid supplementation in pregnancy was based on previously reviewed evidence from a randomized, controlled trial and observational studies reporting reduced NTDs with supplementation and no consistent evidence of harms for multiple gestations, maternal adverse effects, or child respiratory illness.

Chapter 1. Introduction

Purpose

The Agency for Healthcare Research and Quality (AHRQ) requested a limited update to a previous review on folic acid supplementation to prevent neural tube defects (NTDs).[1] The U.S. Preventive Services Task Force (USPSTF) will use this report to update its 2017 recommendation on this topic.[2] Limited updates are intended to support reaffirmations of "A" or "D" recommendations and focus on new evidence since the prior report. The USPSTF guidance notes that the goal of the search for evidence in a reaffirmation evidence update is to find new and substantial evidence sufficient enough to change the recommendation.[3]

Condition Background

Condition Definition

NTDs are major congenital malformations of the brain, spinal cord, and overlying tissues that develop during the first few weeks of gestation as a result of abnormal closure of the embryonic neural tube. The most common NTDs are anencephaly, encephalocele, and spina bifida.[1, 4-7] Anencephaly occurs when the cranial portion of the neural tube does not close; affected infants are born without parts of the brain and skull. Encephaloceles occur when defects along the cranium allow portions of the brain and meninges to protrude. Spina bifida is a diverse group of spinal NTDs that vary in severity from myelomeningocele (protrusion of spinal cord and meninges through a spinal defect) to meningocele (protrusion of meninges through a spinal defect) and spina bifida occulta (spinal defect without any protrusion).[8] Spinal anomalies (e.g., spina bifida) can also co-occur with cranial anomalies (e.g., anencephaly and encephalocele).

Prevalence and Burden of Disease

Based on 2010-2014 data from 39 U.S. population-based birth defects surveillance programs, the Centers for Disease Control and Prevention (CDC) estimated that anencephaly occurred in 2.5 out of 10,000 live births in the United States, encephalocele in 1 out of 10,000, and spina bifida in 3.9 out of 10,000.[9] Estimates of the total burden of NTDs must rely on indirect calculations because of underreporting of pregnancy terminations and fetal deaths. In U.S. studies, from 1988-2000, 30 to 80 percent of pregnancies complicated by spina bifida and anencephaly were terminated after early diagnosis.[10] Using databases that included all prenatally diagnosed NTDs in 1999-2000 regardless of eventual pregnancy outcome, at least 3,000 pregnancies per year in the United States were estimated to be affected by NTDs.[11]

NTDs result in a range of disabilities and death in affected children depending on location and severity of the defect(s). Anencephaly is incompatible with life. Children with encephaloceles have a 50 percent mortality rate, and the majority of survivors have developmental deficits.[12] Disabilities from spina bifida are based on the location of the lesion; the lower the lesion within the spine, the better the prognosis. Common disabilities for survivors of NTDs are paralysis, urinary and fecal incontinence, and ventriculomegaly with placement of ventricular-peritoneal

shunts.[13-15] Some cases of myelomeningocele can be repaired prenatally via fetal surgery to close the NTD during the second trimester of pregnancy, and this appears to improve infant outcomes during the first year of life.[16] The CDC estimated that the total lifetime cost of caring for an infant born with spina bifida is $791,900 based on 2014 dollars.[17] About 18 percent of infants diagnosed with spina bifida in Florida between 1998 and 2007 had more than three hospitalizations in their first year of life.[18] Among children with spina bifida recruited between ages 8 and 15 years old in 2006 in the U.S. Midwest, significant impacts on physical and social quality of life were found, and these increased over time.[19]

Etiology

The neural plate appears at the fifth week of gestation (3 weeks after fertilization) and has completed formation and closure by the sixth week of gestation (28 days after fertilization).[20] Failures in this process are irreversible. Many biological functions are necessary for the neural tube to close properly.[21] The etiology of NTDs is multifactorial and includes a variety of genetic predispositions and environmental factors. The genetic predispositions are likely polygenic in nature involving multiple gene-gene and gene-environmental interactions, many of which have yet to be identified.[21]

Although often used interchangeably, the term "folate" refers to the water-soluble B vitamin (B_9) that occurs in many chemical forms, including naturally in many foods, while "folic acid" is the term applied to the synthetic form of folate that is found in supplements and added to fortified foods.[7] Most NTDs are likely caused by low concentrations of folate stored in the body, which may be due to inadequate dietary intake, poor intestinal absorption, medication use that antagonizes folic acid, and genetic factors that impair folate metabolism. These are called folate-sensitive NTDs and are preventable by consuming adequate amounts of folic acid daily. High levels of folic acid supplementation (4 mg) have been found to reduce the risk of recurrent NTDs by more than 70 percent, and even more modest levels of folic acid supplementation (0.4 mg) reduce the first occurrence of NTDs.[22] The mechanism by which folate reduces the risk of NTDs is not well understood but is likely related to its role in nucleotide synthesis, which is especially important for the rapidly dividing cells in the embryonic neural tube.[23] Without an adequate supply of nucleotides to facilitate deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) replication, the development of neural folds could be impaired. Adequate maternal folate status is important in preventing NTDs, but folic acid supplementation may also prevent NTDs in some individuals with normal folate concentrations who may not metabolize folate in an optimal manner. Furthermore, suboptimal folate status may disproportionately increase the risk of NTDs in specific groups of individuals who have a genetic susceptibility.[20] For example, certain polymorphisms of the methylenetetrahydrofolate reductase (*MTHFR*) gene (e.g., 677C>T) have been associated with lower folate concentrations and a higher risk of NTDs than in the absence of these polymorphisms.[24-26] *MTHFR* is involved in folate metabolism and the transfer of methyl groups used in the synthesis of nucleotides and other substrates including converting homocysteine to methionine. Folic acid supplementation may be particularly important for individuals with these types of genetic predispositions.[27] However, folic acid supplementation at 0.4 mg increases blood folate concentrations, reaching the optimal red blood cell (RBC) folate concentration threshold after 3 to 6 months of supplementation across all MTHFR genotypes.[28, 29]

Risk Factors

In addition to insufficient maternal folate, other risk factors for NTDs include, but are not limited to, a history of previous pregnancy affected by NTDs or a history of NTDs in a first- or second-degree relative; poorly controlled pregestational diabetes[30] or risk for diabetes such as previous gestational diabetes;[31] maternal obesity;[32] malabsorption caused by bariatric procedures; use of folic acid antagonists such as methotrexate, carbamazepine, and valproic acid;[33] specific genetic syndromes (e.g., trisomy 13 and 18); maternal fever in the first trimester;[34] low dietary folate intake; and lack of folic acid supplementation.[35] For diabetes and obesity, these effects may be due in part to genetic differences in glucose homeostasis and the subsequent impact on finely tuned processes in the developing embryo.[36] Socioeconomic risk factors such as maternal education levels, lower income, and lower income of community of residence have been associated with NTDs in some but not all studies.[37, 38] Socioeconomic factors may affect risk as a result of impacts on nutritional status, including supplementation patterns.[39]

Risk of NTDs has been found to be higher in certain ethnic groups such as First Nation groups in Canada and Hispanic persons in California.[40, 41] This finding may be related to a higher risk of genetic polymorphisms among these groups of persons but may also be due to differential folic acid intake. Folic acid fortification of U.S. grain products was found to result in consistent reductions in NTDs across racial and ethnic groups.[42, 43]

Prevention

Rationale for Intervention

NTDs are the second most common group of serious congenital anomalies in the United States, accounting for significant infant morbidity and mortality and costs to affected individuals, their families, and their communities. Many of these NTDS are caused by low folate concentrations in the body. Because NTDs occur very early in pregnancy, often before the pregnancy is even known, and usually results in limited or no chance of complete recovery, strategies that enhance folic acid uptake before pregnancy offer the best chance of prevention.

Intervention Strategies

Two approaches to enhancing folic acid update before pregnancy are available; one relies on folate fortification of the general food supply, and the other relies on individually directed folate supplementation. In keeping with the USPSTF's focus on strategies for prevention that are feasible or relevant for primary care, the focus of this report is on individually directed folate supplementation. However, trends in food fortification provide important context.

In 1998, the U.S. Food and Drug Administration mandated the addition of folic acid to specific enriched cereal grain products. At that time, an immediate drop in the prevalence of NTDs was noted which has been maintained since that time.[42] In 2016, the U.S. Food and Drug Administration began allowing corn masa flour to be voluntarily fortified with folic acid to address known disparities in folic acid intake and NTDs among Hispanic persons. Some experts predict that if there were mandated fortification of corn masa flour, an additional 40 NTDs per

year would be prevented in the United States.[44] Continued surveillance and comparisons of RBC folate concentrations before and after voluntary fortification of masa may help shed light on the effects of masa fortification. One analysis comparing 1 year of data (2017 to 2018) with prior years (2011 to 2016) found no statistically significant differences in RBC folate concentration in Hispanic women of reproductive age but did find that RBC folate concentration increased significantly among lesser acculturated Hispanic women consuming enriched cereal grain products only.[45]

Other potential strategies to prevent NTDs could include reduction of preconception obesity, better control of preconception diabetes, and avoidance of preconception folic acid antagonists. Questions persist regarding the optimal intake of folic acid given food supplementation, individualization of folic acid recommendations based on genetic variants, minimal effective dose, tolerable upper intake, and optimal ways to measure folate concentrations in the body.[46, 47] Questions also persist about potential harms. One proposed potential harm of folic acid supplementation is masking of vitamin B12 deficiency because of a compensatory effect on macrocytic anemia. This compensatory effect has been theorized to lead to a delay in the diagnosis and treatment of vitamin B12 deficiency, thereby causing irreversible neurologic injury. However, a population study using U.S. National Health and Nutrition Examination Survey data measuring serum B12 levels before (1991–1994) and after (2001–2006) food fortification found a lower risk of laboratory-diagnosed B12 deficiency after fortification.[48] Some experts have been concerned about the potential association of folic acid supplementation during pregnancy and autism diagnosis in the resulting children and increase in maternal cancer risk.[49, 50]

Source of Folate and Folic Acid

Folic acid supplementation is usually provided as a single vitamin or part of a multivitamin. Folic acid is converted into folates such as 5,10-methylenetetrahydrofolate or 5-methyltetrahydrofolate.[51] Folate (naturally occurring) and folic acid (synthetic supplement) sources include natural foods such as leafy greens,[52] fruits and fruit juices, nuts, beans, peas, seafood, eggs, dairy products, meat, and poultry;[1, 4, 53, 54] fortified grains and cereals in the United States; and supplements (either as a multivitamin or a single supplement). The bioavailability from supplemental folic acid is estimated to be 1.7 times the bioavailability from food because of the presence of several additional glutamate residues that need to be reduced in naturally occurring folates. Some individuals have suggested using methylfolate supplements for individuals with *MTHFR* variants associated with NTDs, but no data indicate that this supplement reduces the incidence of NTDs.[55]

Measures of Folic Acid Intake and Folate Status

Several measures are used to assess the adequacy of dietary folic acid consumption: recommended daily allowance (RDA), dietary folic equivalent (DFE), and estimated average requirement (**Appendix A Table 1**). It is difficult and imprecise to estimate the intake of folic acid from food sources. Plasma/serum folate can be measured and is a short-term measure of folate status that can vary based on the recency of folic acid intake.[56] No concentration threshold has been established for plasma/serum folate for the prevention of NTDs.

RBC folate concentration is a proxy for tissue stores of folate and is an indicator of long-term folate status. RBC folate concentration is probably the most accurate way to assess optimal body folate concentrations for NTD prevention.[29, 47, 57, 58] Although optimal RBC folate concentrations for NTD prevention have been established at the population level, consensus does not yet exist about whether to or how to routinely use RBC folate concentrations to assess NTD prevention at an individual level.[47, 57] The World Health Organization recommends an RBC folate concentration greater than 400 ng/mL (906 nmol/L) in persons capable of becoming pregnant to achieve the greatest reduction of NTDs.[58] This recommendation is consistent with findings from several recent studies; a dose-dependent response between RBC folate concentrations and NTD risk exists, and an optimal level is around 1,000 nmol/L.[47, 57] Testing is not routinely available at all laboratories, and assays may vary between institutions. The question of how much natural-food folate or folic acid intake is necessary to achieve adequate RBC folate concentration has also not yet been resolved and likely varies between specific populations.[29, 57, 58]

Current Clinical Practice

According to estimates from 2003 to 2006 National Health and Nutrition Examination Survey data, between 15 and 19 percent of reproductive-age women had inadequate folic acid intake when considering diet and supplements,[59] despite folic acid fortification of food and recommended supplementation guidelines. Using survey data from 1998 to 2016, only 20 to about 40 percent of recently pregnant or trying-to-get-pregnant women reported taking periconceptional folic acid supplements and those with unintended pregnancy were four- to fivefold less likely to have taken periconceptional folic acid supplements.[60-62] One source suggests a decrease in multivitamin use during pregnancy between 2006 and 2016.[61] At the same time, the rate of supplementation exceeding the upper level (1,000 µg per day) is low (2.7%).[63] These findings indicate that there is still substantial room for improvement in uptake of periconceptional folic acid supplementation. A recent study reported that the usual intake of folic acid from mandatory fortification is ~115 µg per day, suggesting a continued need for folic acid supplementation.[47]

Major clinical practice guidelines from professional medical and public health organizations consistently recommend a minimum folic acid supplementation daily intake of 400 µg up to 800[64] to 1,000 µg per day for all persons capable of becoming pregnant (**Appendix A Table 2**).[64-71] In addition to folic acid supplementation, organizations also recommend that high-risk persons consult their physicians for additional advice when planning to become pregnant. As noted above, rates of supplementation range from 20 to about 40 percent in individuals capable of pregnancy.[61, 72] According to data from the National Survey of Family Growth from 2011, 45 percent of pregnancies were unintended.[73] Therefore, medical organizations recommend that all persons capable of becoming pregnant should take folic acid supplementation.

Previous USPSTF Recommendation

In 2017, the USPSTF concluded that folic acid supplementation in the periconceptional period has substantial benefits in reducing the risk of NTDs in the developing fetus and reaffirmed its 2009 recommendation[74] that all persons who are planning or capable of pregnancy take a daily

supplement containing 0.4 to 0.8 mg (400 to 800 µg) of folic acid (A recommendation). This recommendation was based on evidence from experimental and observational studies conducted in settings without or before food fortification demonstrating a reduction in NTDs and adequate evidence that folic acid supplementation at usual doses is not associated with harms to the pregnant person or infant. Specifically, the only eligible RCT conducted in Hungary in the 1980s showed a benefit (odds ratio [OR] for NTDs of 0.131 [95% confidence interval {CI}, 0.026 to 0.648]; p=0.013).[75-81] These results were consistent with the results of studies in the United States, with two cohort studies[82-84] and three[33, 85, 86] of four[33, 85-87] case-control studies conducted prior to food fortification showing benefit. Four case-control studies conducted during or after food fortification in the United States did not show a statistically significant benefit.[35, 88-90]

Chapter 2. Methods

Key Questions and Analytic Framework

Using USPSTF methods, the investigators, USPSTF members, and AHRQ Medical Officers developed the scope, Key Questions (KQs), and analytic framework (**Figure 1**) that guided our literature search and limited review. Specifically, our KQs are:

1a. To what extent does folic acid supplementation reduce the risk for NTDs (first occurrence) in persons capable of getting pregnant?
1b. Does the effect of folic acid supplementation on NTDs (first occurrence) differ by race/ethnicity?
1c. Do the benefits of folic acid supplementation differ by dosage, timing, or duration of therapy?
2a. Are harms associated with folic acid supplementation to the pregnant person, fetus, neonate, or child?
2b. Do the harms of folic acid supplementation differ by dosage, timing, or duration of therapy?

Search Strategies

We searched PubMed/MEDLINE®, the Cochrane Library, and Embase for English-language articles published from July 1, 2015, through July 2, 2021. We used Medical Subject Headings as search terms when available and keywords when appropriate, focusing on terms to describe relevant populations, interventions, outcomes, and study designs. **Appendix B** describes the complete search strategies. We conducted targeted searches for unpublished literature by searching ClinicalTrials.gov and the World Health Organization's International Clinical Trials Registry Platform. To supplement electronic searches, we reviewed the reference lists of pertinent review articles meeting our inclusion criteria and added all previously unidentified relevant articles. We also manually reviewed all literature suggested by peer reviewers to incorporate them into the final review as needed. We conducted active surveillance through article alerts and targeted searches of journals to identify major studies published through February 10, 2023, to identify studies that may affect the conclusions or understanding of the evidence and the related USPSTF recommendation.

Study Selection

We selected studies on the basis of inclusion and exclusion criteria developed for each KQ based on the populations, interventions, comparators, outcomes, timing, settings, and study designs briefly described further in this section (detailed description in **Appendix C**). We imported all citations identified through searches and other sources into EndNote X9.2 (Thomson Reuters, New York, NY). Two investigators independently reviewed titles and abstracts. We dually and independently reviewed the full-text articles of abstracts marked for potential inclusion by either

reviewer. We resolved disagreements by discussion and consensus; if necessary, we sought adjudication of conflicts from other experienced team members.

We included studies that focused on the use of folic acid supplementation for the prevention of NTD-affected pregnancies in persons capable of getting pregnant. We did not include studies focusing solely on persons on antiseizure medications, persons with a history of NTDs, or persons not capable of getting pregnant (e.g., persons of biological male sex, prepubertal persons, postmenopausal persons, sterilized persons, or persons with medical conditions rendering them sterile) as these scenarios would be outside of the realm of primary care preventive care recommendations. Studies with mixed samples that included higher-risk persons requiring specialist care were eligible as long as the study also included lower-risk persons. In such studies with mixed samples, when stratified analyses of lower-risk participants were available, we limited our review to these analyses.

We included studies that examined the use of folic acid supplementation. We also included studies that examined supplementation with micronutrients (e.g., multivitamin, iron) in combination with folic acid for the prevention of NTDs.

We included studies that compared interventions with placebo, no treatment, or dietary supplementation only when compared with folic acid supplementation; supplementation with multivitamins not containing folic acid when compared with multivitamin supplementation containing folic acid; or iron supplements not containing folic acid when compared with iron supplementation with folic acid) and food fortification alone when compared with folic acid supplementation with food fortification. For KQs 1c and 2b on dose, we included studies that compared interventions with varying doses of folic acid or micronutrient plus folic acid supplementation. For KQ 1a, we included studies that reported on the benefits of folic acid supplementation initiated before the index pregnancy or in the first trimester of the index pregnancy (to ensure that studies focused on exposure during the critical period for neural tube closure) to prevent NTDs. For KQs 1b, 1c, 2a, and 2b, we included studies that reported on the benefits or harms of folic acid supplementation initiated before the index pregnancy and during the first, second, and third trimesters of pregnancy. Harms such as colorectal cancer or other reported types of cancer, inability to diagnose vitamin B6 or B12 adequately (masking of vitamin B6 or B12 deficiency), autism, asthma or allergies were specified as eligible; additionally, other outcomes that studies described as clinical harms were eligible.

We included studies conducted in the United States or in countries considered *very highly developed* based on the Human Development Index as defined by the United Nations Development Programme in 2020.[91] For KQs 1a, 1b, and 1c, we included randomized, controlled trials (RCTs), controlled trials, cohort studies, and case-control studies. For KQs 2a and 2b, we included RCTs, controlled trials, cohort studies, case-control studies, and registry data.

Quality Assessment and Data Extraction

Two reviewers independently assessed the methodological quality of all studies that met the inclusion criteria as good, fair, or poor using predefined criteria. Disagreements were resolved by discussion and consensus. Studies with "fatal flaws" were rated as having high risk of bias (i.e.,

poor quality). Specific considerations for this topic include the risk of misclassification bias from retrospective recall of dose and timing of exposure; the risk of selection bias from not identifying all cases of the outcome including fetal deaths and pregnancy terminations; and the risk of confounding from not appropriately accounting for relevant factors such as family history of an outcome (e.g., NTD, asthma, autism) or maternal obesity and diabetes (for NTD outcomes only). Other fatal flaws that resulted in poor-quality ratings included high and differential attrition.

For each included study, we abstracted pertinent details about study design, setting, methodology, participant and intervention characteristics, and outcomes. A second team member reviewed all data abstractions for completeness and accuracy.

Data Synthesis and Analysis

This report is a limited systematic review to provide an update of the evidence published since the USPSTF last considered this topic in 2017. The results of newly identified publications are narratively described. Results of studies included in previous evidence reviews are not included in the report. We qualitatively synthesized findings for each KQ by summarizing the characteristics and results of included studies in a narrative format, with accompanying summary tables. A summary table comparing the conclusions of this review with the conclusions of the previous review is provided in Chapter 4.

Expert Review and Public Comment

USPSTF and AHRQ Medical Officers reviewed a draft research plan for this review. The draft research plan was also available for public comment from July 22, 2021, through August 18, 2021. Clarifications to search terms and inclusion and exclusion criteria were made as appropriate. A draft version of this report has been reviewed by content experts, representatives of Federal partners, USPSTF members, and AHRQ Medical Officers and has been revised based on comments, as appropriate. A draft of this report was posted for public comment. In response to public comment, citations were added to support some statements, and the document was edited for clarity.

USPSTF and AHRQ Involvement

AHRQ funded this review under a contract to support the USPSTF. The authors of this review worked with USPSTF liaisons throughout the review process to develop and refine the scope of work, analytic framework, and KQs. AHRQ staff provided project oversight, including reviewing the draft report and assisting in the coordination of an external review of the draft report.

Chapter 3. Results

We screened 3,191 titles and abstracts and 142 full-text articles to identify 12 unique studies from 13 publications and N=1,244,072 for inclusion in this limited update (**Figure 2**).[62] We identified two fair-quality cohort studies[92-94] and one fair-quality case-control study[95] reporting on the benefits of folic acid supplementation to reduce the risk of NTDs (KQ 1). One high-quality RCT,[96] seven fair-quality cohort studies,[97-103] and one fair-quality case-control study[104] assessed the harms of folic acid supplementation (KQ 2). **Appendix D** provides the list of excluded articles that were screened at the full-text stage. **Appendix E** details methodologic quality assessments for all eligible studies. **Appendix F** provides detailed study characteristics and results. The results below focus on newly reported outcomes for this limited update.

KQ 1. Folic Acid Supplementation and Risk Reduction for First Occurrence of NTDs

Summary of Results

Three observational studies, described in four publications, reported on the association between folic acid supplementation and NTDs (N=990,372).[92-95] Two cohort studies were in populations with no food fortification (Norway[92, 93] and Japan[94]). The Norwegian cohort study reported results separately by time periods (1999–2005, 2006–2013, and overall [1999–2013]).[92-95] The authors hypothesized that these periods corresponded to lesser (1999–2005) and greater adherence (2006–2013) to recommendations regarding folic acid supplementation. The study reported a statistically significant reduction in NTDs in women on folic acid supplementation, regardless of timing of intake in the time period hypothesized to correspond to greater adherence (2006 to 2013),[92, 93] but not in other time periods. The Japanese cohort study reported no statistically significant differences associated with folic acid supplementation.[94] The third study, a case-control study set in the United States and Canada in the period following food fortification, focused on participants with pregestational diabetes and prepregnancy obesity and represents a higher-risk cohort than the Norwegian and Japanese cohorts.[95] This study reported no statistically significant associations between daily or less than daily folic acid supplementation compared with no supplementation and NTDs. The same study reported a statistically significant reduction in NTDs in women with prepregnancy obesity taking 0.4 mg to 1 mg folic acid, when compared with women with no supplementation, but this association did not persist in sensitivity analyses that adjusted for planned pregnancy rather than maternal age.[95] Across all three studies, no other statistically significant benefits were reported overall, or by dose (1 study[95]) or timing (1 study[92, 93]). Populations, interventions, and outcomes are described in the section that follows and in **Appendix F Tables 1** through **4**.

Study Characteristics

Three fair-quality studies, described in four publications, reported on the association between folic acid supplementation and the occurrence of NTDs.[92-95] Of these studies, two were cohort studies, set in Norway[92, 93] and Japan,[94] and one was a case-control study drawing from the United States and Canadian Slone Birth Defects Study.[95] The populations in the included studies

were heterogeneous representing different baseline risks for NTDs. Both cohort studies drew from general populations: the Norwegian study drew from the Medical Birth Registry of Norway[92, 93] and the Japanese study drew from the Japan Environment and Children's Study (a nationwide prospective birth cohort).[94] The case-control study from the United States and Canada, however, focused on higher-risk groups (NTD family history, periconceptional antiepileptic drug exposure, pregestational diabetes, and prepregnancy obesity), of which, only the results for participants with pregestational diabetes and prepregnancy obesity are eligible for this review and summarized below.[95]

Norwegian Cohort

Two articles, published in 2016[92] and 2020,[93] reported on the Norwegian cohort study. The 2016 publication reported on 528,220 persons with 880,568 pregnancies and 896,674 live births and stillborn infants; of these infants, 270 had NTDs, indicating a prevalence of 3.07 per 10,000 pregnancies. The International Classification of Diseases-10 (ICD-10) and the International Classification of Diseases-10-British Pediatric Association were used to code birth defects that were identified by pediatric examination in the maternity or neonatal ward. Information on folic acid supplementation in terminated pregnancies was not available so these were not included in the population for the analysis on impacts of folic acid use. When 551 cases of NTDs among terminated pregnancies were added, prevalence of NTDs increased from 3.07 to 9.32 per 10,000 pregnancies, indicating that a significant number of NTDs were not included in the analysis of the impact of folic acid exposure.[92]

The analysis reported on NTDs among live births and stillborn infants from 1999 to 2013 overall and also stratified results into two separate time periods: 1999 to 2005 and 2006 to 2013. The authors performed this stratified analysis because they found that the overall adjusted relative risk (aRR) was affected by year of birth. Although the authors did not list hypotheses to explain differences by time period, they cited several external events of importance in their interpretation of the findings: the introduction of folic acid recommendations in 1999, inclusion of 0.2 mg folic acid in multivitamin supplements from 2004 onward (before 2004, most multivitamins did not include folic acid), and increased compliance with folic acid recommendations in the second half of the time period analyzed (2006–2013). The 2020 publication used a similar but not identical denominator (894,927 births) and the same overall time frame (1999–2013; data on separate time periods were reported in an appendix).[93]

Both publications drew on standard data collection in the Medical Birth Registry of Norway (MBRN) that recorded self-reported maternal exposure to folic acid at 16 weeks and at delivery and defined exposure as occurring before, during, or before and during pregnancy. The type of exposure was recorded as folic acid only, multivitamins only, and folic acid and/or multivitamins. The authors reported that over-the-counter folic acid preparations in Norway contain 0.4 mg. As noted above, multivitamins included no folic acid before 2004 and 0.2 mg thereafter. No measures of adherence were reported. The exposure categories were compared against no use of either folic acid or multivitamins before or during pregnancy.

In the 2016 publication, the outcome of NTDs included anencephaly, encephalocele, and spina bifida and excluded NTDs that were accompanied by chromosomal abnormalities and/or other genetic syndromes.[92] The 2020 publication distinguished between total NTDs and isolated NTDs

and also excluded chromosomal anomalies, genetic syndromes and microdeletions, and teratogenic syndromes.[93]

Both publications associated with this cohort study adjusted for year of birth, maternal age, marital status, parity, maternal smoking, pregestational diabetes, and maternal epilepsy in their analyses. The high proportion of pregnancy terminations due to NTD (551 cases, 67% of all NTDs) compared with live births with NTDs (229 cases, 28% of all NTDs) and stillbirths with NTDs (41 cases, 5% of all NTDs)[92] suggests the potential for selection bias. Overall, the study, across the two publications, was rated as fair quality because of the potential for unmeasured confounding, the potential for recall bias leading to bias in the classification of the intervention, and the potential for selection bias.

Japanese Cohort

The Japanese cohort comprised 92,269 singleton pregnancies occurring between January 2011 and March 2014.[94] Pregnancy outcomes included spontaneous abortion, termination of pregnancy, stillbirth, and live birth. The study recorded 74 unique NTDs (spina bifida, anencephaly, and encephalocele), indicating a prevalence of 8.02 per 10,000 pregnancies. NTD diagnoses and birth outcomes were based on medical records that recorded information diagnosed by obstetricians or gynecologists immediately after delivery and during the first month at a regular checkup.

The study provided information regarding patient-reported supplement use for 1 year before pregnancy confirmation and for 12 weeks after pregnancy confirmation.[94] The study noted that the recommendation intake was 0.4 mg but did not specify the dose from individual participants. The study compared adequate users of folic acid supplements (started before conception) with inadequate users (started after pregnancy recognition or nonuse of folic acid supplements). NTD outcomes included spina bifida, anencephaly, and encephalocele.

The study adjusted for age, smoking habits, body mass index, history or complication of diabetes and gestational diabetes mellitus, valproic acid and other antiepileptic drugs.[94] The study was rated fair quality because of the potential for confounding, bias from recall of folic acid exposure, and lack of information on how missing data were handled.

U.S. and Canadian Case-Control Study

The U.S. and Canadian case-control study identified pregnancies in high-risk groups (diabetes, obesity, NTD family history, periconceptional antiepileptic drug exposure) from tertiary care centers and birth hospitals in Boston, Philadelphia, and Toronto (1976–2005); San Diego (2001–2015), and Nashville (2012–2015); and via birth defect registries in Massachusetts (2003–2015) and parts of New York State (2004–2015); the analyses were restricted to data from 1988 through 2015.[95] We included subgroups of those participants with prepregnancy diabetes and those with prepregnancy obesity in our analyses; as noted earlier, other groups were ineligible for this review. Participants who reported diagnosis of type 1 or 2 diabetes mellitus before the end of the periconceptional period were included in the pregestational diabetes group. Participants whose reported prepregnancy height and weight yielded a body mass index (BMI) ≥ 30 kg/m^2 were included in the prepregnancy obesity group; this group excluded participants

with NTD history, antiepileptic drug use, or pregestational diabetes. The study reported 111 cases and 1,243 controls whose mothers had prepregnancy obesity without diabetes and 12 cases and 63 controls whose mothers had pregestational diabetes with or without obesity.

Cases were defined as pregnancies affected by anencephaly, spina bifida, or encephalocele, resulting in live birth, stillbirth, or elective termination >12 weeks of gestation, based on clinical geneticist review. Cases were identified through arrangements with state birth defect registries and participating institutions.[105] Conjoined twins and infants with amniotic bands, body wall defects, chromosomal anomalies, a known syndrome, or unconfirmed diagnoses were excluded. From 1988 to 1992, controls were pregnancies affected by minor malformations only or by one of several major malformations not known to be associated with folic acid. From 1993 onward, controls were liveborn infants without major structural malformations.

The study ascertained exposure to folic acid supplementation by interview with study participants within 6 months of delivery.[95] Participants reporting daily exposure to a product containing folic acid 28 days before to 28 days after the first day of the last menstrual period were categorized as daily supplement users. Participants reporting exposure but not daily exposure were categorized as than less than daily. Additionally, based on the information provided by participants, the study authors calculated average daily dose and categorized dose as <0.4 mg, 0.4 mg to <.1.0 mg, or ≥1.0 mg. The analyses compared less than daily folic acid supplementation; daily folic acid supplementation; and <0.4 mg, 0.4 mg to <1.0 mg, or ≥1.0 mg of folic acid with no supplements in the periconceptional period.

The study adjusted for maternal age and study center and also adjusted for planned pregnancy instead of maternal age in sensitivity analyses.[95] The study was rated fair quality because of the potential for confounding from failure to account for food fortification changes, unmeasured confounding, and bias from recall of folic acid exposure.

Results of Included Studies

KQ 1a. To What Extent Does Folic Acid Supplementation Reduce the Risk for NTDs (First Occurrence) in Persons Capable of Getting Pregnant?

Table 1 summarizes key results. The Norwegian cohort study reported statistically significant associations with lower NTD risk in the later 2006 to 2013 time period but not in the overall time period 1999 to 2013 or in the earlier time period 1999 to 2005.[92, 93] In the 2006 to 2013 period, the study reported a higher level of compliance with folic acid supplementation recommendations than in previous time points and inclusion of folic acid in multivitamin supplements. The use of folic acid and/or multivitamins resulted in a statistically significant lower aRR of NTDs when taken before pregnancy (aRR, 0.54 [95% CI, 0.31 to 0.91]).[92] NTDs were diagnosed after live or stillbirth by expert examiners. The study also reported statistically significant associations between folic acid or multivitamin supplementation during pregnancy only or both before and during pregnancy. No analyses in the first time period (1999–2005) or in the overall time period (1999–2013) yielded statistically significant associations.

The Japanese cohort study reported no statistically significant associations between adequate use of folic acid supplementation (initiated before conception) when compared with inadequate use

(started after pregnancy recognition or nonuse of folic acid supplements).[94] The study reported an adjusted odds ratio (aOR) of 0.62 (95% CI, 0.23 to 1.71). Analyses by type of NTD (spina bifida, anencephaly, encephalocele) also showed no statistically significant associations. NTDs were diagnosed after live birth or stillbirth by expert examiners.

The U.S. and Canadian case-control study reported no statistically significant associations between most measures of exposure (less than daily; daily; and <0.4 mg, 0.4 mg to <1.0 mg, or ≥1.0 mg of folic acid supplementation) and NTDs.[95] The authors reported multiple aORs, depending on variables adjusted in the model. For daily supplementation compared with no supplementation, aORs ranged from 0.65 to 0.69 depending on variables included in the model; CIs spanned the null. For women with pregestational diabetes, aORs ranged from 0.25 to 0.37; CIs spanned the null. Nearly all other measures of exposure (less than daily, <0.4 mg, 0.4 mg to <1.0 mg, or ≥1.0 mg of folic acid supplementation) similarly reported no statistically significant associations between folic acid supplementation and NTDs. The only exception was participants with prepregnancy obesity taking supplements of 0.4 mg to 1 mg. Among this group, the OR for NTDs was significantly lower in analyses that adjusted for maternal age and study center (aOR, 0.54 [95% CI, 0.29 to 0.95]) but not in sensitivity analyses that adjusted for planned pregnancy instead of maternal age and study center (aOR, 0.57 [95% CI, 0.30 to 1.02]). The authors did not report adjusting the CIs for multiple comparisons. NTDs in this study were from a birth defects registry that used an active surveillance program with trained personnel evaluating reports of birth defects based on physical exam at the time of delivery. NTDs from pregnancy terminations or early fetal loss were not included. Notably, the study included data in a period of time (1988–2015) spanning the introduction of food fortification in the United States and Canada in 1998 and consequent attenuation of the effect of individual supplementation, but the study did not stratify the analyses accordingly.

KQ 1b. Does the Effect of Folic Acid Supplementation on NTDs (First Occurrence) Differ by Race and Ethnicity?

Differences in NTD prevalence by race and ethnicity could not be evaluated because no studies reported on these data.

KQ 1c. Do the Benefits of Folic Acid Supplementation Differ by Dosage, Timing, or Duration of Therapy?

As noted previously, one study in Norway, reported in two publications, presented data on a population cohort relevant to NTD risk and the timing (before only, during only, before and during) of supplementation.[92, 93] The results were consistent in demonstrating no effect of folic acid supplementation in the overall time period (1999–2013) and the first time period (1999–2005), regardless of the timing of folic acid supplementation (before, during, or before and during pregnancy). In the second time period, the results were consistent in demonstrating benefit of folic acid supplementation regardless of timing (before pregnancy only: aRR, 0.54 [95% CI, 0.31 to 0.91];[92] before and during pregnancy: aRR, 0.49 [95% CI, 0.29 to 0.83];[93] during pregnancy only: aRR, 0.62 [95% CI, 0.39 to 0.97][93]).

One case-control study of participants with prepregnancy obesity reported statistically significantly reduced association between NTD risk and exposure of 0.4 to <1.0 mg of folic acid

supplementation daily (aOR, 0.54 [95% CI, 0.29 to 0.95]) but not for exposures of <0.4 mg (aOR, 1.29 [95% CI, 0.40 to 3.37]) or ≥1.0 mg (aOR, 0.84 [95% CI, 0.38 to 1.68]).[95] As noted previously, the finding of a statistically significant difference for the 0.4 to <1.0 mg did not persist in sensitivity analyses adjusting for planned pregnancy instead of maternal age (aOR, 0.57 [95% CI, 0.30 to 1.02]), in which all exposures, regardless of dose, did not demonstrate a statistically significant association with NTDs. Notably, these subgroups of participants had as few as four to 14 cases; a single statistically significant result may have arisen by chance.

KQ 2. Harms of Folic Acid Supplementation

Summary of Results

One trial reported no association between doses of folic acid supplementation (4 mg vs. 0.4 mg) and twin delivery (N=431).[96]

Six cohort studies[98-103] and one case-control study[104] examined the association between folic acid supplementation and autism spectrum disorder (ASD) (N=761,125). In addition, one cohort study assessed association between folic acid supplementation and maternal cancer (N=429,004).[97] No study reported a statistically significant association for either of these two harms.

Two cohort studies reported some statistically significant benefits associated with folic acid supplementation and ASD,[100, 103] but other analyses in the similar geographic settings[104] or even the same population[102] that used different measures of exposure[102, 104] or comparator[104] did not report these benefits.

Two cohort studies and one case-control study examined associations between folic acid supplementation and ASD by dose[98, 100, 104] and found effects with overlapping CIs, suggesting no differences by dose. Two cohort studies assessed associations between folic acid supplementation and ASD by timing.[99, 100] Neither reported harm, but one reported a statistically significant benefit associated with folic acid supplementation initiation in weeks 5 to 8 of the pregnancy alone.[100] Populations, interventions, and outcomes are described below in detail and in **Appendix F Tables 5** through **11**.

Study Characteristics: Twinning

A single high-quality RCT, conducted in Italy between 2009 and 2014, compared outcomes following randomization of 1,060 women age 18 to 44 years and planning a pregnancy to 4 mg vs. 0.4 mg of folic acid supplementation.[96] After exclusions for early interruptions (e.g., withdrawal of consent, adverse events, or other reasons) (N=167), loss to followup (N=137), lack of conception at 1 year (N=251), delayed or unclear timing of the start of folic acid supplementation relative to conception (N=44) or assisted reproductive technology conceptions (N=30), 431 natural conceptions were retained for analysis.

Results of Included Studies: Twinning

KQ 2a. Are Harms Associated With Folic Acid Supplementation to the Pregnant Person, Fetus, Neonate, or Child?

No studies reported on risk of overall risk of twinning.

KQ 2b. Do the Harms of Folic Acid Supplementation Differ by Dosage, Timing, or Duration of Therapy?

One trial reported no differences between an exposure of 4 mg vs. 0.4 mg of folic acid on twin deliveries (3/227 [1.3%] vs. 6/204 [2.9%]; RR, 0.45 [95% CI, 0.11 to 1.77]). No studies reported on variation in harms by duration or timing of therapy.

Study Characteristics: Autism Spectrum Disorder

Six fair-quality cohort studies[98-103] and one fair-quality case-control study[104] examined the association between folic acid supplementation and the incidence of ASD. Two of these studies were set in Israel,[103, 104] one in Sweden,[101] two in Denmark,[98, 99] and two in Norway.[100, 102] Given similarities within countries (and differences across countries) in secular trends in supplementation and typical doses of folic acid in over-the-counter supplements, the analysis below summarizes results by country.

Israeli Studies

The two studies set in Israel drew from independent populations of the Maccabi Healthcare Service organization (Sharman Moser et al, 2019,[104] case-control study from 2000 to 2013, N=21,895 children, including 2,009 with ASD) and the Meuhedet healthcare organization[103] (retrospective controlled cohort study of births from 2003 to 2007, N=45,300 children, including 572 with ASD).

The Sharman Moser case-control study relied on ASD diagnoses made after a multidisciplinary assessment (pediatric neurology, development, and psychology) and concurrence between the physician and the psychologist that Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria were met.[104] ASD patients were randomly matched with ASD-free children by birth year (within 2 years), maternal age (within 2 years), sex, residential area, and socioeconomic status. The study measured folic acid exposure from dispensing data in medical records and categorized exposure as low supplemented (0.2 to <0.4 mg/day), typically supplemented (0.4 to <1 mg/day), high supplemented (1 to <3 mg/day), and very high supplemented (>3 mg/day) and compared these results to not supplemented or very low supplemented (median daily dose of <0.2 mg).[104] In Israel, multivitamins are available through the health system at a lower cost than outside the health system ($9 for 100 tablets of 0.4 mg vs. $12).[104]

In the Levine cohort study, folic acid supplement doses were not specified but were recorded from prescription registers as occurring before or during pregnancy when compared with no exposure in that time interval.[103] This study followed participants from birth to 15 years. ASD

was ascertained by a developmental behavioral pediatrician; authors used 10th revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-I0) codes to define the condition. These diagnoses happened after children with probable ASD were evaluated by an expert panel comprising social workers, a psychologist, and either a trained psychiatrist, developmental behavioral pediatrician, or child neurologist.[103] This study included all participants with ASD and one-third of all live births.[103]

The Levine cohort study adjusted for birth year, sex, socioeconomic status (high vs. low), a maternal and paternal psychiatric diagnosis at childbirth (present or absent), maternal and paternal age at childbirth, and parity.[103] The Sharman Moser case-control study matched participants by birth year, maternal age, sex, residential area, and socioeconomic status and adjusted results for maternal age, subfertility, number of physician or obstetrics visits in the 15 months before index date, birth order, and total number of children in the family but not birth order of the child; sensitivity analyses focused on first-order births.[104] We rated both studies as fair quality because of potential for bias from unmeasured confounding and the potential for bias in measurement of exposure from prescriptions or medical records. Neither study evaluated adherence to the supplements.[103, 104]

Swedish Cohort

One cohort study drew from the Stockholm youth cohort, which includes children between 4 and 15 years of age living in Stockholm County, Sweden, for at least 4 years between 2001 and 2011 (N=94,864, including 2,123 with ASD).[101] Clinicians recorded self-reported supplement use during pregnancy at the first antenatal visit; dose was not reported in the study. The comparison was no use of multivitamins, iron, or folic acid. The outcome of ASD was ascertained following structured diagnostic assessment by specialists and was recorded in medical records using ICD-10 and DSM-IV codes. The analysis adjusted for child characteristics (sex, birth year, and years resided in Stockholm County), socioeconomic indicators (education, family income, and maternal birth country), maternal characteristics (age, BMI, parity, smoking status), medication use during pregnancy (antidepressants or antiepileptics), and maternal neuropsychiatric conditions (anxiety disorders, autism, bipolar disorder, depression, epilepsy, intellectual disability, nonaffective psychotic disorders, and stress disorders). We rated the study as fair quality because of the risk of bias from unmeasured confounding and attrition. The study conducted sensitivity analyses using matched siblings and propensity score–adjusted models.

Danish Cohort

Two publications of the same cohort, the Danish National Birth Cohort (DNBC), reported on the association between folic acid exposure and ASD.[98, 99] The database, comprising more than 100,000 pregnancies, collected data from 1996 to 2002. The study organizers changed the recruitment form midway through the study to allow greater specificity in exposure to supplements. The 2016 DNBC publication by Virk et al. selected 19,042 (total N with ASD, autism, or Asperger's syndrome were not reported) women with singleton offspring with exposure to folic acid or no exposure to supplements from 2000 to 2002.[99] A later publication by Strom and colleagues, in 2018, took advantage of ongoing computerization of the early recruitment forms and selected 87,210 participants (1,234 with ASD and 312 with childhood autism) with singleton offspring who were born at 32 weeks or more and weighed 2,500 grams

or more.[98] The exposure was reporting having taken folic acid in at least 2 weeks in the period that began 4 weeks before the last menstrual period and continued 8 weeks after the last menstrual period. Most folic acid supplements available in Norway during this period contained 0.4 mg. Periconception folic acid use was defined as any use of a supplement containing folic acid during one or more of the following periods: −4 to −1 weeks, 1 to 4 weeks, and 5 to 8 weeks. The two publications varied in their reference categories: the Virk (2016) publication defined the reference group of unexposed women as women who indicated no supplement use during the −4- to 8-week period.[99] The Strom (2018) publication created a reference category of no use for each separate time period of exposure.[98] The Strom (2018) publication also provided dose information from a mid-pregnancy measure of folic acid use of <0.4 mg and ≥0.4 mg compared with no use. Virk et al mentioned an average of 9.6 years (8.1−11.4 years) followup.[99]

The Virk (2016) study obtained outcome data from the National Hospital Register and included ICD-10-CM codes for autistic disorder, Asperger's syndrome, pervasive developmental disorder, and ASD (the most inclusive).[99] The Strom (2018) study identified 1,234 cases of ASD and 312 cases of childhood autism that had been diagnosed with ICD-10 diagnosis codes for childhood autism and entered in at least one of two national registries.[98] The only category in which the case definition is identical in the two studies was autistic disorder/childhood autism.

We rated both studies as fair quality because of the risk from unmeasured confounding and attrition. The 2016 publication adjusted for maternal age, household socioeconomic status, maternal smoking during pregnancy, maternal alcohol consumption during pregnancy, maternal BMI, maternal mental health history, and socioeconomic status.[99] The 2018 publication adjusted for the following covariates: maternal age, paternal age, parity, maternal smoking during pregnancy, maternal education, family socioeconomic status, pregnancy intendedness, maternal BMI, and sex of child.[98]

Norwegian Cohort

Two publications drew from a pregnancy cohort in Norway consisting of pregnant persons who were recruited in the second trimester. Suren et al selected participants from the Norwegian Mother and Child Cohort Study (MoBa) and its substudy specific to autism, the Autism Birth Cohort study.[100] Nilsen et al drew from the same source but sought to examine the potential for selection bias, by comparing the MoBa cohort with a nationwide registry, the Medical Birth Registry of Norway (MBRN).[102] All pregnancies lasting more than 12 weeks in Norway are required to be recorded in the MBRN by law, but only a subset of women volunteered to participate in MoBa. In addition to these differences in data sources and purposes, the publications varied in other respects as well. Suren et al selected 85,176 children (270 with ASD) born between 2002 and 2008;[100] Nilsen et al selected 89,836 children (234 with ASD) born from the MoBa cohort between 1999 and 2007 and 507,856 children (2,072 with ASD) born in the same period from the MBRN.[102] The two data sources had an overlap of 234 ASD cases.

The MBRN population was younger, more likely to be single, and more likely to deliver at a larger hospital, more likely to smoke and less likely to use folic acid supplements compared with pregnant persons in the MoBa cohort. Participants in the MoBa cohort were likelier to have healthier lifestyles and higher socioeconomic status than the nationwide population, suggesting a more selective population.

Importantly, the two analyses also differed in their source of data for use of folic acid supplements. Suren et al relied on MoBa supplement data collected at 18 weeks of gestation. Women were asked to record their intake of vitamins and supplements but not asked to specify exact amounts; those who took folic acid as part of a multivitamin supplement may have received less than 0.4 mg. Suren et al further recorded the initiation of exposure from weeks −4 to –1, 1 to 4, 5 to 8, and 9 to 16, using no exposure to vitamins or minerals in weeks −4 to 8 as the referent. The Suren publication also reported on exposure based on self-reported use of supplements in week 22 of pregnancy and recorded folic acid intake as 0.001 to 0.399 mg and 0.4 mg and more. The analysis of folic acid exposure used no folic acid in week 22 as the referent.[100] Nilsen et al relied on the MBRN data, specifically use was determined by an item on a participant questionnaires at the beginning and the end of the pregnancy about "any use of maternal folic acid supplements before and/or during pregnancy."[102]

The two publications also varied in outcome measurement. Suren et al reported that cases were identified through a variety of means, but all cases had either been individually assessed using validated tools and diagnosed according to DSM-IV criteria (N=135) or via specialist diagnosis ICD-10-CM in patient registry (N=135) for ASD including autism, Asperger's, and pervasive developmental disorder.[100] In the Nilsen publication, authors diagnosed ASD by linkage with a national administrative database with mandatory reporting via ICD-10-CM for autism, atypical autism, Asperger's, pervasive developmental disorder, and unspecified and other pervasive developmental disorder. The cases in the cohort were validated against DSM-IV criteria and were found to be accurate in 97 percent of cases.[102]

Suren et al reported ORs that were adjusted for year of birth, maternal education, and parity.[100] Nilsen et al reported adjusting effect estimates for year of birth, maternal and paternal age, marital status, parity, and hospital size.[102] We rated these studies as fair quality because of the potential for unmeasured confounding; additionally, the measurement of exposure (any use vs. no use) in the Nilsen et al study does not account for dose or adherence.[102]

Results of Included Studies: Autism Spectrum Disorder

KQ 2a. Are Harms Associated With Folic Acid Supplementation to the Pregnant Person, Fetus, Neonate, or Child?

Table 2 presents summary results across the included studies.

Both studies set in Israel reported no harms. The Levine cohort reported benefits: exposure to folic acid supplements either before (aRR, 0.56 [95% CI, 0.42 to 0.74]) or during pregnancy (aRR, 0.32 [95% CI, 0.26 to 0.41]) was associated with a lower risk of ASD compared with no exposure.[103] By contrast, the Sharman Moser case-control study found ORs ranging from 1.01 to 1.15 for the four levels of folic acid exposure with no statistically significant associations and no dose response observed.[104] Differences in the definition of exposure and comparator may explain these differences: the Sharman Moser cohort study stratified exposure by dose and compared it with no or low exposure,[104] whereas the Levine cohort study did not stratify exposure and compared exposure with no exposure.[103]

The Swedish cohort study found no statistically significant associations between folic acid exposure and ASD with (aOR, 1.20 [95% CI, 0.71 to 2.01]) or without (aOR, 1.29 [95% CI, 0.99 to 1.67]) intellectual disability.[101] However, the association between folic acid supplementation and these outcomes put together, for ASD with or without intellectual disability, was statistically significant (aOR, 1.27 [95% CI, 1.01 to 1.30]). Sensitivity analyses of a matched cohort of siblings (aOR, 1.48 [95% CI, 0.87 to 2.51]) and propensity score models (aOR, 1.17 [95% CI, 0.89 to 1.51]) both resulted in nonsignificant effects, suggesting a lack of evidence of association.

Two analyses with some overlap in time and participants from Denmark reported consistent results.[98, 99] The 2016 analysis found no statistically significant associations between folic acid exposure between pregnancy weeks −4 to 8 and ASD, autism, Asperger's syndrome, or pervasive developmental disorder not otherwise specified. Reported aRRs ranged from 0.85 to 1.18 (see **Appendix F Table 6** for more details).[99] The 2018 analysis,[98] using a larger and overlapping dataset but that restricted the outcomes to only ASD or childhood autism, also found no statistically significant associations between exposure to folic acid supplements between pregnancy weeks −4 to 8 and these outcomes; adjusted hazard ratio (HR) ranged from 1.06 to 1.09 (see **Appendix F Table 6** for more details).[98] The studies reported no associations between dose or time intervals of exposure and autism.

Two analyses (Suren et al[100] and Nilsen et al[102]) conducted among participants in Norway, with some overlap in populations, but differences in measurement of exposure and outcomes, found no harms. The Suren et al analysis reported that prepregnancy and early pregnancy exposure to folic acid supplementation resulted in lower odds of autism (aOR 0.61 [95% CI, 0.41 to 0.90]) but no statistically significant associations for Asperger's syndrome (aOR 0.65 [95% CI, 0.36 to 1.16]) or pervasive developmental disorder (aOR, 1.04 [95% CI, 0.66 to 1.63]). Notably, sample sizes for all these analyses were small, leaving open the potential for chance findings

The Nilsen et al analysis sought to understand bias in outcome measurement using two data sources and looked at ASD only and found an aOR of 0.86 (95% CI, 0.78 to 0.95) for the MBRN population and 0.85 (95% CI, 0.65 to 1.11) for the MoBa cohort.[102] Nilsen and coauthors attributed differences between the population and the cohort to lack of precise data in the MBRN on timing, dose, and frequency of folic acid supplement use compared with the MoBa.

KQ 2b. Do the Harms of Folic Acid Supplementation Differ by Dosage, Timing, or Duration of Therapy?

Three studies, set in Israel (Sharman Moser[104]), Denmark (Strom et al[98]), and Norway (Suren et al[100]), reported on dosage. Two studies used similar exposure and comparator variables: the Danish[98] and Norwegian[100] studies reported aORs or HRs ranging from 0.89 to 1.06 for folic acid doses (<0.4 mg and ≥0.4 mg vs. no use) in mid-pregnancy and autism, with overlapping CIs, suggesting no differences by dose. The Sharman Moser case-control study reported no statistically significant associations across the various exposure categories defined in this study compared with no or very low exposure with aORs ranging from 1.01 to 1.15.[104]

Two studies, set in Denmark (Virk et al,[99]) and Norway (Suren et al[100]), used similar categories for timing of folic acid exposure and similar comparators. Both found aRR or HR ranging from

0.44 to 1.39 for various exposures when compared with no exposure. The Norwegian study focused on initiation in the specified time period, whereas the Danish study focused on use of supplements within the time period. With the exception of initiation in weeks 5 to 8 in the Norwegian study (14/16,184 vs. 32/14,721, aOR, 044 [95% CI, 0.23 to 0.83]), no statistically significant associations were identified.

No studies reported on associations between duration of exposure and autism.

Study Characteristics: Maternal Cancer

Norway Cohort

A single fair-quality cohort study, drawing from multiple population registries in Norway between 1999 and 2010, examined the association between exposure to folic acid supplementation during pregnancy and incidence of maternal cancer (3,781 cases) among 429,004 persons over an average of 7 years (range 0.04 to 12 years).[97] Information on folic acid exposure came from the MBRN notification form. This information was used to characterize folic acid exposure as no use, before and/or during one pregnancy, and before and/or during two or more pregnancies.

The outcome was defined as the first incidence of any cancer diagnosis; additionally cancers were presented by type. Breast cancer was the single most commonly reported cancer (30% of all cancers). Other relatively frequently occurring cancer types included melanoma (13%), cervical and uterine cancer (12%), central nervous system cancers (9%), and other cancers (9%). Other reported cancers included colorectal, lung and trachea, non-melanoma skin, ovarian, thyroid, other endocrine, Hodgkin's lymphoma, non-Hodgkin's lymphoma, and leukemia. The analyses adjusted for maternal age, maternal age at first childbirth, maternal year of birth, parity, marital status, education, occupation, multivitamin use, and smoking. We rated the study as fair quality because of the potential for unmeasured confounding and potential for bias in the classification of the intervention from recall bias and lack of information on dose and adherence.

Results of Included Studies: Maternal Cancer

KQ 2a. Are Harms Associated With Folic Acid Supplementation to the Pregnant Person, Fetus, Neonate, or Child?

The one study we identified adjusted for maternal age, age at first childbirth, year of birth, parity, marital status, education, occupation, and smoking status; it found no statistically significant differences in the risk of developing any cancer after use of folic acid in one (HR, 1.08 [95% CI, 1.00 to 1.18]) or two or more pregnancies (HR, 1.06 [95% CI, 0.91 to 1.22]), when compared with women with no exposure to folic acid in pregnancy.[97] However, the study did not report the total timing of folic acid exposure in months and years as this amount of time could vary between individuals who take longer or shorter to conceive. The study also did not report statistically significant associations for any individual cancer type, for example, breast cancer (HR, 0.96 to 1.10, with CIs widely spanning the null). The range of HRs for other cancers spanned from 0.26 to 2.41 with wide CIs that included the null. Sixteen percent of the sample

was missing information on smoking. The authors performed multiple imputation for smoking status and reported that they found no substantial changes in the risk estimates.

KQ 2b. Do the Harms of Folic Acid Supplementation Differ by Dosage, Timing, or Duration of Therapy?

No studies reported on variation in harms by dosage, duration, or timing of therapy.

Chapter 4. Discussion

Summary of Evidence

Since the previous review on this topic, new observational studies were published on the effects of folic acid supplementation on NTDs (3 analyses), maternal cancer (1 analysis), and ASD (7 analyses). This update was limited to summarizing only new evidence and does not incorporate the foundational evidence leading the USPSTF's current A recommendation for this topic. This recommendation was based on experimental and observational studies conducted in settings without or before food fortification demonstrating a reduction in NTDs and no evidence of harms at usual doses.

Once the benefits of folic acid supplementation were widely known and fortification of the food supply was initiated in some countries, ethical and logistical constraints have precluded the conduct of trials comparing folic acid supplementation with no supplementation. Since then, studies have relied on cohort and case-control designs with greater potential bias than in randomized trials, both from selection bias (e.g., inability to include pregnancy terminations, other unmeasured confounding) and potential misclassification of exposure (from poor or differential recall of exposure). In the prior review, the results from these observational studies inconsistently demonstrated benefit but have not demonstrated any harms. The overall conclusions from this limited update are consistent with those of the prior review in demonstrating some evidence of benefit for NTDs and no evidence of harm (**Table 3**) for autism and maternal cancer; we found no newly eligible studies on previously reported harms of multiple gestation, childhood respiratory conditions, and maternal adverse events. We found no evidence to suggest that benefits for NTDs varied by timing, duration, and dose of folic acid exposure.

Studies identified in this review measured the impact of folic acid supplementation among women who eventually became pregnant. However, the guidance focuses on "women who are planning or capable of pregnancy." There are two potential inconsistencies to consider. One is that individuals who are capable of becoming pregnant do not always identify as women; therefore, gendered language is not inclusive of their experience.[106] Second, the language of the recommendation may be taken to assume that every reproductive-age individual who could theoretically carry a pregnancy should take folic acid supplementation. In all cases, the people involved in the identified studies were "women" who were planning a pregnancy or who had already become pregnant. No studies evaluated the use of folic acid supplementation among all people with a uterus who could theoretically conceive. Because a significant proportion of pregnancies in the United States are unintended, patient-centered counseling by primary care physicians about preconception folic acid supplementation may need to include a discussion on the likelihood of an individual becoming pregnant based on their actual behaviors, including sexual activity, sexual partners, and consistency of contraceptive use. No studies evaluated this type of approach to recommending folic acid supplementation.

Limitations of the Review

Our scoping decisions serve as limitations to our conclusions. We restricted interventions to folic acid supplementation and did not consider food fortification, counseling to increase dietary intake, or screening for NTDs. We did not systematically examine the benefits of folic acid supplementation on benefits other than averted NTDs. We focused our limited update on the direct association between folic acid supplementation and NTDs, which is the focus of the prior review. A future updated review may need to consider the impact of folic acid supplementation on RBC folate concentrations, which may be a less biased measure of folate exposure than self-report of intake and would reflect adherence. We did not systematically evaluate the effect of folic acid supplementation among high-risk populations such as women with previous pregnancies with NTDs or exposure to antiepileptic drugs. These populations may respond to folic acid supplementation in different ways. We did not evaluate the impact of various clinical and public health strategies to improve the uptake of folic acid recommendations, which has been previously reviewed by the Community Preventive Services Task Force.[107] We did not evaluate the impact of folic acid supplementation on the risk of early pregnancy loss or preconception outcomes such as ovulation patterns and infertility, although there is growing evidence that folate status and supplementation may have an impact on these outcomes as well.[108]

Limitations of the Evidence

The state of the science also serves as a limitation to the evidence. Although we searched for evidence on subgroups of interest such as *MTHFR* status, we found no eligible studies. All new evidence is observational with limited ability to control for confounding, selection bias, recall bias, and attrition. One important limitation was that few studies were able to include pregnancies ending by termination, preimplantation, or early loss, thereby limiting the ability to account for all NTDs in the population. As a result, included studies have inherent uncertainty regarding case ascertainment (for NTDs and harms) and degree of exposure (dose, timing, and duration) to folic acid supplementation. Mandatory food fortification practices vary by geography and time period of investigation and contribute to heterogeneity across studies. Furthermore, failure to account for changes in food fortification practices over time within studies creates the potential for confounding. Heterogeneity in measuring adherence to folic acid supplementation and harms such as autism also limit the potential for causal inference.

Future Research Needs

Studies suggested that folic acid supplementation of 400 µg resulted in an optimal RBC folate concentration threshold after 3 to 6 months of supplementation across all *MTHFR* genotypes,[28, 29] and that folic acid supplementation in populations known to have high rates of *MTHFR* TT resulted in a reduction in NTDs in that population.[109] No trials have explored the effect of folic acid supplementation on NTDs by genotype. Future reviews could evaluate the risk of NTDs based on RBC folate concentrations and whether screening for RBC folate concentration preconception could help identify individuals who need modified folic acid supplementation of different doses or formulations before conception. Future reviews could also investigate whether

folic acid supplementation affects other preconception and early pregnancy outcomes, including fertility, miscarriage, and early fetal loss. Given that these outcomes may lead to differential rates of NTDs, understanding the impact of folic acid supplementation on these outcomes would add further context to the question of how folic acid supplementation affects NTDs. These questions were outside the scope of this limited update.

Conclusion

New evidence from observational studies provides continued evidence of benefit of folic acid supplementation for preventing NTDs and no evidence of harms related to autism or maternal cancer and is consistent with the previously reviewed evidence on this topic. The 2017 USPSTF recommendation supporting folic acid supplementation in pregnancy was based on previously reviewed evidence from an RCT and observational studies reporting reduced NTDs with supplementation and no consistent evidence of harms for multiple gestations, maternal adverse effects, or child respiratory illness.

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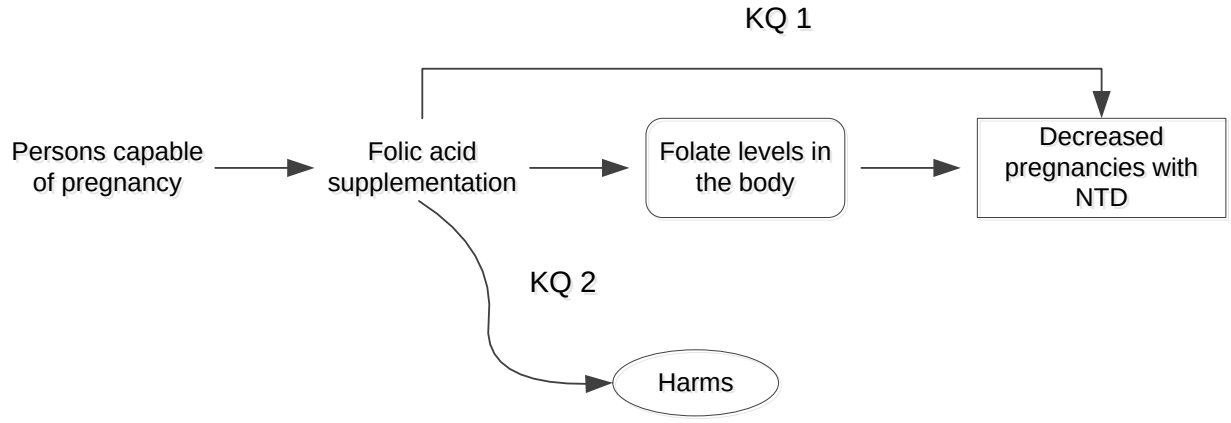
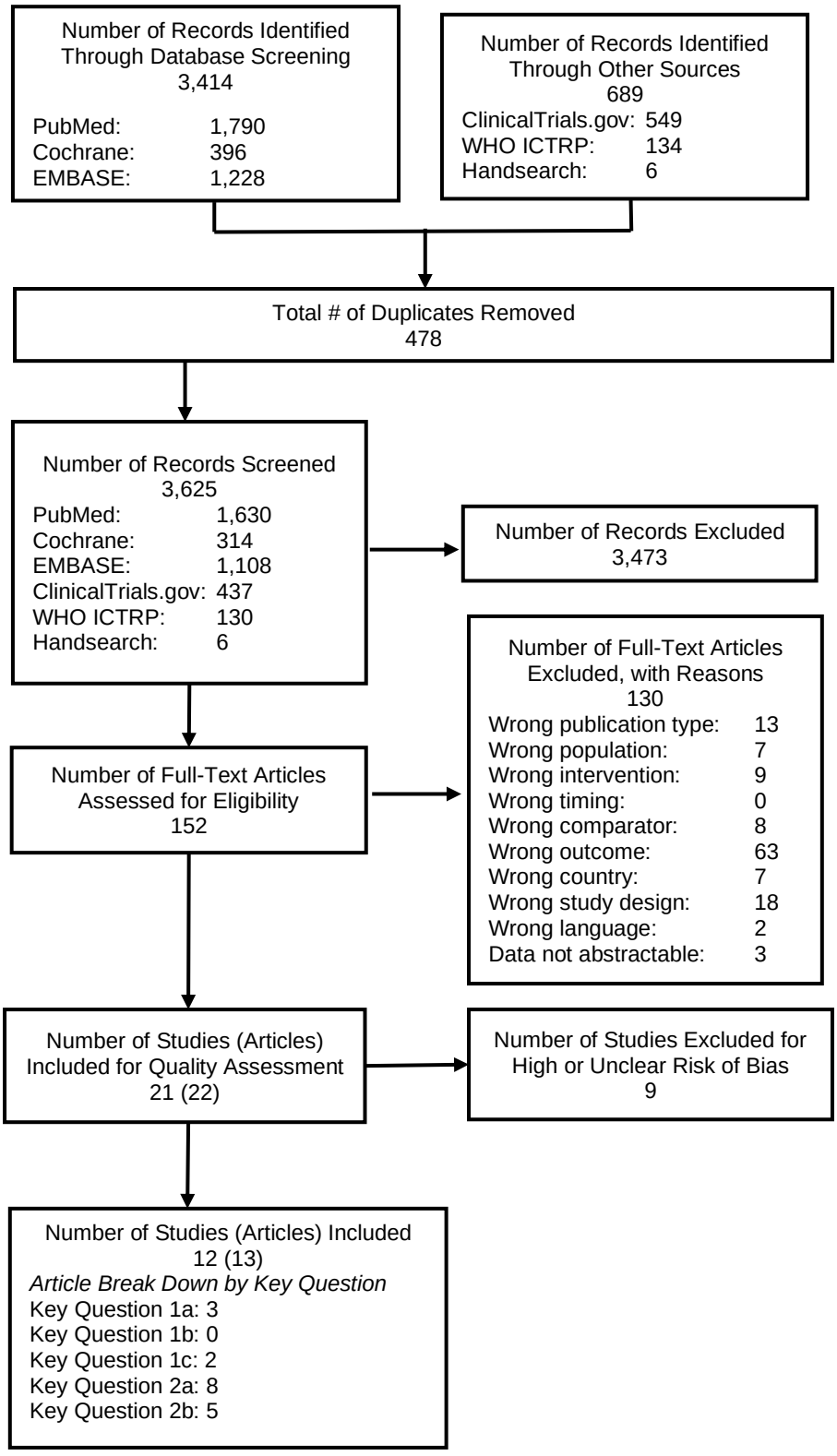
Figure 1. Analytic Framework



Abbreviations: KQ=key question; NTD=neural tube defect.

Figure 2. Flow Diagram of Studies



Abbreviations: ICTRP=International Clinical Trials Registry Platform; WHO=World Health Organization.

Table 1. Folic Acid Supplementation and Neural Tube Defect Outcomes

Cohort	Supplementation Group	Comparator	Outcome: (N With Event/N Exposed vs. N With Event/N Nonexposed)	Effect on NTD Incidence (95% CI)
Gildestad et al, 2016[92] Gildestad et al, 2020[93] (Norwegian cohort)*	Folic acid and/or multivitamins before pregnancy	No use of folic acid or multivitamins before or during pregnancy	Time period 1999–2013: 44/189,217 (0.02%) vs. 141/380,273 (0.04%) Time period 1999–2005: 22/101,977 (0.04%) vs. 95/242,696 (0.04%) Time period 2006–2013: 22/134,515 (0.02%) vs. 46/137,577 (0.03%)	Time period 1999–2013, aRR, 0.76 (0.53 to 1.10) Time period 1999–2005, aRR, 1.02 (0.63 to 1.65) Time period 2006–2013, aRR, 0.54 (0.31 to 0.91)
Nishigori et al, 2019[94] (Japanese cohort)	Adequate use of folic acid supplements (started before conception)	Inadequate use (started folic acid supplements after pregnancy recognition or nonuse of folic acid supplements)	4/7,634 (0.05%) vs. 70/84,635 (0.08%)	aOR, 0.62 (0.23 to 1.71)
Petersen et al, 2019[95] (U.S. and Canadian case-control study)	Daily exposure to a product containing folic acid 28 days before to 28 days after the first day of the last menstrual period	No supplements in the periconceptional period	NA (case-control study)	Prepregnancy obesity,† aORs adjusting for maternal age: 0.65 (95% CI, 0.40 to 1.04); aOR adjusting for planned pregnancy: 0.69 (95% CI, 0.42 to 1.10) Pregestational diabetes, aOR adjusting for maternal age: 0.25 (95% CI, 0.04 to 1.05); aOR adjusting for planned pregnancy: 0.37 (95% CI, 0.06 to 1.65)

* Data presented for use of folic acid supplement or multivitamins containing folic acid before pregnancy only, data limited to folic acid supplements only and for use during pregnancy and combined before and during pregnancy is available in **Appendix F Table 2**.

† Additional results for various levels of folic acid exposure are in **Appendix F Table 2**.

Abbreviations: aOR=adjusted odds ratio; aRR=adjusted relative risk; CI=confidence interval; NA=not applicable; N=number; NTD=neural tube defect; U.S.=United States; vs.=versus.

Table 2. Folic Acid Supplementation and Autism Spectrum Disorder Outcomes

Country	First Author, Year	Supplementation Groups	Comparator	Outcome: (N With Event/N Exposed vs. N With Event/N Nonexposed)	Effect on Autism Incidence (95% CI)
Israel	Levine et al, 2018[103]	Folic acid supplement exposure before pregnancy	No folic acid supplement exposure before pregnancy	ASD: NR by arm	aRR, 0.56 (95% CI, 0.42 to 0.74)
		Folic acid supplement exposure during pregnancy	No folic acid supplement exposure during pregnancy	ASD: NR by arm	aRR, 0.32 (95% CI, 0.26 to 0.41)
	Sharman Moser et al, 2019[104]	0.2 <0.4 mg/day	<0.2 mg/day	ASD: NA (case-control study)	aOR, 1.27 (95% CI, 0.98 to 1.65)
		0.4–<1 mg/day	<0.2 mg/day	ASD: NA (case-control study)	aOR, 1.12 (95% CI, 0.91 to 1.39)
		1–<3 mg/day	<0.2 mg/day	ASD: NA (case-control study)	aOR, 1.18 (95% CI, 0.89 to 1.56)
		>3 mg/day	<0.2 mg/day	ASD: NA (case-control study)	aOR, 1.08 (95% CI, 0.44 to 2.64)
Sweden	DeVilbiss et al, 2017[101]	Folic acid supplement use during pregnancy	No use of folic acid, multivitamins, or iron during pregnancy	ASD with intellectual disability: 15/2,789 (0.54%) vs. 430/91,895 (0.47%)	aOR, 1.2 (95% CI, 0.71 to 2.01)
		Folic acid supplement use during pregnancy	No use of folic acid, multivitamins, or iron during pregnancy	ASD without intellectual disability: 63/2,789 (2.26%) vs. 1,615/91,895 (1.76%)	aOR, 1.29 (95% CI, 0.99 to 1.67)
		Folic acid supplement use during pregnancy	No use of folic acid, multivitamins, or iron during pregnancy	ASD with or without intellectual disability: 72/2,789 (2.58%) vs. 2045/91,895 (2.23%)	aOR, 1.27 (95% CI, 1.01 to 1.6)
		Folic acid supplement use during pregnancy	No use of folic acid, multivitamins, or iron during pregnancy	ASD with or without intellectual disability: 72/2,789 (2.58%) vs. 2045/91,895 (2.23%)	aOR (sensitivity analysis: propensity score model): 1.17 (95% CI, 0.89 to 1.51)
		Folic acid supplement use during pregnancy	No use of folic acid, multivitamins, or iron during pregnancy	ASD with or without intellectual disability: 72/2,789 (2.58%) vs. 2045/91,895 (2.23%)	aOR (sensitivity analysis: sibling controls): 1.48 (95% CI, 0.87 to 2.51)
Denmark	Virk et al, 2016[99]	Folic acid use weeks −4 to −1	No folic acid use between weeks −4 to 8	ASD: NR by arm	aRR, 1.06 (95% CI, 0.83 to 1.36)
		Folic acid use weeks 1 to 4	No folic acid use between weeks −4 to 8	ASD: NR by arm	aRR, 0.98 (95% CI, 0.77 to 1.24)
		Folic acid use weeks 5 to 8	No folic acid use between weeks −4 to 8	ASD: NR by arm	aRR, 0.99 (95% CI, 0.8 to 1.22)
		Folic acid use weeks −4 to 8	No folic acid use between weeks −4 to 8	ASD: NR by arm	aRR, 1.00 (95% CI, 0.82 to 1.22)

Table 2. Folic Acid Supplementation and Autism Spectrum Disorder Outcomes

Country	First Author, Year	Supplementation Groups	Comparator	Outcome: (N With Event/N Exposed vs. N With Event/N Nonexposed)	Effect on Autism Incidence (95% CI)
Denmark (continued)	Strom et al, 2018[98]	Folic acid use weeks −4 to −1	No folic acid use between weeks −4 to 8	ASD: 749/52822 (1.42%) vs. 485/34,388 (1.41%);	aHR: 1.03 (95% CI, 0.92 to 1.16)
		Folic acid use between weeks −4 and 1	No folic acid use between weeks −4 and 1	ASD: 414/28295 (1.46%) vs. 820/58,315 (1.41%)	aHR: 1.02 (95% CI, 0.91 to 1.15)
		Folic acid use between weeks 1 and 4	No folic acid use between weeks 1 and 4	ASD: 545/38,326 (1.42%) vs. 689/48,884 (1.41%)	aHR: 1.01 (95% CI, 0.9 to 1.13)
		Folic acid use between weeks 5 and 8	No folic acid use between weeks 5 and 8	ASD: 732/51559 (1.42%) vs. 502/35,651 (1.41%)	aHR: 1.03 (95% CI, 0.92 to 1.15)
Norway	Suren et al, 2013[100]	Any folic acid use weeks −4 to 8	No folic acid use between weeks −4 and 8	Autistic disorder: 64/61,042 (0.10%) vs. 50/21,134 (0.24%)	aOR, 0.61 (95% CI, 0.41 to 0.90)
		Folic acid initiation weeks −4 to −1	No folic acid use between weeks −4 and 8	Autistic disorder: 32/28,061 (0.11%) vs. 32/14,721 (0.22%)	aOR, 0.67 (95% CI, 0.4 to 1.14)
		Folic acid initiation weeks 1 to 4	No folic acid use between weeks −4 and 8	Autistic disorder: 18/16,797 (0.11%) vs. 32/14,721 (0.22%)	aOR, 0.58 (95% CI, 0.32 to 1.05)
		Folic acid initiation weeks 5 to 8	No folic acid use between weeks −4 and 8	Autistic disorder: 14/16,184 (0.09%) vs. 32/14,721 (0.22%)	aOR, 0.44 (95% CI, 0.23 to 0.83)
		Folic acid initiation weeks 9 to 16	No folic acid use between weeks −4 and 8	Autistic disorder: 18/9395 (0.19%) vs. 32/14,721 (0.22%)	aOR, 0.87 (95% CI, 0.49 to 1.57)
	Nilsen et al, 2013[102]	Prenatal folic acid use	No prenatal folic acid use	ASD: NR by arm	aOR, 0.86 (95% CI, 0.78 to 0.95) (MBRN population)
		Prenatal folic acid use	No prenatal folic acid use	ASD: NR by arm	aOR, 0.85 (95% CI, 0.65 to 1.11) (MoBa cohort)

Abbreviations: aHR=adjusted hazard ratio; aOR=adjusted odds ratio; aRR=adjusted relative risk; ASD=autism spectrum disorder; CI=confidence interval; MBRN=Medical Birth Registry of Norway; MoBa=Mother and Child Cohort Study; NA=not applicable; N=number; NR=not reported; vs.=versus.

Table 3. Summary of Evidence

Key Question	Foundational Evidence: N of Studies (Study Designs); N of Participants	Rationale and Foundational Evidence	Limitations of the Foundational Evidence	New Evidence: N of Studies (Study Designs); N of Participants	New Evidence Findings	Limitations of New Evidence	Consistency of New Evidence With Foundational Evidence
KQ1a: Effects of folic acid supplements on the risk of NTDs	12 (1 RCT,[75-81] 2 cohort studies,[82-84] 8 case-control studies,[33, 35, 85-90, 110] 1 previous review);[110, 111] N>41,802	Generally consistent evidence within the prefortification (indicating benefit) and postfortification eras (no statistically significant differences), inconsistent over time One RCT (prefortification): Peto OR for NTD, 0.131 (95% CI, 0.026 to 0.648); p=0.013[75-81] Two cohort studies (prefortification): aOR for NTD, 0.11 (95% CI, 0.01 to 0.91);[82] OR, 0.27 (95% CI, 0.11 to 0.63)[83, 84] Four case-control publications (prefortification): aOR for NTD, 0.7 (95% CI, 0.5 to 0.8);[33] RR for NTD, 0.6 (95% CI, 0.4 to 0.8);[85] OR for NTD, 0.65 (95% CI, 0.45	No new trials can be conducted on this topic. New studies must rely on observational data with inherent risks of case ascertainment bias (in prospective cohort studies) or exposure recall bias (in retrospective studies)	Three studies (2 cohort studies [3 publications publications[92-94]], 1 case-control study[95]); N=990,372	Norwegian cohort (no mandatory fortification) study reported no statistically significant associations in overall analysis (1999–2013) or the first period (1999–2005) with low compliance with folic acid supplementation recommendations; statistically significant associations from 2006–2013 with higher compliance with folic acid recommendations: before pregnancy only (aRR, 0.54 [95% CI, 0.31 to 0.91]);[92] during pregnancy only (aRR, 0.62 [95% CI, 0.39 to 0.97]);[93] and before and during pregnancy (aRR, 0.49 [95% CI, 0.29 to 0.83])[93] No consistently and statistically significant associations in	Heterogenous populations with different levels of food fortification and diet patterns; method-ological limitations in foundational evidence also apply	New studies have some evidence of benefit for reducing NTDs and do not change conclusions from foundational evidence.

Table 3. Summary of Evidence

Key Question	Foundational Evidence: N of Studies (Study Designs); N of Participants	Rationale and Foundational Evidence	Limitations of the Foundational Evidence	New Evidence: N of Studies (Study Designs); N of Participants	New Evidence Findings	Limitations of New Evidence	Consistency of New Evidence With Foundational Evidence
KQ1a: Effects of folic acid supplements on the risk of NTDs (continued)		to 0.94);[86] OR, 1.00 (95% CI, 0.73 to 1.40); p=0.97[87] One case-control study (spanning pre- and postfortification): aOR for NTD, 1.12 (95% CI, 0.22 to 5.78)[88] Three case-control studies (postfortification): OR for NTD, 1.11 (95% CI, 0.74 to 1.65) for consistent users;[89] aOR for NTD (anencephaly+spina bifida), 0.93 (95% CI, 0.82 to 1.06);[35] aOR (anencephaly), 1.2 (95% CI, 0.8 to 1.9);[90] aOR (spina bifida), 1.4 (95% CI, 1.0 to 1.8)[90]			Japanese cohort of general population (no mandatory fortification) (aOR, 0.62 [95% CI, 0.23 to 1.71])[94] or U.S. and Canadian case-control study (postfortification study) of participants with prepregnancy diabetes or pregestational obesity for exposures measured as less than daily, daily, <0.4 mg, 0.4 mg to <0.1.0 mg[95]		
KQ 1b: Differences in effect of folic acid supplements on NTDs by race/ ethnicity	Three (3 case-control studies);[86, 89, 90] N=11,154	Inconsistent and imprecise findings from fair-quality studies suggesting no differences No effect in first study;[90] higher risk in second	Small numbers in each comparison, differences in direction of estimate of effect effects possibly due to chance	No new evidence	NA	NA	NA

Table 3. Summary of Evidence

Key Question	Foundational Evidence: N of Studies (Study Designs); N of Participants	Rationale and Foundational Evidence	Limitations of the Foundational Evidence	New Evidence: N of Studies (Study Designs); N of Participants	New Evidence Findings	Limitations of New Evidence	Consistency of New Evidence With Foundational Evidence
KQ 1b: Differences in effect of folic acid supplements on NTDs by race/ ethnicity (continued)		(aOR for Hispanic women with consistent use compared with nonuse, 2.20 [95% CI, 0.98 to 4.92]);[89] less protective effect in third (OR for Hispanic women, 0.96 [95% CI, 0.44 to 2.10]) vs. 0.62 [95% CI, 0.35 to 1.10]) for non-Hispanic White womenvs. 0.54 [95% CI, 0.09 to 3.20] for Black women)[86]					
KQ 1c: Differences in effect of folic acid supplements on NTDs by dosage, duration, and timing	Dosage: Four (1 cohort study,[83, 84] 3 case-control studies[85-87]); N=26,791 Duration: 0 Timing: Five (1 cohort study, [83, 84] 4 case-control studies[86, 88-90]); N=26,808	No indication of dose response in 3 of 4 studies.[83-87] One study showed lower odds for daily use vs. less than daily use (OR, 0.57 [95% CI, 0.35 to 0.93])[83-87] Duration: None Timing: Calculated OR from cohort study for use weeks 1–6 vs. weeks 7 and later: 0.29 [95% CI, 0.14 to 0.60].[83, 84] Older studies consistently	Small numbers in each comparison, effects possibly due to chance, studies used different measures of dose and timing	Dosage: 1 case-control study of women with prepregnancy obesity; N=1,429[95] Timing: One cohort (2 publications[92, 93]); N=896,674	Dosage: Statistically significantly reduced association between NTD risk and exposure of 0.4 to <1.0 mg of folic acid supplementation daily (aOR, 0.54 [95% CI, 0.29 to 0.95]) but not for exposures of <0.4 mg (aOR, 1.29 [95% CI, 0.40 to 3.37]) or <0.4 mg or ≥1.0 mg (aOR, 0.84 [95% CI, 0.38 to 1.68]);[95] differences did not	Small numbers in each comparison, effects possibly due to chance	New studies do not change conclusions regarding dosage or timing

Table 3. Summary of Evidence

Key Question	Foundational Evidence: N of Studies (Study Designs); N of Participants	Rationale and Foundational Evidence	Limitations of the Foundational Evidence	New Evidence: N of Studies (Study Designs); N of Participants	New Evidence Findings	Limitations of New Evidence	Consistency of New Evidence With Foundational Evidence
KQ 1c: Differences in effect of folic acid supplements on NTDs by dosage, duration, and timing (continued)		showed no effect of timing;[86, 88] one new study (postfortification) showed a protective effect of use before pregnancy vs. initiation in the first month of pregnancy on anencephaly but not spina bifida[90] The other new study did not find a protective effect for spina bifida for consistent periconceptional use vs. initiation in the first month of pregnancy.[89]			persist in sensitivity analysis Timing: Consistent benefits regardless of timing in 1 of 3 time periods examined (2006–2013) (aRR before pregnancy only [0.54, 95% CI, 0.31 to 0.91];[92] during pregnancy only [0.62 [95% CI, 0.39 to 0.97];[93] and before and during pregnancy [0.49 {95% CI, 0.29 to 0.83}]][93]; consistently no statistically significant differences for the other time periods (1999–2013, 1999–2005)		
KQ 2a: Harms associated with folic acid supplements: multiple gestation (twinning)	2 (1 trial,[77] 1 cohort[112]); N=7,387	Trial found no statistically significant differences in twin pregnancy rate (RR, 1.4 [95% CI, 0.87 to 2.26]).[77] Cohort found higher risk of twin birth for folate use (OR, 1.59 [95% CI, 1.41 to 1.78]) was	Low event rate, wide CIs	No new evidence	NA	NA	NA

Table 3. Summary of Evidence

Key Question	Foundational Evidence: N of Studies (Study Designs); N of Participants	Rationale and Foundational Evidence	Limitations of the Foundational Evidence	New Evidence: N of Studies (Study Designs); N of Participants	New Evidence Findings	Limitations of New Evidence	Consistency of New Evidence With Foundational Evidence
KQ 2a: Harms associated with folic acid supplements: multiple gestation (twinning) (continued)		attenuated once potential misclassification was accounted for (OR, 1.04 [95% CI, 0.91 to 1.18])[112]					
KQ 2a: Harms associated with folic acid supplements: childhood asthma, allergy, wheezing	Childhood asthma, wheezing, allergy (3 SRs,[113-115] 8 observational studies[116-123]); N>14,438	No effect for a large majority of comparisons and outcomes[113-123]	Variable measures of outcomes and exposure, all observation studies with risks of bias from case ascertainment and recall	No new evidence	NA	NA	NA
KQ 2a: Harms associated with folic acid supplements: other adverse events in women	1 RCT; N=4,862[81]	Increased risk for weight gain, diarrhea, constipation; reduced risk for irregular defecation; no difference for increased appetite, lack of appetite, exanthema, heartburn, and vertigo[81]	Low event rate, wide confidence intervals	No new evidence	NA	NA	NA
KQ 2a: Harms associated with folic acid supplements: autism	No eligible evidence	NA	NA	Six fair-quality cohort studies[98-103] and one fair-quality case-control study[104]; N=761,125	Seven studies set in four countries (Israel, Sweden, Denmark, Norway); varied measures of exposure, comparator, and outcomes; generally no statistically significant	No study reported harm but differences in statistically significant associations (benefits vs. no evidence of difference) may stem from differences in	NA

Table 3. Summary of Evidence

Key Question	Foundational Evidence: N of Studies (Study Designs); N of Participants	Rationale and Foundational Evidence	Limitations of the Foundational Evidence	New Evidence: N of Studies (Study Designs); N of Participants	New Evidence Findings	Limitations of New Evidence	Consistency of New Evidence With Foundational Evidence
KQ 2a: Harms associated with folic acid supplements: autism (continued)					associations; three publications of two populations in Israel,[103] and Norway[100, 102] respectively, reported some benefits[103]	measurement of exposure, choice of comparator, and controls for confounding	
KQ 2a: Harms associated with folic acid supplements: maternal cancer	No eligible evidence	NA	NA	One cohort study;[97] N=429,004	HR for one pregnancy with exposure to folic acid supplementation vs. no exposure in pregnancy: 1.08 [95% CI, 1.00 to 1.18][97] HR for two or more pregnancies with exposure to folic acid supplementation vs. no exposure in pregnancy: 1.06 [95% CI, 0.91 to 1.22][97]	Potential for unmeasured confounding and recall bias in the classification of the intervention	NA
KQ 2b: Differences in harms associated with folic acid supplements by dosage, timing, and duration: twinning	No eligible evidence	NA	NA	One trial;[96] N=431	RR for twin deliveries with exposure to 4 mg folic acid supplementation vs. exposure to 0.4 mg folic acid supplementation; boths groups	Applicability uncertain to unplanned pregnancies	NA

Table 3. Summary of Evidence

Key Question	Foundational Evidence: N of Studies (Study Designs); N of Participants	Rationale and Foundational Evidence	Limitations of the Foundational Evidence	New Evidence: N of Studies (Study Designs); N of Participants	New Evidence Findings	Limitations of New Evidence	Consistency of New Evidence With Foundational Evidence
KQ 2b: Differences in harms associated with folic acid supplements by dosage, timing, and duration: twinning (continued)					exposed before conception and through 12 weeks gestation: 0.45; 95% CI, 0.11 to 1.77		
KQ 2b: Differences in harms associated with folic acid supplements by dosage, timing, and duration: childhood asthma, allergy, wheezing	Dosage: One SR,[115] one observational study[117]; N=484 Duration: 0 Timing of asthma, wheezing, allergy (2 SRs,[114, 115] 3 observational studies[116, 118, 119]); N varies by outcome	Dosage: No consistent increase in the risk of childhood asthma, wheeze, or allergies by dosage[115, 117] Duration: None Timing: No consistent increase in the risk of childhood asthma, wheeze, or allergies by timing[114-116, 118, 119]	Variable measures of outcomes and exposure, all observation studies with risks of bias from case ascertainment and recall	No new evidence	NA	NA	NA
KQ 2b: Differences in harms associated with folic acid supplements by dosage, timing, and duration: autism	No eligible evidence	NA	NA	Dose: Three (2 cohort studies,[98, 100] 1 case-control study),[104] N=194,281 Timing: Two cohort studies,[99, 100] N=120,235 Duration: 0, N=NA	Dose: Overlap in CIs with exposure to folic acid supplementation in different doses vs. no or very low exposure to folic acid supplementation in pregnancy, all not statistically significant	Potential for unmeasured confounding and recall bias in the classification of the intervention	NA

Table 3. Summary of Evidence

Key Question	Foundational Evidence: N of Studies (Study Designs); N of Participants	Rationale and Foundational Evidence	Limitations of the Foundational Evidence	New Evidence: N of Studies (Study Designs); N of Participants	New Evidence Findings	Limitations of New Evidence	Consistency of New Evidence With Foundational Evidence
KQ 2b: Differences in harms associated with folic acid supplements by dosage, timing, and duration: autism (continued)					Timing: Overlap in CIs with exposure to folic acid supplementation in different time intervals vs. no exposure to folic acid supplementation in pregnancy, all but one estimate not statistically significant; initiation in weeks 5 to 8 associated with benefit (14/16,184 vs. 32/14,721, aOR, 044 [95% CI, 0.23 to 0.83])[100]		

Abbreviations: aOR=adjusted odds ratio; aRR=adjusted relative risk; CI=confidence interval; HR=hazard ratio; KQ=key question; N=number; NA=not applicable; NTD=neural tube defect; OR=odds ratio; RCT=randomized, controlled trial; RR=relative risk; SR=systematic review; U.S.=United States; vs.=versus.

Appendix A Table 1. Measures and Definitions

Measure	Definition
Recommended Daily Allowance (RDA)[124]	The RDA is the average daily dietary nutrient intake level sufficient to meet the nutrient requirement of nearly all (97% to 98%) healthy individuals in a particular life stage and gender group. The primary indicators to determine RDA are RBC folate, plasma homocysteine, and folate concentration levels. If the standard deviation is available and the data are normally distributed, the RDA = estimated average requirement (EAR) + 2 SD of EAR. If data about variability in requirements are insufficient to calculate an SD, a coefficient of variation for the EAR of 10% is assumed. The resulting equation for the RDA is then RDA = 1.2 × EAR. The RDA for folate is set by assuming a coefficient of variation of 10% because information is not available on the standard deviation of the requirement for folate; the RDA is defined as equal to the EAR plus twice the CV to cover the needs of 97% to 98% of the individuals in the group. For folate, the RDA is 120% of the EAR. The U.S. Institute of Medicine (IOM) recommends an RDA for men, women, and adolescents of 14–18 is 400 µg/day. The IOM recommended RDA for pregnant women is 600 µg/day. The recommendation for women capable of becoming pregnant exceeds the RDA. They are recommended to consume 400 µg/day of folate from supplements and/or fortified foods and to consume naturally occurring food folate from a varied diet. The IOM relied on case reports (from 1947 to 1990, of 1 to 48 cases) of progression of neurological disorders of vitamin B12-deficient patients who were receiving oral doses of folate to identify the lowest observed adverse effect level. They observed that an exposure 5 mg/day was associated with more than 100 reported cases of neurological progression, whereas lower exposures (0.33 to 2.5 mg/day) were associated with 8 cases. The threshold of 5 mg/day was divided by an uncertainty factor of 5 to arrive at an upper level of 1 mg/day.[68]
Dietary Folic Equivalent (DFE)	1 µg DFE = 1 µg food folate = 0.6 µg folic acid from fortified food or as a supplement consumed with food = 0.5 µg of a folic acid supplement taken on an empty stomach[68]
Estimated Average Requirement (EAR)	The EAR is the average daily nutrient intake level estimated to meet the requirement of half the healthy individuals in a particular life stage and gender group. The U.S. IOM EAR for females 19–50: 320 µg/day of DFE[68] The IOM EAR for pregnancy is 520 µg/day of DFE. The 320 DFE is based on one study of five patients who were fed a diet of 319 DFE.[125] Of these women, three had RBC folate <305 nmol/L, suggesting that with 320 DFE half would have RBC folate over 305 nmol/L. The threshold of 305 nmol/L (140 ng/mL) of folate was chosen as the cutoff point for adequate folate status based on evidence that lower levels were associated with the appearance of hypersegmented neutrophils (1 case[126]; 2 cases[127] and its association with megaloblastic anemia (40 patients with megaloblastic anemia also had RBC folate <305 nmol/L[128]; 238 pregnant women with RBC <327 nmol/L had megoblastic marrow[129] or chromosomal damage (8 patients with RBC folate <305 nmol/L had a threefold higher frequency of cellular micronuclei (suggesting DNA and chromosomal damage) than 14 control patients.
Plasma/serum folate concentration	Concentration of folate in the circulation based on recent intake of folate from natural-food sources, foods fortified with folic acid, and folic acid supplementation. It is estimated that steady state is achieved after 12–14 weeks of supplementation once cellular folate stores have been saturated. Low folate levels (<6.8 nmol/L) at single time points may reflect only transient changes in intake versus true deficiency and must be combined with other markers of deficiency.[68, 130, 131] No threshold value for plasma/serum folate concentration to prevent NTDs.
Red Blood Cell (RBC) Folate Concentrations	Reflects tissue stores of folate; therefore, considered to be the most reliable biomarker of folate. Folate is incorporated during their maturation in the bone marrow and remains at the same level throughout their 120-day life span. RBC folate levels can be assessed with microbiological assays or commercial protein-binding assays on automated clinical analyzers. In women of reproductive age, RBC folate concentrations should be above 400 ng/mL (906 nmol/L).[58] There is a dose response relationship with RBC folate levels and folate intake from diet and supplements, but there are not yet clear guidelines incorporating RBC folate levels into individualized folic acid supplementation recommendations.[29, 58]

Appendix A Table 1. Measures and Definitions

Measure	Definition
Red Blood Cell (RBC) Folate Concentrations (continued)	

Abbreviations: CV= coefficient of variation; DFE=dietary folic equivalent; DNA=deoxyribonucleic acid; EAR=estimated average requirement; IOM=Institute of Medicine; NTD=neural tube defect; RBC=red blood cell; RDA=recommended daily allowance; SD=standard deviation; U.S.=United States; vs.=versus.

Appendix A Table 2. Current Guidelines for Folic Acid Supplementation

Organization (Year)	Definition of Treatment Population	Guideline
American College of Obstetrics and Gynecology (2019, reaffirmed in 2020)[65]	General population: Women capable of becoming pregnant	All women of reproductive age (15–45 years) should take folic acid supplementation. For average-risk women, supplementation with 400 µg per day is adequate. Women at increased risk of NTDs, including women with a prior pregnancy with an NTD or women with seizure disorders, should be counseled to take 4 mg of folic acid daily
American Academy of Pediatrics (1999)[66], reaffirmed January 2007	General population: Women with no history of a previous pregnancy affected by an NTD	All women of childbearing age, capable of becoming pregnant, and having no history of a previous pregnancy affected by an NTD should consume 400 µg (0.4 mg) of folic acid daily.
Centers for Disease Control (1993)[64]	General population: Women of childbearing age in the United States	Women of childbearing age in the United States who are capable of becoming pregnant should consume 0.4 mg of folic acid per day to reduce the risk of having a pregnancy affected with spina bifida or other NTDs. Because the effects of high intakes are not well known but include complicating the diagnosis of vitamin B12 deficiency, care should be taken to keep total folate consumption at less than 1 mg per day, except under the supervision of a physician.
American Academy of Family Physicians (2014)[67, 71]*	Women planning pregnancy	Folic acid supplementation should be recommended early, preferably before conception. Folic acid, 400 mcg daily, started before pregnancy and continued until 6 to 12 weeks' gestation reduces the rate of NTDs by nearly 75%.
Institute of Medicine (1998)[68]	Women capable of becoming pregnant	400 µg of folic acid daily from fortified foods, supplements, or both in addition to consuming food folate from a varied diet.
National Institute for Health and Care Excellence (United Kingdom, 2014)[69, 70]	General population: Women who may become pregnant and women in early pregnancy	A daily dose of 400 µg of folic acid before pregnancy and throughout the first 12 weeks is recommended.

* The American Academy of Family Physicians cites the 2017 USPSTF guidance.[132]

Abbreviation: NTD=neural tube defect.

7/01/2021 PubMed Benefits Search

Search	Query	Items Found
1	"5-Me-THF*" OR "5-Me-H4F" OR "5-methyltetrahydrofolate" OR "5-methyltetrahydropteroylpentaglutamate"[Supplementary Concept] OR "5-methyltetrahydrofolate triglutamate"[Supplementary Concept] OR "folacin"[tw] OR "folate"[tw] OR "folic acid"[MeSH] OR "folic acid"[tw] OR "folvite"[tw] OR MTHFR[tw] OR "Methylenetetrahydrofolate reductase"[tw] OR "vitamin b 9"[tw] OR "vitamin b9"[tw] OR "vitamin m"[tw] OR "Pteroylglutamic Acid"[tw]	68,749
2	multivitamin OR multivitamins OR "prenatal vitamin"[all fields] OR "prenatal vitamins"[all fields] OR "vitamin supplement"[all fields] OR "vitamin supplements"[all fields]	5,916
3	#1 OR #2	73,336
4	Acrania[tw] OR Acranias[tw] OR Craniorachischisis[tw] OR Craniorachischises[tw] OR Diastematomyelia[tw] OR Diastematomyelias[tw] OR Exencephaly[tw] OR Exencephalies[tw] OR Iniencephalies[tw] OR "neural tube defects"[MeSH] OR "neural tube damage"[All Fields] OR "neural tube defect"[All Fields] OR "neural tube defects"[All Fields] OR "neural tube disorders"[All Fields] OR "Neural tube defect, folate-sensitive"[Supplementary Concept] OR "Neurenteric Cyst"[tw] OR "Neurenteric Cysts"[tw] OR "Neuroenteric Cyst"[tw] OR "Neuroenteric Cysts"[tw] OR "Occult Spinal Dysraphism"[tw] OR "Occult Spinal Dysraphisms"[tw] OR "spina bifida"[All Fields] OR "Spinal Cord Myelodysplasia"[tw] OR "Spinal Cord Myelodysplasias"[tw] OR "Tethered Cord Syndrome"[tw] OR "Tethered Cord Syndromes"[tw] OR "Tethered Spinal Cord Syndrome"[tw]	34,623
5	#3 AND #4	3,695
6	#5 AND ("2015/07/01"[Date - Publication] : "3000"[Date - Publication])	772
7	"Female"[MeSH] OR "Preconception Care"[MeSH] OR Pregnancy[MeSH] OR "Pregnant Women"[MeSH] OR "Prenatal Care"[MeSH] OR "Women"[MeSH:noexp] OR childbearing[tw] OR child-bearing[tw] OR gestation*[tiab] OR conception*[tw] OR maternal[tw] OR nonpregnant[tw] OR non-pregnant[tw] OR periconception*[tw] OR preconception*[tw] OR prenatal*[tw] OR pregnancy[tw] OR pregnancies[tw] OR pregnant[tw] OR ((women[tiab] OR woman[tiab]) AND reproduct*[tiab])	9,290,701
8	#6 AND #7	651
9	address[pt] OR "autobiography"[pt] OR "bibliography"[pt] OR "biography"[pt] OR "case report"[tw] OR "case reports"[tw] OR "comment"[pt] OR "comment on"[All Fields] OR congress[pt] OR "dictionary"[pt] OR "directory"[pt] OR "editorial"[pt] OR "festschrift"[pt] OR "historical article"[pt] OR "interview"[pt] OR lecture[pt] OR "legal case"[pt] OR "legislation"[pt] OR letter[pt] OR "news"[pt] OR "newspaper article"[pt] OR "patient education handout"[pt] OR "periodical index"[pt] OR ("Animals"[Mesh] NOT "Humans"[Mesh]) OR rats[tw] OR cow[tw] OR cows[tw] OR chicken[tw] OR chickens[tw] OR horse[tw] OR horses[tw] OR mice[tw] OR mouse[tw] OR bovine[tw] OR sheep OR ovine OR murine OR murinae	10,674,879
10	#8 NOT #9	531
11	#8 NOT #9	508

Appendix B. Search Strategy

Search	Query	Items Found
12	"Systematic Review"[pt] OR ("review"[Publication Type] AND "systematic"[tiab]) OR "systematic review"[All Fields] OR ("review literature as topic"[MeSH] AND "systematic"[tiab]) OR "meta-analysis"[Publication Type] OR "meta-analysis as topic"[MeSH Terms] OR "Systematic Reviews as Topic"[Mesh] OR "meta-analysis"[tiab] OR "meta-analyses"[tiab] OR "meta-synthesis"[tiab] OR "meta-syntheses"[tiab] OR "Umbrella Review"[tiab]	381,030
13	#11 AND #12	42
14	#11 NOT #13	466

Appendix B. Search Strategy

7/02/2021 PubMed Harms Search

Search	Query	Items Found
1	"5-Me-THF*" OR "5-Me-H4F" OR "5-methyltetrahydrofolate" OR "5-methyltetrahydropteroylpentaglutamate"[Supplementary Concept] OR "5-methyltetrahydrofolate triglutamate"[Supplementary Concept] OR "folacin"[tw] OR "folate"[tw] OR "folic acid"[MeSH] OR "folic acid"[tw] OR "folvite"[tw] OR MTHFR[tw] OR "Methylenetetrahydrofolate reductase"[tw] OR "vitamin b 9"[tw] OR "vitamin b9"[tw] OR "vitamin m"[tw] OR "Pteroylglutamic Acid"[tw]	68,769
2	multivitamin OR multivitamins OR "prenatal vitamin"[all fields] OR "prenatal vitamins"[all fields] OR "vitamin supplement"[all fields] OR "vitamin supplements"[all fields]	5,917
3	#1 OR #2	73,357
4	"adverse effect"[tiab] OR "adverse effects"[Subheading] OR "adverse effects"[tiab] OR "adverse event"[tiab] OR "adverse events"[tiab] OR "Asthma"[Mesh] OR asthma* OR atopy OR allerg* OR "Bronchial Hyperreactivity"[Mesh] OR "Colorectal Neoplasms"[Mesh] OR complication[tiab] OR complications[tiab] OR "Drug-Related Side Effects and Adverse Reactions"[Majr] OR harm[tiab] OR harms[tiab] OR Hypersensitivity[MeSH] OR "Patient Harm"[Majr] OR "Pregnancy Complications"[Mesh] OR "Pregnancy, Twin"[Mesh] OR "reactive airway*" OR respiratory OR "Respiratory Sounds"[Majr:NoExp] OR twinning OR "Twins"[Mesh] OR twins OR "Vitamin B 6 Deficiency"[Mesh] OR ("vitamin b 6"[MeSH] AND deficien*) OR "Vitamin B 12 Deficiency"[Mesh] OR ("vitamin b 12"[MeSH] AND deficien*) OR wheez*	4,834,660
5	#3 AND #4	24,469
6	#5 AND ("2015/07/01"[Date - Publication] : "3000"[Date - Publication])	4,963
7	#5 AND ("2015/07/01"[Date - Publication] : "3000"[Date - Publication])	4,710
8	address[pt] OR "autobiography"[pt] OR "bibliography"[pt] OR "biography"[pt]OR "case report"[tw] OR "case reports"[tw] OR "comment"[pt] OR "comment on"[All Fields] OR congress[pt] OR "dictionary"[pt] OR "directory"[pt] OR "editorial"[pt] OR "festschrift"[pt] OR "historical article"[pt] OR "interview"[pt] OR lecture[pt] OR "legal case"[pt] OR "legislation"[pt] OR letter[pt] OR "news"[pt] OR "newspaper article"[pt] OR "patient education handout"[pt] OR "periodical index"[pt] OR ("Animals"[Mesh] NOT "Humans"[Mesh]) OR rats[tw] OR cow[tw] OR cows[tw] OR chicken[tw] OR chickens[tw] OR horse[tw] OR horses[tw] OR mice[tw] OR mouse[tw] OR bovine[tw] OR sheep OR ovine OR murine OR murinae	10,651,998
9	#7 NOT #8	3,677
10	"Preconception Care"[MeSH] OR Pregnancy[MeSH] OR "Pregnant Women"[MeSH] OR "Prenatal Care"[MeSH] OR "Women"[MeSH:noexp] OR childbearing[tw] OR child-bearing[tw] OR gestation*[tiab] OR conception*[tw] OR maternal[tw] OR nonpregnant[tw] OR non-pregnant[tw] OR periconception*[tw] OR preconception*[tw] OR prenatal*[tw] OR pregnancy[tw] OR pregnancies[tw] OR pregnant[tw] OR ((women[tiab] OR woman[tiab]) AND reproduct*[tiab])	1,297,068
11	#9 AND #10	1,130
12	"Systematic Review"[pt] OR ("review"[Publication Type] AND "systematic"[tiab]) OR "systematic review"[All Fields] OR ("review literature as topic"[MeSH] AND "systematic"[tiab]) OR "meta-analysis"[Publication Type] OR "meta-analysis as topic"[MeSH Terms] OR "Systematic Reviews as Topic"[Mesh] OR "meta-analysis"[tiab] OR "meta-analyses"[tiab] OR "meta-synthesis"[tiab] OR "meta-syntheses"[tiab] OR "Umbrella Review"[tiab]	381,072
13	#11 AND #12	125
14	#11 NOT #13	1005

7/02/2021 Cochrane Library Benefits Search

Search	Query	Items Found
1	"5-Me-THF*" OR "5-Me-H4F" OR "5-methyltetrahydrofolate" OR "5-methyltetrahydropteroylpentaglutamate" OR "5-methyltetrahydrofolate triglutamate" OR folacin OR folate OR [mh "folic acid"] OR "folic acid" OR folvite OR MTHFR OR "Methylenetetrahydrofolate reductase" OR "vitamin b 9" OR "vitamin b9" OR "vitamin m" OR "Pteroylglutamic Acid"	8219
2	multivitamin OR multivitamins OR "prenatal vitamin" OR "prenatal vitamins" OR "vitamin supplement" OR "vitamin supplements"	1994
3	#1 OR #2	9735
4	Acrania OR Acranias OR Craniorachischisis OR Craniorachischises OR Diastematomyelia OR Diastematomyelias OR Exencephaly OR Exencephalies OR Iniencephaly OR Iniencephalies OR "neural tube damage" OR [mh "neural tube defects"] OR "neural tube defect" OR "neural tube defects" OR "neural tube disorders" OR "Neural tube defect, folate-sensitive" OR "Neurenteric Cyst" OR "Neurenteric Cysts" OR "Neuroenteric Cyst" OR "Neuroenteric Cysts" OR "Occult Spinal Dysraphism" OR "Occult Spinal Dysraphisms" OR "spina bifida" OR "Spinal Cord Myelodysplasia" OR "Spinal Cord Myelodysplasias" OR "Tethered Cord Syndrome" OR "Tethered Cord Syndromes" OR "Tethered Spinal Cord Syndrome"	625
5	#3 AND #4	261
6	#5 with Cochrane Library publication date Between Jul 2015 and Dec 2021	131
7	[mh Female] OR [mh "Preconception Care"] OR [mh Pregnancy] OR [mh "Pregnant Women"] OR [mh "Prenatal Care"] OR [mh ^Women] OR childbearing:ti,ab,kw OR child-bearing:ti,ab,kw OR gestation*:ti,ab,kw OR conception*:ti,ab,kw OR maternal:ti,ab,kw OR nonpregnant:ti,ab,kw OR non-pregnant:ti,ab,kw OR periconception*:ti,ab,kw OR preconception*:ti,ab,kw OR prenatal*:ti,ab,kw OR pregnancy:ti,ab,kw OR pregnancies:ti,ab,kw OR pregnant:ti,ab,kw OR ((women:ti,ab OR woman:ti,ab) AND reproduct*:ti,ab)	524,998
8	#6 AND #7	108
9	Address:pt OR "autobiography":pt OR "bibliography":pt OR "biography":pt OR "case report" OR "case reports" OR "comment":pt OR "comment on" OR congress:pt OR "dictionary":pt OR "directory":pt OR "editorial":pt OR "festschrift":pt OR "historical article":pt OR "interview":pt OR lecture:pt OR "legal case":pt OR "legislation":pt OR letter:pt OR "news":pt OR "newspaper article":pt OR "patient education handout":pt OR "periodical index":pt OR ([mh "Animals"] NOT [mh "Humans"]) OR rats OR cow OR cows OR chicken OR chickens OR horse OR horses OR mice OR mouse OR bovine OR sheep OR ovine OR murine OR murinae	55,043
10	#8 NOT #9	82

7/01/2021 Cochrane Library Harms Search

Search	Query	Items Found
1	"5-Me-THF*" OR "5-Me-H4F" OR "5-methyltetrahydrofolate" OR "5-methyltetrahydropteroylpentaglutamate" OR "5-methyltetrahydrofolate triglutamate" OR folacin OR folate OR [mh "folic acid"] OR "folic acid" OR folvite OR MTHFR OR "Methylenetetrahydrofolate reductase" OR "vitamin b 9" OR "vitamin b9" OR "vitamin m" OR "Pteroylglutamic Acid"	8219
2	multivitamin OR multivitamins OR "prenatal vitamin" OR "prenatal vitamins" OR "vitamin supplement" OR "vitamin supplements"	1994
3	#1 OR #2	9735
4	"adverse effect":ti,ab OR "adverse effects":ti,ab OR "adverse event":ti,ab OR "adverse events":ti,ab OR [mh "Asthma"] OR asthma* OR atopy OR allerg* OR [mh "Bronchial Hyperreactivity"] OR [mh "Colorectal Neoplasms"] OR complication:ti,ab OR complications:ti,ab OR [mh "Drug-Related Side Effects and Adverse Reactions"[mj]] OR harm:ti,ab OR harms:ti,ab OR [mh Hypersensitivity] OR [mh "Patient Harm"[mj]] OR [mh "Pregnancy Complications"] OR [mh "Pregnancy, Twin"] OR "reactive airway*" OR respiratory OR [mh ^"Respiratory Sounds"[mj]] OR twinning OR [mh "Twins"] OR twins OR [mh "Vitamin B 6 Deficiency"] OR ([mh "vitamin b 6"] AND deficien*) OR [mh "Vitamin B 12 Deficiency"] OR ([mh "Vitamin B 12"] AND deficien*) OR wheez*	370952
5	#3 AND #4	3526
6	#5 with Cochrane Library publication date Between Jul 2015 and Dec 2021	1831
7	Address:pt OR "autobiography":pt OR "bibliography":pt OR "biography":pt OR "case report" OR "case reports" OR "comment":pt OR "comment on" OR congress:pt OR "dictionary":pt OR "directory":pt OR "editorial":pt OR "festschrift":pt OR "historical article":pt OR "interview":pt OR lecture:pt OR "legal case":pt OR "legislation":pt OR letter:pt OR "news":pt OR "newspaper article":pt OR "patient education handout":pt OR "periodical index":pt OR ([mh "Animals"] NOT [mh "Humans"]) OR rats OR cow OR cows OR chicken OR chickens OR horse OR horses OR mice OR mouse OR bovine OR sheep OR ovine OR murine OR murinae	55,043
8	#6 NOT #7	1431
9	[mh "Preconception Care"] OR [mh Pregnancy] OR [mh "Pregnant Women"] OR [mh "Prenatal Care"] OR [mh ^Women] OR childbearing:ti,ab,kw OR child-bearing:ti,ab,kw OR gestation*:ti,ab,kw OR conception*:ti,ab,kw OR maternal:ti,ab,kw OR nonpregnant:ti,ab,kw OR non-pregnant:ti,ab,kw OR periconception*:ti,ab,kw OR preconception*:ti,ab,kw OR prenatal*:ti,ab,kw OR pregnancy:ti,ab,kw OR pregnancies:ti,ab,kw OR pregnant:ti,ab,kw OR ((women:ti,ab OR woman:ti,ab) AND reproduct*:ti,ab)	91,731
10	#8 AND #9	364

7/02/2021 EMBASE Benefits Search

Search	Query	Items Found
1	'5 me thf' OR '5 me h4f' OR '5 methyltetrahydrofolate' OR '5 methyltetrahydropteroylpentaglutamate' OR '5-methyltetrahydrofolate triglutamate' OR 'folacin'/exp OR folacin OR 'folate'/exp OR 'folate' OR 'folic acid'/exp OR 'folic acid' OR 'folvite'/exp OR folvite OR mthfr OR 'methylenetetrahydrofolate reductase' OR 'vitamin b 9' OR 'vitamin b9' OR 'vitamin m'/exp OR 'vitamin m' OR 'pteroylglutamic acid'/exp OR 'pteroylglutamic acid'	98,009
2	'multivitamin'/exp OR multivitamin OR multivitamins OR 'prenatal vitamin' OR 'prenatal vitamins' OR 'vitamin supplement' OR 'vitamin supplements'	14,517
3	#1 OR #2	109,353
4	acrania OR acranias OR craniorachischisis OR craniorachischises OR 'diastematomyelia'/exp OR diastematomyelia OR diastematomyelias OR 'exencephaly'/exp OR exencephaly OR exencephalies OR 'iniencephaly'/exp OR iniencephaly OR iniencephalies OR 'neural tube damage' OR 'neural tube defect'/exp OR 'neural tube defect' OR 'neural tube defects'/exp OR 'neural tube defects' OR 'neural tube defect, folate-sensitive' OR 'neural tube disorder' OR 'neural tube disorders' OR 'neurenteric cyst' OR 'neurenteric cysts' OR 'neuroenteric cyst' OR 'neuroenteric cysts' OR 'occult spinal dysraphism'/exp OR 'occult spinal dysraphism' OR 'occult spinal dysraphisms' OR 'spina bifida' OR 'spinal cord myelodysplasia' OR 'spinal cord myelodysplasias' OR 'spinal dysraphism'/exp OR 'spinal dysraphism' OR 'tethered cord syndrome'/exp OR 'tethered cord syndrome' OR 'tethered cord syndromes' OR 'tethered spinal cord syndrome'	42,652
5	#3 AND #4	5,788
6	#5 AND [1-7-2015]/sd NOT [02-7-2021]/sd	1,346
7	'female'/exp OR 'prepregnancy care'/exp OR 'pregnancy'/exp OR 'pregnant woman'/exp OR 'prenatal care'/exp OR childbearing:ti,ab,kw OR child-bearing:ti,ab,kw OR gestation*:ti,ab,kw OR conception*:ti,ab,kw OR maternal:ti,ab,kw OR nonpregnant:ti,ab,kw OR non-pregnant:ti,ab,kw OR periconception*:ti,ab,kw OR preconception*:ti,ab,kw OR prenatal*:ti,ab,kw OR pregnancy:ti,ab,kw OR pregnancies:ti,ab,kw OR pregnant:ti,ab,kw OR ((women:ti,ab OR woman:ti,ab) AND reproduct*:ti,ab)	10,828,926
8	#6 AND #7	1,129
9	#8 NOT ([conference review]/lim OR [editorial]/lim OR [letter]/lim OR [note]/lim)	1.056
10	#9 NOT (([animal cell]/lim OR [animal experiment]/lim OR [animal model]/lim OR [animal tissue]/lim OR 'nonhuman'/exp OR rats OR cow OR cows OR chicken OR chickens OR horse OR horses OR mice OR mouse OR bovine OR sheep OR ovine OR murine OR murinae) NOT 'human'/exp)	979
11	#9 NOT (([animal cell]/lim OR [animal experiment]/lim OR [animal model]/lim OR [animal tissue]/lim OR 'nonhuman'/exp OR rats OR cow OR cows OR chicken OR chickens OR horse OR horses OR mice OR mouse OR bovine OR sheep OR ovine OR murine OR murinae) NOT 'human'/exp) AND [english]/lim	943
12	#9 NOT (([animal cell]/lim OR [animal experiment]/lim OR [animal model]/lim OR [animal tissue]/lim OR 'nonhuman'/exp OR rats OR cow OR cows OR chicken OR chickens OR horse OR horses OR mice OR mouse OR bovine OR sheep OR ovine OR murine OR murinae) NOT 'human'/exp) AND [english]/lim AND ([medline]/lim OR [pubmed-not-medline]/lim)	621
13	#11 NOT #12	322
14	'systematic review'/exp OR 'systematic review (topic)'/exp OR 'meta analysis'/exp OR 'meta analysis (topic)'/exp OR 'systematic literature review':ti,ab OR 'this systematic review':ti,ab OR 'umbrella review':ti,ab OR 'meta-analysis':ti,ab OR 'meta-analyses':ti,ab OR 'meta-synthesis':ti,ab OR 'meta-syntheses':ti,ab	516,396

Appendix B. Search Strategy

Search	Query	Items Found
15	#13 AND #14	12
16	#13 NOT #15	310

7/02/2021 EMBASE Harms Search

Search	Query	Items Found
1	'5 me thf' OR '5 me h4f' OR '5 methyltetrahydrofolate' OR '5 methyltetrahydropteroylpentaglutamate' OR '5-methyltetrahydrofolate triglutamate' OR 'folacin'/exp OR folacin OR 'folate'/exp OR 'folate' OR 'folic acid'/exp OR 'folic acid' OR 'folvite'/exp OR folvite OR mthfr OR 'methylenetetrahydrofolate reductase' OR 'vitamin b 9' OR 'vitamin b9' OR 'vitamin m'/exp OR 'vitamin m' OR 'pteroylglutamic acid'/exp OR 'pteroylglutamic acid'	97,677
2	'multivitamin'/exp OR multivitamin OR multivitamins OR 'prenatal vitamin' OR 'prenatal vitamins' OR 'vitamin supplement' OR 'vitamin supplements'	14,516
3	#1 OR #2	109,020
4	'abnormal respiratory sound'/exp OR 'adverse drug reaction'/exp OR 'adverse drug reaction' OR 'adverse effect' OR 'adverse effects' OR 'adverse event' OR 'adverse events' OR 'asthma'/exp OR asthma OR 'atopy'/exp OR atopy OR 'allergic reaction'/exp OR 'allergy'/exp OR allerg* OR (b12 AND deficien*) OR ('b 12' AND deficien*) OR (b6 AND deficien*) OR ('b 6' AND deficien*) OR 'bronchus hyperreactivity'/exp OR 'bronchial hyperreactivity':ti,ab OR 'colorectal tumor'/exp OR 'colorectal tumor':ti,ab OR 'colorectal cancer':ti,ab OR complication OR complications OR 'cyanocobalamin deficiency'/exp OR harm OR harms OR 'patient harm'/exp/mj OR 'pregnancy complication'/exp OR 'pregnancy complication' OR 'pregnancy complications' OR 'pyridoxine deficiency'/exp OR 'reactive airway*' OR respiratory:ti,ab OR 'twin pregnancy'/exp OR 'twins'/exp OR twin OR twins OR twinning OR 'wheezing'/exp OR wheez*:ti,ab	6,375,031
5	#3 AND #4	34,224
6	#5 AND [1-7-2015]/sd NOT [2-7-2021]/sd	9,764
7	#5 AND [1-7-2015]/sd NOT [2-7-2021]/sd AND [english]/lim	9,537
8	#7 NOT ([conference review]/lim OR [editorial]/lim OR [letter]/lim OR [note]/lim)	9,088
9	#8 NOT (([animal cell]/lim OR [animal experiment]/lim OR [animal model]/lim OR [animal tissue]/lim OR 'nonhuman'/exp OR rats OR cow OR cows OR chicken OR chickens OR horse OR horses OR mice OR mouse OR bovine OR sheep OR ovine OR murine OR murinae) NOT 'human'/exp)	8,737
10	#8 NOT (([animal cell]/lim OR [animal experiment]/lim OR [animal model]/lim OR [animal tissue]/lim OR 'nonhuman'/exp OR rats OR cow OR cows OR chicken OR chickens OR horse OR horses OR mice OR mouse OR bovine OR sheep OR ovine OR murine OR murinae) NOT 'human'/exp) AND ([medline]/lim OR [pubmed-not-medline]/lim)	4,730
11	#9 NOT #10	4,007
12	'prepregnancy care'/exp OR 'pregnancy'/exp OR 'pregnant woman'/exp OR 'prenatal care'/exp OR childbearing:ti,ab,kw OR child-bearing:ti,ab,kw OR gestation*:ti,ab,kw OR conception*:ti,ab,kw OR maternal:ti,ab,kw OR nonpregnant:ti,ab,kw OR non-pregnant:ti,ab,kw OR periconception*:ti,ab,kw OR preconception*:ti,ab,kw OR prenatal*:ti,ab,kw OR pregnancy:ti,ab,kw OR pregnancies:ti,ab,kw OR pregnant:ti,ab,kw OR ((women:ti,ab OR woman:ti,ab) AND reproduct*:ti,ab)	1,439,087
13	#11 AND #12	867
14	'systematic review'/exp OR 'systematic review (topic)'/exp OR 'meta analysis'/exp OR 'meta analysis (topic)'/exp OR 'systematic literature review':ti,ab OR 'this systematic review':ti,ab OR 'umbrella review':ti,ab OR 'meta-analysis':ti,ab OR 'meta-analyses':ti,ab OR 'meta-synthesis':ti,ab OR 'meta-syntheses':ti,ab	516,061
15	#13 AND #14	52
16	#13 NOT #15	815

Grey Literature Searches, 6-30-2021 and 7-02-2021

WHO International Clinical Trials Registry Platform (ICTRP), 06/30/2021
Searched Beta Advanced Search portal (**ICTRP Search Portal Advanced Search (ictrptest.azurewebsites.net)** because existing site:
https://apps.who.int/trialsearch/AdvSearch.aspx was not working.

Benefits search: 102 results, **102** imported

In Condition box –

No wildcards, let synonym search in the database handle synonyms:

spina bifida OR neural tube OR Craniorachischis OR Diastematomyelia OR Tethered Cord OR Occult Spinal Dysraphism OR Iniencephaly OR Neurenteric Cyst OR Neuroenteric Cyst OR Myelodysplasia OR Acrania OR Exencephaly

Recruitment status: ALL
Date registered between July 1, 2015 – June 30, 2021

Harms search: 11 results, **11** imported

Intervention box - folic acid OR folvite OR folacin OR folate

Condition box: drug-related side effects OR adverse reaction OR harm OR harms OR adverse effect OR adverse effects OR adverse event OR adverse events OR Complication OR Complications OR asthma OR atopy OR allerg* OR reactive airway OR respiratory OR wheez*

Recruitment status: ALL
Date registered between July 1, 2015 – June 30, 2021

ClinicalTrials.gov, 7-02-2021, all limited to Last Updated date of 07/01/2015 – 07/02/2021.

Benefits Advanced Search:

In Condition box – ("neural tube defects" OR "spina bifida" OR "neural tube damage" OR "neural tube defect" OR "neural tube disorders" OR Craniorachischisis OR Craniorachischises OR Diastematomyelia OR Diastematomyelias OR "Tethered Cord Syndrome" OR "Tethered Cord Syndromes" OR "Tethered Spinal Cord Syndrome" OR "Occult Spinal Dysraphism" OR "Occult Spinal Dysraphisms" OR Iniencephaly OR Iniencephalies OR "Neurenteric Cyst" OR "Neurenteric Cysts" OR "Neuroenteric Cyst" OR "Neuroenteric Cysts" OR "Spinal Cord Myelodysplasia" OR "Spinal Cord Myelodysplasias" OR Acrania OR Acranias OR Exencephaly OR Exencephalies)

In Intervention box – ("folic acid" OR "vitamin b9" OR "vitamin m" OR "Pteroylglutamic Acid" OR folvite OR folacin OR folate OR multivitamin OR multivitamins OR "prenatal vitamin" OR "prenatal vitamins" OR "vitamin supplement" OR "vitamin supplements" OR '5 me thf' OR '5 me h4f' OR 5-methyltetrahydrofolate OR mthfr OR 'methylenetetrahydrofolate reductase')

All put together in Expert search: 14 results, **14** imported

AREA[ConditionSearch] (“neural tube defects” OR “spina bifida” OR “neural tube damage” OR “neural tube defect” OR “neural tube disorders” OR Craniorachischisis OR Craniorachischises OR Diastematomyelia OR Diastematomyelias OR “Tethered Cord Syndrome” OR “Tethered Cord Syndromes” OR “Tethered Spinal Cord Syndrome” OR “Occult Spinal Dysraphism” OR “Occult Spinal Dysraphisms” OR Iniencephaly OR Iniencephalies OR “Neurenteric Cyst” OR “Neurenteric Cysts” OR “Neuroenteric Cyst” OR “Neuroenteric Cysts” OR “Spinal Cord Myelodysplasia” OR “Spinal Cord Myelodysplasias” OR Acrania OR Acranias OR Exencephaly OR Exencephalies) AND AREA[InterventionSearch] (“folic acid” OR “vitamin b9” OR “vitamin m” OR “Pteroylglutamic Acid” OR folvite OR folacin OR folate OR multivitamin OR multivitamins OR “prenatal vitamin” OR “prenatal vitamins” OR “vitamin supplement” OR “vitamin supplements” OR ‘5 me thf’ OR ‘5 me h4f’ OR 5-methyltetrahydrofolate OR mthfr OR ‘methylenetetrahydrofolate reductase’) AND AREA[LastUpdatePostDate] EXPAND[Term] RANGE[07/01/2015, 07/02/2021]

ClinicalTrials.gov Harms Advanced Search

Search 1:

Intervention box - (“folic acid” OR “vitamin b9” OR “vitamin m” OR “Pteroylglutamic Acid” OR folvite OR folacin OR folate OR multivitamin OR multivitamins OR “prenatal vitamin” OR “prenatal vitamins” OR “vitamin supplement” OR “vitamin supplements” OR ‘5 me thf’ OR ‘5 me h4f’ OR 5-methyltetrahydrofolate OR mthfr OR ‘methylenetetrahydrofolate reductase’)

Other terms box – (Pregnancy OR pregnancies OR prepregnancy OR pregnant OR prenatal* OR childbearing OR child-bearing OR gestation* OR conception OR maternal* OR nonpregnant OR non-pregnant OR periconception* OR preconception* OR ((woman OR women) AND reproduct*))

Condition box: (“drug-related side effects” OR “adverse reaction” OR harm OR harms OR “adverse effect” OR “adverse effects” OR “adverse event” OR “adverse events” OR Complication OR Complications OR asthma OR atopy OR allerg* OR ‘reactive airway’ OR respiratory OR wheez*)

Expert search box: 234 results, 234 imported

(Pregnancy OR pregnancies OR prepregnancy OR pregnant OR prenatal* OR childbearing OR child-bearing OR gestation* OR conception OR maternal* OR nonpregnant OR non-pregnant OR periconception* OR preconception* OR ((woman OR women) AND reproduct*)) AND AREA[ConditionSearch] (“drug-related side effects” OR “adverse reaction” OR harm OR harms OR “adverse effect” OR “adverse effects” OR “adverse event” OR “adverse events” OR Complication OR Complications OR asthma OR atopy OR allerg* OR ‘reactive airway’ OR respiratory OR wheez*)AND AREA[InterventionSearch] (“folic acid” OR “vitamin b9” OR “vitamin m” OR “Pteroylglutamic Acid” OR folvite OR folacin OR folate OR multivitamin OR multivitamins OR “prenatal vitamin” OR “prenatal vitamins” OR “vitamin supplement” OR “vitamin supplements” OR ‘5 me thf’ OR ‘5 me h4f’ OR 5-methyltetrahydrofolate OR mthfr OR

'methylenetetrahydrofolate reductase') AND AREA[LastUpdatePostDate] EXPAND[Term] RANGE[07/01/2015, 07/02/2021]

Search 2:

Intervention box - ("folic acid" OR "vitamin b9" OR "vitamin m" OR "Pteroylglutamic Acid" OR folvite OR folacin OR folate OR multivitamin OR multivitamins OR "prenatal vitamin" OR "prenatal vitamins" OR "vitamin supplement" OR "vitamin supplements" OR '5 me thf' OR '5 me h4f' OR 5-methyltetrahydrofolate OR mthfr OR 'methylenetetrahydrofolate reductase')

Other terms box – Other terms box – (Pregnancy OR pregnancies OR prepregnancy OR pregnant OR prenatal* OR childbearing OR child-bearing OR gestation* OR conception OR maternal* OR nonpregnant OR non-pregnant OR periconception* OR preconception* OR ((woman OR women) AND reproduct*)) **AND** ("drug-related side effects" OR "adverse reaction" OR harm OR harms OR "adverse effect" OR "adverse effects" OR "adverse event" OR "adverse events" OR Complication OR Complications OR asthma OR atopy OR allerg* OR 'reactive airway' OR respiratory OR wheez*)

Expert search box: 364 results, 129 imported

(Pregnancy OR pregnancies OR prepregnancy OR pregnant OR prenatal* OR childbearing OR child-bearing OR gestation* OR conception OR maternal* OR nonpregnant OR non-pregnant OR periconception* OR preconception* OR ((woman OR women) AND reproduct*)) **AND** ("drug-related side effects" OR "adverse reaction" OR harm OR harms OR "adverse effect" OR "adverse effects" OR "adverse event" OR "adverse events" OR Complication OR Complications OR asthma OR atopy OR allerg* OR 'reactive airway' OR respiratory OR wheez*) AND AREA[InterventionSearch] ("folic acid" OR "vitamin b9" OR "vitamin m" OR "Pteroylglutamic Acid" OR folvite OR folacin OR folate OR multivitamin OR multivitamins OR "prenatal vitamin" OR "prenatal vitamins" OR "vitamin supplement" OR "vitamin supplements" OR '5 me thf' OR '5 me h4f' OR 5-methyltetrahydrofolate OR mthfr OR 'methylenetetrahydrofolate reductase') AND AREA[LastUpdatePostDate] EXPAND[Term] RANGE[07/01/2015, 07/02/2021]

Appendix C. Inclusion/Exclusion Criteria

Criteria	Include	Exclude
Populations	KQ 1: Persons capable of getting pregnant KQ 2: Persons capable of getting pregnant; fetus, neonate, or child from index pregnancy	KQ 1: Persons not capable of getting pregnant (i.e., biological male sex, prepubertal persons, postmenopausal persons, sterilized persons, or persons with medical conditions rendering them sterile, persons on antiseizure medications); persons with history of NTDs
Interventions	Folic acid supplementation*, with or without food fortification or naturally occurring folate, for the prevention of NTDs and other birth defects Supplementation with micronutrients (e.g., multivitamins, iron) in combination with folic acid	Food fortification only Naturally occurring folate only Counseling to improve dietary supplementation
Comparisons	KQs 1a, 1b, 1c (timing, duration), 2a: Placebo or no treatment or diet only (when compared with folic acid supplementation); supplementation with prenatal vitamins without folic acid (when compared with prenatal vitamin supplementation with folic acid); iron supplements without folic acid (when compared with iron supplementation with folic acid) Food fortification alone when compared folic acid supplementation with food fortification‡ KQs 1c, 2b (dose): Different doses of folic acid or micronutrient plus folic acid supplementation	Folic acid vs. other active comparators (e.g., multivitamins) KQs 1a, 1b, 1c (timing, duration), 2a: Lower vs. higher doses of folic acid supplementation
Outcomes	Neonatal outcomes: NTDs Harms from treatment: Colorectal cancer or other reported types of cancer Inability to diagnose vitamin B6 or B12 adequately (masking of vitamin B6 or B12 deficiency) Autism Asthma or allergies Other reported child, neonatal, fetal, or maternal harms	Benefits not specified in inclusion criteria
Timing	KQs 1a: Supplementation initiated before index pregnancy and in the first month of pregnancy KQs 1 b, c, 2a, 2b: All timing	KQs 1a: Supplementation initiated after the first month of pregnancy
Study designs	Efficacy (KQ 1): Randomized, controlled trials; controlled clinical trials; cohort or case-control studies Harms (KQ 2): RCTs, controlled clinical trials, or observational studies (case-control, cohort, registry data)	Systematic reviews, case reports, case series
Publication type	Original research	Commentaries, editorials

Appendix C. Inclusion/Exclusion Criteria

Criteria	Include	Exclude
Setting	Countries ranked as very high on the Human Development Index as defined by the United Nations Development Programme in 2020†	All other countries
Sample size	All	None
Quality	Good and fair quality	Poor quality
Language	English	Non-English studies

* Terms for folic acid are broad and include folate, folic acid, folvite, folacin, vitamin B, and methyltetrahydrofolate among others.

† Countries designated as very high on the Human Development Index include Andorra, Argentina, Australia, Austria, Bahamas, Bahrain, Barbados, Belarus, Belgium, Brunei, Bulgaria, Canada, Chile, Costa Rica, Croatia, Cyprus, Czech Rep., Denmark, Estonia, Finland, France, Georgia, Germany, Greece, Hong Kong, Hungary, Iceland, Ireland, Israel, Italy, Japan, Kazakhstan, Kuwait, Latvia, Liechtenstein, Lithuania, Luxembourg, Malaysia, Malta, Mauritius, Montenegro, Netherlands, New Zealand, Norway, Oman, Palau, Panama, Poland, Portugal, Qatar, Romania, Russian Federation, Saudi Arabia, Serbia, Singapore, Slovakia, Slovenia, South Korea, Spain, Sweden, Switzerland, Taiwan, Turkey, UAE, UK, Uruguay, USA.

ǂ Listed incorrectly in the protocol as "Folic acid supplementation alone when compared folic acid supplementation with food fortification"; the intent of this comparison was to include studies that allowed attribution of effects to folic acid supplementation alone.

Abbreviations: KQ=key question; NTD=neural tube defect; RCT=randomized, controlled trial; vs.=versus.

Appendix D. Excluded Studies

List of Exclusion Codes:

X 1: Wrong publication type (editorials, letters, opinions, or commentaries to the editor with no primary data)
X 2: Wrong population (persons not capable of getting pregnant, persons with history NTDs)
X 3: Wrong intervention (food fortification only, naturally occurring folate only, counseling to improve dietary supplementation)
X 4: Wrong timing (supplementation initiated after the first month of pregnancy for benefits only)
X 5: Wrong comparator (folic acid vs. active comparators, lower vs. higher doses of folic acid supplementation for timing and duration only)
X 6: Wrong outcome (benefits other than NTDs)
X 7: Wrong country (countries with Human Development Index of low to high)
X 8: Wrong study design (case reports, case series, systematic reviews)
X 9: Wrong Language (non-English)
X 10: Data not abstractable (insufficient evidence reported in conference abstract, full-text irretrievable)

1. The use of folic acid in pregnancy and prevalence of neural tube defects in Europe. *Geneesmiddelenbulletin*. 2016;50(11):125-6. Exclusion Code: X 1.
2. Impact of folic acid supplementation during pregnancy on cognitive performance of children at age 11 years: preliminary results from the FASSTT Offspring study. *Proceedings of the Nutrition Society*. 2018;77(OCE3)doi: 10.1017/S0029665118001027. PMID: CN-01993569. Exclusion Code: X 6.
3. Early life primary prevention against infant bronchial asthma: a 3-year follow-up. *International journal of clinical and experimental medicine*. 2020;13(3):2009-15. PMID: CN-02147346. Exclusion Code: X 7.
4. Abe M. Impact of season of birth and maternal folic acid supplementation on food allergy in children. *Allergy: European Journal of Allergy and Clinical Immunology*. 2016;71:369-70. doi: 10.1111/all.12974. Exclusion Code: X 10.
5. Abe M. The effects of egg and folic acid intake on food allergy onset by the birth season of children. *Allergy: European Journal of Allergy and Clinical Immunology*. 2018;73:650-1. doi: 10.1111/all.13539. Exclusion Code: X 10.
6. Abe M. Effect of folic acid supplementation to pregnant women on food hypersensitivities in children in which neither parent had any allergies. *Allergy: European Journal of Allergy and Clinical Immunology*. 2020;75(SUPPL 109):530-1. doi: 10.1111/all.14508. Exclusion Code: X 10.
7. Alfonso VH, Bandoli G, von Ehrenstein O, et al. Early Folic Acid Supplement Initiation and Risk of Adverse Early Childhood Respiratory Health: A Population-based Study. *Matern Child Health J*. 2018 Jan;22(1):111-9. doi: 10.1007/s10995-017-2360-6. PMID: 28887720. Exclusion Code: X 5.
8. Alfonso VH, von Ehrenstein O, Bandoli G, et al. The Influence of Pre-natal Supplement Initiation on Preterm Birth Among Majority Hispanic Women in Los Angeles County: The Role of Nativity. *Matern Child Health J*. 2016 Sep;20(9):1861-8. doi: 10.1007/s10995-016-1990-4. PMID: 27060911. Exclusion Code: X 6.
9. Alotbi FKS, al-Dulaimy AFJ, Jameel GH. Carcinoembryonic Antigen, Alpha Fetoprotein And Folic Acid Levels In Pregnant Women And Fetuses With Anencephaly. *Biochemical and Cellular Archives*. 2019;19(1):1713-6. Exclusion Code: X 7.
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Appendix E Table 1. Quality Assessments for All Included Studies

Good: Meets all criteria: Comparable groups are assembled initially and maintained throughout the study (followup at least 80%; reliable and valid measurement instruments are used and applied equally to the groups; interventions are spelled out clearly; all important outcomes are considered; and appropriate attention to confounders in analysis. In addition, for RCTs intention to treat analysis is used.
Fair: Studies will be graded "fair" if any or all of the following problems occur
without the fatal flaws noted in the "poor" category below: Generally comparable groups are assembled initially but some question remains whether some (although not major) differences occurred with followup; measurement instruments are acceptable (although not the best) and generally applied equally; some but not all important outcomes are considered; and some but not all potential confounders are accounted for. Intention to treat analysis is done for RCTs.
Poor: Studies will be graded "poor" if any of the following fatal flaws exists: Groups assembled initially are not close to being comparable or maintained throughout the study; unreliable or invalid measurement instruments are used or not applied at all equally among groups (including not masking outcome assessment); and key confounders are given little or no attention. For RCTs intention to treat analysis is lacking

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Abe et al, 2019[133]	Yes	No information	No information	No information	No information	No information	No information	No information	Uncertain because no information	Adjusted for birth order, egg intake, breast-feeding, and caesarean; unable to adjust for everything and not sure if other other variables were controlled
Alfonso et al, 2018[134]	Yes	No	NA	Probably yes	Probably yes	Probably no	NA	NA	Some concerns	Potential for unmeasured confounding. Adjusted for mother's race/ethnicity (non-Hispanic White, Hispanic White, African American/ Black, Asian/Other) and nativity (U.S. born,

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Alfonso et al, 2018[134] (continued)										foreign born), mother's age at pregnancy (<20, 20–24, 25–29, 30+), mother's education at the time of pregnancy (≤11, 12, ≥13 years), use of preconcep-tion vitamins (yes, no), initiation of prenatal care (first trimester, after first trimester/ never), alcohol use during pregnancy (yes, no), home environ-mental tobacco smoke during

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Alfonso et al, 2018[134] (continued)										pregnancy; yes,no), pre-pregnancy BMI (<24.9, ≥25.0 kg/m), marital status (married/ cohabitating, single/ divorced/ separated), primary source of payment for prenatal care (private health insurance, other/none), parity (only child, ≥1 sibling), and birth outcome (preterm, term birth). Maternal history of atopy (including eczema,

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Alfonso et al, 2018[134] (continued)										asthma, and hay fever), duration of exclusive breastfeeding (<3 months, ≥3 months), child attendance to daycare or preschool (yes, no), infection during pregnancy (yes, no), and housing characteri-stics (mold or pests in the home in the past year)
Bjork et al, 2018[135]	Yes	No	NA	Probably no	Probably yes	Probably no	NA	NA	Some concerns	Partially adjusted for confounding: maternal age, parental socio-economic status (single

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Bjork et al, 2018[135] (continued)										mother, low educational attainment [9 y], low household income [€42 404/y or 399 999 kr/y; $49 336/y]), parity (prior pregnancies in past 21 gestational weeks), smoking (any), alcohol use (number of units per month from conception to week 19, depression
Dekker et al, 2017[136]	Yes	No	NA	Probably yes	Probably yes	Probably no	NA	NA	Some concerns	Partially adjusted for confounding. Models were adjusted for maternal age and BMI at

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Dekker et al, 2017[136] (continued)										intake, parity, history of asthma or atopy, educational level, smoking or alcohol use during pregnancy, child's gestational age at birth, birthweight, and ethnicity
DeVilbiss et al, 2017[101]	Yes	No	NA	Probably no	Probably yes	Probably no	NA	NA	Some concerns	Partially adjusted for confounding: child character-istics (sex, birth year, and years resided in Stockholm County), socio-economic indicators (education,

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DeVilbiss et al, 2017[101] (continued)										family income, and maternal birth country), maternal character-istics (age, BMI, parity, smoking status), medication use during pregnancy (antidepres-sants or anti-epileptics), and maternal neuro-psychiatric conditions (anxiety disorders, autism, bipolar disorder, depression, epilepsy, intellectual disability, nonaffective

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DeVilbiss et al, 2017[101] (continued)										psychotic disorders, and stress disorders)
Gildestad et al, 2016[92]	Yes	No	NA	Probably no	NA	No	NA	NA	Some concerns	Potential for residual confounding, Adjusted for year of birth, maternal age, marital status, parity, maternal smoking, pre-gestational diabetes, maternal epilepsy
Gildestad et al, 2020[93]	Yes	No	NA	Probably no	Probably yes	Probably no	NA	NA	Some concerns	Potential for residual confounding, did not control for history of NTDs
Hoang et al, 2019[137]	Yes	No	NA	No	NA	No	NA	NA	High	No adjustment for confounding

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Jenkins et al, 2017[138]	Yes	No	NA	No	NA	No	NA	NA	High	No adjustment for confounding for analyses overall (the results were stratified and adjusted for sample collection status)
Kondo et al, 2015[139]	Yes	No	NA	Probably no	NA	No	NA	NA	High	Potential for residual confounding, did not control for epilepsy or diabetes because "had nothing to do with an increase in maternal supplement use" but the reasoning behind the asssertion is not clear.

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Kondo et al, 2015[139] (continued)										Adjusted only for year and place of birth
Levine et al, 2018[103]	Yes	No	NA	Probably no	Probably yes	Probably no	NA	NA	Some concerns	Partially adjusted for confounding (birth year, sex, SES (high vs. low), a maternal and paternal psychiatric diagnosis at childbirth (present or absent), maternal and paternal age at childbirth, and parity)
Mortensen et al, 2015[97]	Yes	No	NA	Probably no	Probably yes	Probably no	NA	NA	Some concerns	Partially adjusted for confounding: adjusted for maternal age (<20, 20–24, 25–29, 30–34, 35–39, ≥40 years) at first childbirth

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Mortensen et al, 2015[97] (continued)										in the study period 1999–2010), maternal year of birth (1949–59, 1960–69, 1970–79, 1980–89, 1990–96), parity (1, 2, 3, ≥4), marital status (unmarried, married/ registered partner/ cohabitant, divorced/ widowed), education (compulsory [1st–7th class level], intermediate [8th–12th class level],tertiary [14th–20th class level]),

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Mortensen et al, 2015[97] (continued)										occupation (armed forces/ unspecified, legislators, senior officials/ managers, professionals, technicians/ associate professionals, clerks, service workers/shop workers/ market sales workers, agricultural/ forestry/ fishery workers, craft/related trades workers, plant/ machine operators, assemblers/ elementary

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Mortensen et al, 2015[97] (continued)										occupations), and smoking (never, intermittent, 10 cigarettes daily, >10 cigarettes daily, daily smoking–unknown number of cigarettes). For total cancer and breast cancer the model was also adjusted for maternal age at first childbirth (<20, 20–24, 25–29, 30–34, 35–39, ≥40 years) prior to start of followup.

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Nilsen et al, 2013[102]	Yes	No	NA	Probably no	Probably yes	Probably no	NA	NA	Some concerns	Partially adjusted for confounding: maternal age (<25, 25–29, 30–34, ≥35 years), parity (0, 1, ≥2 previous deliveries), hospital size (<500, 500–1,499, 1,500–2,999, ≥3,000 births per year), smoking. Marital status, paternal age, and year of birth and sex were poorly associated with covariates and so excluded from the analysis.

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Nishigori et al, 2019[94]	Yes	No	NA	Probably no	Yes	No	NA	NA	Some concerns	Potential for residual confounding, did not control for history of NTDs, adjusted for age, smoking habits, BMI, history or complication of diabetes and gestational diabetes mellitus, valproic acid and other antiepileptic drugs
Ozer et al, 2016[140]	Yes	No	NA	No	NA	No	NA	NA	High	No adjustment for confounding
Petersen et al, 2019[95]	Yes	No	NA	Probably no	Probably yes	Probably no	NA	NA	Some concerns	Potential for residual confounding and failure to account for

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Petersen et al, 2019[95] (continued)										time period (which includes pre- and post-fortification), adjusted for age and study center, planned pregnancy
Sharman Moser et al, 2019[104]	Yes	No	NA	Probably no	Probably yes	Probably no	NA	NA	Some concerns	Partially adjusted for confounding: age of mother at birth of child, number of family physician and obstetric visits in the 15 months before index date, sub-fertility, and number of children in family, birth order

Appendix E Table 2. Individual Study Quality Assessment Based on the ROBINS-I Tool, Part 1

Author Year	Is there potential for confounding of the effect of intervention in this study	Was the analysis based on splitting participants' followup time according to intervention received?	Were intervention discontinuations or switches likely to be related to factors that are prognostic for the outcome?	Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	Did the authors control for any post-intervention variables that could have been affected by the inter-vention?	Did the authors use an appropriate analysis method that adjusted for all the important confounding domains and for time varying con-founding?	Were confounding domains that were adjusted for measured validly and reliably by the variables available in this study?	Overall Bias due to Confounding	Justification/ Comments
Socha-Banasiak et al, 2018[141]	Yes	No	NA	No	NA	No	NA	NA	High	No adjustment for confounding
Strom et al, 2018[98]	Yes	No	NA	Probably no	Probably yes	Probably no	NA	NA	Some concerns	Partially adjusted for confounding: maternal age, paternal age, parity, maternal smoking during pregnancy, maternal education, family socio-economic status, whether the pregnancy was planned, maternal pre-pregnancy BMI and sex of the child
Suren et al, 2013[100]	Yes	No	NA	Probably no	Probably yes	Probably no	NA	NA	Some concerns	Partially adjusted for confounding:

Appendix E Table 2. Individual Study Quality Assessment Based on the ROBINS-I Tool, Part 1

Author Year	Is there potential for confounding of the effect of intervention in this study	Was the analysis based on splitting participants' followup time according to intervention received?	Were intervention discontinuations or switches likely to be related to factors that are prognostic for the outcome?	Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	Did the authors control for any post-intervention variables that could have been affected by the inter-vention?	Did the authors use an appropriate analysis method that adjusted for all the important confounding domains and for time varying con-founding?	Were confounding domains that were adjusted for measured validly and reliably by the variables available in this study?	Overall Bias due to Confounding	Justification/ Comments
Suren et al, 2013[100] (continued)										maternal age, paternal age, parity, maternal smoking during pregnancy, maternal education, family socio-economic status, whether the pregnancy was planned, maternal pre-pregnancy BMI and sex of the child
Tsai et al, 2018[142]	Yes	No information	No information	No information	No information	No information	No information	No information	Uncertain because no information	Adjusted for sex, age, number of older siblings, breast-feeding duration, maternal smoking during pregnancy,

Appendix E Table 2. Individual Study Quality Assessment Based on the ROBINS-I Tool, Part 1

Author Year	Is there potential for confounding of the effect of intervention in this study	Was the analysis based on splitting participants' followup time according to intervention received?	Were intervention discontinuations or switches likely to be related to factors that are prognostic for the outcome?	Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	Did the authors control for any post-intervention variables that could have been affected by the inter-vention?	Did the authors use an appropriate analysis method that adjusted for all the important confounding domains and for time varying con-founding?	Were confounding domains that were adjusted for measured validly and reliably by the variables available in this study?	Overall Bias due to Confounding	Justification/ Comments
Tsai et al, 2018[142] (continued)										maternal allergy, maternal education level, maternal age, and socio-economic status
Virk et al, 2016[99]	Yes	No	NA	Probably no	Probably yes	Probably no	NA	NA	Some concerns	Partially adjusted for confounding: maternal age (≤24, 25–29, 30–34, ≥35 years); household socio-economic status (higher grade professionals, middle-grade professionals, skilled work, unskilled work, student, unemployed >1year,

Appendix E Table 2. Individual Study Quality Assessment Based on the ROBINS-I Tool, Part 1

Author Year	Is there potential for confounding of the effect of intervention in this study	Was the analysis based on splitting participants' followup time according to intervention received?	Were intervention discontinuations or switches likely to be related to factors that are prognostic for the outcome?	Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	Did the authors control for any post-intervention variables that could have been affected by the inter-vention?	Did the authors use an appropriate analysis method that adjusted for all the important confounding domains and for time varying con-founding?	Were confounding domains that were adjusted for measured validly and reliably by the variables available in this study?	Overall Bias due to Confounding	Justification/ Comments
Virk et al, 2016[99] (continued)										unclassified); maternal smoking (never, ≤9 cigarettes/ day, >9 cigarettes/ day); and alcohol consumption during pregnancy (never, 0–1 glasses per week, 2–4 glasses per week, >4 glasses per week)

Abbreviations: BMI=body mass index; NA=not applicable; NTD=neural tube defects; ROBINS-I=Risk Of Bias In Non-randomized Studies of Interventions; SES=socioeconomic status; vs-versus.

Appendix E Table 3. Individual Study Quality Assessment Based on the ROBINS-I Tool, Part 2

Author Year	Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention?	Were the post-intervention variables that influenced selection likely to be associated with intervention?	Were the post-intervention variables that influenced selection likely to be influenced by the outcome or a cause of the outcome?	Do start of followup and start of intervention coincide for most participants?	Were adjustment techniques used that are likely to correct for the presence of selection biases?	Overall Bias in Selection of Participants into the Study	Justification/Comments
Abe et al, 2019[133]	No information	No information	No information	No information	No information	Uncertain because no information	NA
Alfonso et al, 2018[134]	Probably no	NA	NA	Yes	NA	Low	NA
Bjork et al, 2018[135]	Probably no	NA	NA	Yes	NA	Low	NA
Dekker et al, 2017[136]	Probably no	NA	NA	Yes	NA	Low	NA
DeVilbiss et al, 2017[101]	Probably no	NA	NA	Yes	NA	Low	NA
Gildestad et al, 2016[92]	Probably yes	Probably yes	Probably yes	Yes	NA	Some concerns	The study included only live births and stillbirths and excluded pregnancy terminations due to fetal anomaly. The study reported NTDs by birth outcome. The high proportion of terminations with NTDs (67%) compared with live births with NTDs (28%) and stillbirths with NTDs (5%) suggests the potential for selection bias.
Gildestad et al, 2020[93]	Probably no	NA	NA	Yes	NA	Low	NA
Hoang et al, 2019[137]	Yes	Yes	Yes	no	no	High	Case control of infants with and without NTDs, did not account for pregnancy losses
Jenkins et al, 2017[138]	Yes	Yes	Yes	no	no	High	Case control of infants with and without NTDs,

Appendix E Table 3. Individual Study Quality Assessment Based on the ROBINS-I Tool, Part 2

Author Year	Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention?	Were the post-intervention variables that influenced selection likely to be associated with intervention?	Were the post-intervention variables that influenced selection likely to be influenced by the outcome or a cause of the outcome?	Do start of followup and start of intervention coincide for most participants?	Were adjustment techniques used that are likely to correct for the presence of selection biases?	Overall Bias in Selection of Participants into the Study	Justification/Comments
Jenkins et al, 2017[138] (continued)							did not account for pregnancy losses
Kondo et al, 2015[139]	Yes	Yes	Yes	Yes	no	High	Study included live births only, did not account for pregnancy losses. Additionally, the sample frame for controls was changed (time and region) during the study and it is not clear if there were regional or temporal practice differences.
Levine et al, 2018 [103]	Probably no	NA	NA	Yes	NA	Low	NA
Mortensen et al, 2015[97]	Probably no	NA	NA	Yes	NA	Low	NA
Nilsen et al, 2013[102]	Probably no	NA	NA	Yes	NA	Low	NA
Nishigori et al, 2019[94]	Probably no	NA	NA	Yes	NA	Low	NA
Ozer et al, 2016[140]	Yes	Yes	Yes	No	No	High	Case control of infants with and without NTDs, did not account for pregnancy losses
Petersen et al, 2019[95]	Probably no	NA	NA	Yes	NA	Low	NA
Sharman Moser et al, 2019[104]	Probably no	NA	NA	Yes	NA	Low	NA
Socha-Banasiak et al, 2018[141]	Probably no	NA	NA	Yes	NA	Low	NA
Strom et al, 2018[98]	Probably no	NA	NA	Yes	NA	Low	NA

Appendix E Table 3. Individual Study Quality Assessment Based on the ROBINS-I Tool, Part 2

Author Year	Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention?	Were the post-intervention variables that influenced selection likely to be associated with intervention?	Were the post-intervention variables that influenced selection likely to be influenced by the outcome or a cause of the outcome?	Do start of followup and start of intervention coincide for most participants?	Were adjustment techniques used that are likely to correct for the presence of selection biases?	Overall Bias in Selection of Participants into the Study	Justification/Comments
Suren et al, 2013[100]	Probably no	NA	NA	Yes	NA	Low	NA
Tsai et al, 2018[142]	No information	No information	No information	No information	No information	Uncertain because no information	NA
Virk et al, 2016[99]	Probably no	NA	NA	Yes	NA	Low	NA

Abbreviations: NA=not applicable; NTDs=neural tube defects; ROBINS-I=Risk Of Bias In Non-randomized Studies of Interventions.

Appendix E Table 4. Individual Study Quality Assessment Based on the ROBINS-I Tool, Part 3

Author Year	Were intervention groups clearly defined?	Was the information used to define intervention groups recorded at the start of the intervention?	Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	Overall Bias in Classification of Intervention	Justification/Comments
Abe et al, 2019[133]	No information	No information	No information	Uncertain because no information	Intake based on recall
Alfonso et al, 201 [134]	Probably no	No	Probably yes	Some concerns	Meaured at 3–6 months postpartum; some potential for recall bias but low likelihood of awareness of outcome influencing recall of exposure differentially; no information on dose, adherence, timing
Bjork et al, 2018[135]	Probably yes	No	No	Low	NA
Dekker et al, 2017[136]	Probably no	No	No	Some concerns	Meaured at 18 weeks of gestation, some potential for recall bias but no reason to expect differential recall bias before outcome; actual intake (dose/adherence) unclear based on measurement
DeVilbiss et al, 2017[101]	Probably no	No	Probably no	Some concerns	Cohort based on registration at 9 to 12 weeks gestation, dose/adherence and timing unclear
Gildestad et al, 2016[92]	No	No	Probably no	Some concerns	Although measurement of exposure was not prospective, it was based on a registry of data collected at 12 weeks gestation, so it is unlikely to have a recall bias issue; the dose is implicit in the source, but adherence/level of exposure is unknown

Appendix E Table 4. Individual Study Quality Assessment Based on the ROBINS-I Tool, Part 3

Author Year	Were intervention groups clearly defined?	Was the information used to define intervention groups recorded at the start of the intervention?	Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	Overall Bias in Classification of Intervention	Justification/Comments
Gildestad et al, 2020[93]	No	No	Probably no	Some concerns	Although measurement of exposure was not prospective, it was based on a registry of data collected at 12 weeks gestation, so it is unlikely to have a recall bias issue; the dose is implicit in the source, but adherence/level of exposure is unknown
Hoang et al, 2019[137]	Probably no	Probably no	Probably yes	Some concerns	Participants reported on any use of folic acid 3 months before pregnancy through the first month. Although the exposure period was correct, the measurement did not control for level of exposure and the information was obtained by recall, leading to the potential for biased recall and misclassification
Jenkins et al, 2017[138]	No	Probably no	Probably yes	High	Potential for recall bias because participants were asked to recall exposure from 6 to 24 months after delivery, unclear if the folic acid recall pertained to the period of NTD occurrence, dose and level of adherence unclear
Kondo et al, 2015[139]	No	Probably no	Probably yes	Some concerns	Potential for recall bias because participants were asked to recall exposure from 6 to 12 years ago, unclear that the folic acid

Appendix E Table 4. Individual Study Quality Assessment Based on the ROBINS-I Tool, Part 3

Author Year	Were intervention groups clearly defined?	Was the information used to define intervention groups recorded at the start of the intervention?	Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	Overall Bias in Classification of Intervention	Justification/Comments
Kondo et al, 2015[139] (continued)					recall pertained to the period of NTD occurrence, dose and level of adherence unclear
Levine et al, 2018[103]	Probably no	Yes	No	Some concerns	Based on prescriptions so actual intake unclear, but appears to assume that each dispensation was 1 pill, accounts for type of dispenstation (folic acid or multivitamin) and timing
Mortensen et al, 2015[97]	Probably no	No	Probably no	Some concerns	Cohort based on compulsory notiifcation at week 16, dose/adherence unclear, timing is measured as before/after pregnancy
Nilsen et al, 2013[102]	Probably no	No	Probably no	Some concerns	Cohort based on compulsory notifcation at week 18, dose/adherence unclear, timing includes questions about preconceptional exposure, also asked about brands, amounts, and period of exposure. However, midway through recruitment, the method of data collection changed because 14% of the information was not computerized.Authors note that the new version included a table where the women ticked off which weeks (from gestation week −4 to 14) they had taken the supplement and

Appendix E Table 4. Individual Study Quality Assessment Based on the ROBINS-I Tool, Part 3

Author Year	Were intervention groups clearly defined?	Was the information used to define intervention groups recorded at the start of the intervention?	Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	Overall Bias in Classification of Intervention	Justification/Comments
Nilsen et al, 2013[102] (continued)					asked her to write the average number of units taken per week. Then the task of making the data from the first version of the recruitment form electronically available was taken up, which implied interpretation and coding of electronical text variables. For a smaller proportion of pregnancies, for which the first version of the recruitment form had not been computerized, the original questionnaires had to be manually processed. Although this 2.3% of population was missing data on exposure and was excluded from the sample, but there is no mention of sensitivity analyses of missing data
Nishigori et al, 2019[94]	Probably no	Probably no	Probably yes	Some concerns	Some potential for recall bias (folic acid supplements, valproic acid, and other antiepileptic drugs used were investigated for 1 year before pregnancy confirmation and for an additional 12 weeks after pregnancy confirmation), timing recorded, dose and adherence unclear
Ozer et al, 2016[140]	No information	No information	No information	Uncertain because no information	Authors noted that patients were identified and

Appendix E Table 4. Individual Study Quality Assessment Based on the ROBINS-I Tool, Part 3

Author Year	Were intervention groups clearly defined?	Was the information used to define intervention groups recorded at the start of the intervention?	Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	Overall Bias in Classification of Intervention	Justification/Comments
Ozer et al, 2016[140] (continued)					grouped retrospectively according to the receipt of periconceptional folate supplementation so the accuracy of classification of the intervention is unclear
Petersen et al, 2019[95]	Probably yes	Probably no	Probably yes	Some concerns	Potential for recall bias, partially addressed level of exposure by asking about daily vs. less than daily supplements, categorized by dose, asked about timing
Sharman Moser et al, 2019[104]	Probably no	Yes	No	Some concerns	Based on prescriptions so actual intake unclear, dose is listed, timing and adherence unclear, also did not include over-the-counter medications
Socha-Banasiak et al, 2018[141]	Probably no	No	No	Some concerns	Meaured at 2 to 72 months of child's age, potential for recall bias, timing measured as before or in each trimester of pregnancy, dose calculated from package, adherence unclear
Strom et al, 2018[98]	Probably no	No	Probably no	Some concerns	Folic acid supplement at -4, -1, 1–4, and 5–8 and -4–8 weeks; recall at 6–10 weeks pregnant; brand name and period taken
Suren et al, 2013[100]	Probably no	No	Probably no	Some concerns	Cohort based on compulsory notiifcation at week 18, dose/adherence unclear, timing is

Appendix E Table 4. Individual Study Quality Assessment Based on the ROBINS-I Tool, Part 3

Author Year	Were intervention groups clearly defined?	Was the information used to define intervention groups recorded at the start of the intervention?	Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	Overall Bias in Classification of Intervention	Justification/Comments
Suren et al, 2013[100] (continued)					measured within 4-week intervals from before the start of the pregnancy
Tsai et al, 2018[142]	No information	No information	No information	Uncertain because no information	NA
Virk et al, 2016[99]	Probably no	No	Probably no	Some concerns	Cohort based on registration around 12 weeks gestation, asked about timing and use each week, adherence unclear

Abbreviations: NA= not applicable; NTDs=neural tube defects; ROBINS-I=Risk Of Bias In Non-randomized Studies of Interventions; vs.=versus.

Appendix E Table 5. Individual Study Quality Assessment Based on the ROBINS-I Tool, Part 4

Author Year	Were there deviations from the intended intervention beyond what would be expected in usual practice?	Were these deviations from intended intervention unbalanced between groups and likely to have affected the outcome?	Overall Bias due to Deviation From Intended Intervention
Abe et al, 2019[133]	No information	No information	Uncertain because no information
Alfonso et al, 2018[134]	No information	NA	Uncertain because no information
Bjork et al, 2018[135]	No information	NA	Uncertain because no information
Dekker et al, 2017[136]	No information	NA	Uncertain because no information
DeVilbiss et al, 2017[101]	No information	NA	Uncertain because no information
Gildestad et al, 2016[92]	No information	NA	Uncertain because no information
Gildestad et al, 2020[93]	No information	NA	Uncertain because no information
Hoang et al, 2019[137]	No information	NA	Uncertain because no information
Jenkins et al, 2017[138]	No information	NA	Uncertain because no information
Kondo et al, 2015[139]	No information	NA	Uncertain because no information
Levine et al, 2018[103]	No information	NA	Uncertain because no information
Mortensen et al, 2015[97]	No information	NA	Uncertain because no information
Nilsen et al, 2013[102]	No information	NA	Uncertain because no information
Nishigori et al, 2019[94]	No information	NA	Uncertain because no information
Ozer et al, 2016[140]	No information	NA	Uncertain because no information
Petersen et al, 2019[95]	No information	NA	Uncertain because no information
Sharman Moser et al, 2019[104]	No information	NA	Uncertain because no information
Socha-Banasiak et al, 2018[141]	No information	NA	Uncertain because no information
Strom et al, 2018[98]	No information	NA	Uncertain because no information
Suren et al, 2013[100]	No information	NA	Uncertain because no information
Tsai et al, 2018[142]	No information	No information	Uncertain because no information
Virk et al, 2016[99]	No information	NA	Uncertain because no information

Abbreviations: NA=not applicable; ROBINS-I=Risk Of Bias In Non-randomized Studies of Interventions.

Appendix E Table 6. Individual Study Quality Assessment Based on the ROBINS-I Tool, Part 5

Author Year	Were outcome data available for all, or nearly all, participants?	Were participants excluded due to missing data on intervention status?	Were participants excluded due to missing data on other variables needed for the analysis?	Are the proportion of participants and reasons for missing data similar across interventions?	Is there evidence that results were robust to the presence of missing data?	Overall Bias due to Missing Data	Justification/Comments
Abe et al, 2019[133]	No information	No information	No information	No information	No information	Uncertain because no information	NA
Alfonso et al, 2018[134]	No	No information	No information	No information	No information	High	About 50% response rate to outcomes survey. At baseline, folic acid initation in the first trimester was 85%; at followup, it was 87%. Race, ethnicity, age, and education influenced censoring, and after adjustment, folic acid initation was not associated with censoring. Authors conducted inverse probability censoring weights in sensitivity analyses, with results that were consistent with the main analysis.
Bjork et al, 2018[135]	No	No information	No information	No information	No information	High	No information on differential attition; overall retention at 18 months was 67% and at 36 months was 54%
Dekker et al, 2017[136]	No	Yes	Yes	No information	Probably no	High	25% of sample was missing and sensitivity analyses suggested differences between those who dropped out and those who were retained, leading to the potential for bias from missing data; 19.9% of

Appendix E Table 6. Individual Study Quality Assessment Based on the ROBINS-I Tool, Part 5

Author Year	Were outcome data available for all, or nearly all, participants?	Were participants excluded due to missing data on intervention status?	Were participants excluded due to missing data on other variables needed for the analysis?	Are the proportion of participants and reasons for missing data similar across interventions?	Is there evidence that results were robust to the presence of missing data?	Overall Bias due to Missing Data	Justification/Comments
Dekker et al, 2017[136] (continued)							the sample was excluded for missing folic acid supplementation exposure and 16.7% was missing current asthma outcomes; neither was imputed
DeVilbiss et al, 2017[101]	No	No information	No information	Probably no	No information	Some concerns	6% of the eligible sample was missing from the medical birth register, no sensitivity analyses done
Gildestad et al, 2016[92]	Probably yes	No information	No information	NA	NA	Low	NA
Gildestad et al, 2020[93]	Probably yes	No information	No information	NA	NA	Low	NA
Hoang et al, 2019[137]	No information	No information	No information	No information	No information	Uncertain because no information	NA
Jenkins et al, 2017[138]	No information	No information	No information	No information	No information	Uncertain because no information	NA
Kondo et al, 2015[139]	No information	No information	No information	No information	No information	Uncertain because no information	NA
Levine et al, 2018[103]	No information	No information	No information	No information	No information	Uncertain because no information	NA
Mortensen et al, 2015[97]	Probably yes	No	Yes	No information	No information	Some concerns	16% of the sample was missing information on smoking, multiple imputation was performed and showed no substantial changes in the risk estimates. Data were also missing on maternal age at first birth,

Appendix E Table 6. Individual Study Quality Assessment Based on the ROBINS-I Tool, Part 5

Author Year	Were outcome data available for all, or nearly all, participants?	Were participants excluded due to missing data on intervention status?	Were participants excluded due to missing data on other variables needed for the analysis?	Are the proportion of participants and reasons for missing data similar across interventions?	Is there evidence that results were robust to the presence of missing data?	Overall Bias due to Missing Data	Justification/Comments
Mortensen et al, 2015[97] (continued)							education, occupation, marital status
Nilsen et al, 2013[102]	No	No information	No information	Probably no	No information	Some concerns	2.3% of the eligible sample was missing exposure data, and no sensitivity analyses were provided.
Nishigori et al, 2019[94]	No	No information	No information	No information	No information	Some concerns	Excluded 2,885 people for lack of information on exposure and 1,322 for lack of information on outcomes, but no information provided on differential exclusion and no sensitivity analysis
Ozer et al, 2016[140]	No information	No information	No information	No information	No information	Uncertain because no information	NA
Petersen et al, 2019[95]	No information	No information	No information	No information	No information	Uncertain because no information	NA
Sharman Moser et al, 2019[104]	No information	No information	No information	No information	No information	Uncertain because no information	NA
Socha-Banasiak et al, 2018[141]	No information	No information	No information	No information	No information	Uncertain because no information	NA
Strom et al, 2018[98]	Probably yes	Probably yes	No information	No information	No information	Some concerns	87,210 mother-child pairs out of 92,676 included. Approximately 2,000 missing supplement data Education missing for 28%.
Suren et al, 2013[100]	No	No information	No information	Probably no	No information	Some concerns	Approximately 80% of sample had available data; differential attrition

Appendix E Table 6. Individual Study Quality Assessment Based on the ROBINS-I Tool, Part 5

Author Year	Were outcome data available for all, or nearly all, participants?	Were participants excluded due to missing data on intervention status?	Were participants excluded due to missing data on other variables needed for the analysis?	Are the proportion of participants and reasons for missing data similar across interventions?	Is there evidence that results were robust to the presence of missing data?	Overall Bias due to Missing Data	Justification/Comments
Suren et al, 2013[100] (continued)							on screening questionnaires
Tsai et al, 2018[142]	No information	No information	No information	No information	No information	Uncertain because no information	NA
Virk et al, 2016[99]	No	No information	No information	Probably no	No information	Some concerns	20% of the eligible sample was missing from the eligible population because of a change in the recruitment forms, no sensitivity analyses done. Authors noted that women who were excluded due to missing reported weeks of supplement use were similar to those who reported weeks of use

Abbreviations: NA=not applicable; ROBINS-I=Risk Of Bias In Non-randomized Studies of Interventions.

Appendix E Table 7. Individual Study Quality Assessment Based on the ROBINS-I Tool, Part 6

Author Year	Could the outcome measure have been influenced by knowledge of the intervention received?	Were outcome assessors aware of the intervention received by study participants?	Were the methods of outcome assessment comparable across intervention groups?	Were any systematic errors in measurement of the outcome related to intervention received?	Overall Bias in Measurement of Outcomes	Justification/Comments
Abe et al, 2019[133]	No information	No information	No information	No information	Uncertain because no information	NA
Alfonso et al, 2018[134]	Probably no	No information	Probably yes	Probably no	Low	NA
Bjork et al, 2018[135]	Probably no	No information	Probably yes	Probably no	Low	NA
Dekker et al, 2017[136]	Probably no	No information	Probably yes	Probably no	Low	NA
DeVilbiss et al, 2017[101]	Probably no	No information	Probably yes	Probably no	Low	NA
Gildestad et al, 2016[92]	Probably no	No information	Probably yes	Probably no	Low	NA
Gildestad et al, 2020[93]	Probably no	No information	Probably yes	Probably no	Low	NA
Hoang et al, 2019[137]	Probably no	No information	Probably yes	Probably no	Low	NA
Jenkins et al, 2017[138]	Probably no	No information	No	Probably yes	Some concerns	No information about control infants and whether their charts were reviewed to determine if they could have had a congenital anomaly. Only the charts of the cases were reviewed. This would have been unlikely to be missed given they were using registries, and most of the time, these birth defects would need to be reported, but methods are dissimilar.
Kondo et al, 2015[139]	Probably no	No information	Probably yes	Probably no	Low	NA
Levine et al, 2018[103]	Probably no	No information	Probably yes	Probably no	Low	NA
Mortensen et al, 2015[97]	No	No information	Yes	Probably no	Low	NA

Appendix E Table 7. Individual Study Quality Assessment Based on the ROBINS-I Tool, Part 6

Author Year	Could the outcome measure have been influenced by knowledge of the intervention received?	Were outcome assessors aware of the intervention received by study participants?	Were the methods of outcome assessment comparable across intervention groups?	Were any systematic errors in measurement of the outcome related to intervention received?	Overall Bias in Measurement of Outcomes	Justification/Comments
Nilsen et al, 2013[102]	Probably no	No information	Probably yes	Probably no	Low	NA
Nishigori et al, 2019[94]	Probably no	No information	Probably yes	Probably no	Low	NA
Ozer et al, 2016[140]	Probably no	No information	Probably yes	Probably no	Low	NA
Petersen et al, 2019[95]	Probably no	No information	Probably yes	Probably no	Low	NA
Sharman Moser et al, 2019[104]	Probably no	No information	Probably yes	Probably no	Low	NA
Socha-Banasiak et al, 2018 [141]	Probably no	No information	Probably yes	Probably no	Low	NA
Strom et al, 2018[98]	No	No information	Yes	Probably no	Low	NA
Suren et al, 2013[100]	Probably no	No information	Probably yes	Probably no	Low	NA
Tsai et al, 2018[142]	No information	No information	No information	No information	Uncertain because no information	NA
Virk et al, 2016[99]	Probably no	No information	Probably yes	Probably no	Low	NA

Abbreviations: NA= not applicable; ROBINS-I=Risk Of Bias In Non-randomized Studies of Interventions.

Appendix E Table 8. Individual Study Quality Assessment Based on the ROBINS-I Tool, Part 7

Author Year	Is the reported effect estimate likely to be selected, on the basis of the results, from multiple outcome measurements within the outcome domain?	Is the reported effect estimate likely to be selected, on the basis of the results, from multiple analyses of the intervention outcome relationship?	Is the reported effect estimate likely to be selected, on the basis of the results, from different subgroups?	Overall Bias in Selection of the Reported Result
Abe et al, 2019[133]	No information	No information	No information	Uncertain because no information
Alfonso et al, 2018[134]	No information	No information	No information	Uncertain because no information
Bjork et al, 201 [135]	No information	No information	No information	Uncertain because no information
Dekker et al, 2017[136]	No information	No information	No information	Uncertain because no information
DeVilbiss et al, 2017[101]	No information	No information	No information	Uncertain because no information
Gildestad et al, 2016[92]	No information	No information	No information	Uncertain because no information
Gildestad et al, 2020[93]	No information	No information	No information	Uncertain because no information
Hoang et al, 2019[137]	No information	No information	No information	Uncertain because no information
Jenkins et al, 2017[138]	No information	No information	No information	Uncertain because no information
Kondo et al, 2015[139]	No information	No information	No information	Uncertain because no information
Levine et al, 2018[103]	No information	No information	No information	Uncertain because no information
Mortensen et al, 2015[97]	No information	No information	No information	Uncertain because no information
Nilsen et al, 2013[102]	No information	No information	No information	Uncertain because no information
Nishigori et al, 2019[94]	No information	No information	No information	Uncertain because no information
Ozer et al, 2016[140]	No information	No information	No information	Uncertain because no information
Petersen et al, 2019[95]	No information	No information	No information	Uncertain because no information
Sharman Moser et al, 2019[104]	No information	No information	No information	Uncertain because no information

Appendix E Table 8. Individual Study Quality Assessment Based on the ROBINS-I Tool, Part 7

Author Year	Is the reported effect estimate likely to be selected, on the basis of the results, from multiple outcome measurements within the outcome domain?	Is the reported effect estimate likely to be selected, on the basis of the results, from multiple analyses of the intervention outcome relationship?	Is the reported effect estimate likely to be selected, on the basis of the results, from different subgroups?	Overall Bias in Selection of the Reported Result
Socha-Banasiak et al, 2018[141]	No information	No information	No information	Uncertain because no information
Strom et al, 2018[98]	No information	No information	No information	Uncertain because no information
Suren et al, 2013[100]	No information	No information	No information	Uncertain because no information
Tsai et al, 2018[142]	No information	No information	No information	Uncertain because no information
Virk et al, 2016[99]	No information	No information	No information	Uncertain because no information

Abbreviations: ROBINS-I=Risk Of Bias In Non-randomized Studies of Interventions.

Appendix E Table 9. Individual Study Quality Assessment Based on the ROBINS-I Tool Overall Risk of Bias, Part 8

Author, Year	Overall Rating Justification/ Comments	Overall Rating Justification/Comments	Does rating of study vary by outcome?
Abe et al, 2019[133]	Uncertain because no information	Abstract only, so very limited information; adjusted for some outcomes but unable to adjust for everything and unsure what other variables were controlled for; intervention status based on recall, no information on how missing data were handled; no information on how reported results were selected; no information on measure of outcomes	No
Alfonso et al, 2018[134]	High	Potential for bias from unmeasured confounding, potential for bias from attrition	No
Bjork et al, 2018[135]	High	Potential for bias from unmeasured confounding, potential for bias from attrition	No
Dekker et al, 2017[136]	High	Potential for bias from attrition, residual confounding, and recall	No
DeVilbiss et al, 2017[101]	Some concerns	Potential for bias from unmeasured confounding, potential for bias from attrition	No
Gildestad et al, 2016[92]	Some concerns	Potential for bias from unmeasured confounding, potential for recall bias, potential for selection bias	No
Gildestad et al, 2020[93]	Some concerns	Potential for bias from unmeasured confounding, potential for recall bias	No
Hoang et al, 2019[137]	High	No adjustment for potential confounding, risk of selection bias because pregnancy losses were not included	No
Jenkins et al, 2017[138]	High	Risk of bias from confounding, selection, and recall	No
Kondo et al, 2015[139]	High	Potential for bias from selection, potential for recall bias	No
Levine et al, 2018[103]	Some concerns	Potential for bias from unmeasured confounding, potential for bias in measurement of exposure from prescriptions	No
Mortensen et al, 2015[97]	Some concerns	Potential for bias from unmeasured confounding	No
Nilsen et al, 2013[102]	Some concerns	Potential for bias from unmeasured confounding, potential for bias from attrition and measurement of exposure	No
Nishigori et al, 2019[94]	Some concerns	Potential for confounding, intervention status based on recall, no information on how missing data were handled	No
Ozer et al, 2016[140]	High	Risk of bias from confounding and selection	No
Petersen et al, 2019[95]	Some concerns	Potential for bias from unmeasured confounding and confounding from changes in food fortification, potential for recall bias	No

Appendix E Table 9. Individual Study Quality Assessment Based on the ROBINS-I Tool Overall Risk of Bias, Part 8

Author, Year	Overall Rating Justification/ Comments	Overall Rating Justification/Comments	Does rating of study vary by outcome?
Sharman Moser et al, 2019[104]	Some concerns	Potential for bias from unmeasured confounding, potential for bias in measurement of exposure from prescriptions	Yes
Socha-Banasiak et al, 2018[141]	High	Potential for bias from confounding	No
Strom et al, 2018[98]	Some concerns	Confounding, missing data	No
Suren et al, 2013[100]	Some concerns	Potential for bias from unmeasured confounding, potential for bias from attrition	No
Tsai et al, 2018[142]	Uncertain because no information	Abstract only, so very limited information; adjusted for some outcomes but unable to adjust for everything; very little information on intervention, no information on how missing data were handled, no information on how results were selected	No
Virk et al, 2016[99]	Some concerns	Potential for bias from unmeasured confounding, potential for bias from attrition	No

Abbreviations: ROBINS-I=Risk Of Bias In Non-randomized Studies of Interventions.

Appendix E Table 10. Individual Study Quality Assessment Based on Cochrane RoB 2.0

Author, Year	Domain 1 RoB	Domain 2 RoB	Domain 3 RoB	Domain 4 RoB	Domain 5 RoB	Overall RoB	Justification/Comments
Bortolus et al, 2021 [96]	Low	Low	Low	Low	Low	Low	An appropriate analysis was not used to compare groups. No information on missing data regarding adverse events

Abbreviations: RoB=risk of bias**.**

Appendix F Table 1. Study Characteristics of Included Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Authors, Year Study Name Design Risk of Bias Sample Size	Population	Inclusion Exclusion Criteria	Timing and Setting	Supplementation Groups	Age	% Non-White
Petersen et al, 2019[95] Slone Birth Defects Study Case-control Medium (fair quality) N= 1,429	Women in higher-risk groups for NTDs (pregestational diabetes and prepregnancy obesity)	Inclusion: Major malformation resulting in a live birth, stillbirth, or elective termination >12 weeks; after 1993 nonmalformed pregnancies were also included. Cases included NTDs (anencephaly, spina bifida, or encephalocele). Controls included minor malformations not associated with folic acid (1988–1992) and live births without major malformation (1993 and after) Exclusions: Conjoined twins and infants with amniotic bands, body wall defects, chromosomal anomalies, a known syndrome, or unconfirmed diagnoses	1988–2015 Tertiary care centers and birth hospitals; birth defect registries	Daily folic acid supplementation 28 days before and 28 days after last menstrual period Less than daily folic acid supplementation 28 days before and 28 days after last menstrual period No folic acid supplementation Daily dose categorized as <0.4 mg, 0.4 mg to <1.0 mg, or ≥1.0 mg	Pregestational diabetes Cases N (%) <25: 1 (8) 25 to <35: 7 (58) 35+: 4 (33) Controls N (%) <25: 12 (19) 25 to <35: 31(50) 35+: 19 (31) Unknown: 1 Prepregnancy obesity Cases N (%) <25: 22(20) 25 to <35: 72 (65) 35+: 17 (15) Controls N (%) <25: 291 (23) 25 to <35: 730 (59) 35+: 220 (18) Unknown: 2	Pregestational diabetes Cases N (%) White non-Hispanic: 5 (42) Black non-Hispanic: 4 (33) Hispanic: 2 (17) Asian non-Hispanic: 0 (0) Other: 0 (0) Unknown: 1 (8) Controls N (%) White non-Hispanic: 39 (62) Black non-Hispanic: 12 (19) Hispanic: 9 (14) Asian non-Hispanic: 2 (3) Other: 1 (2) Prepregnancy obesity Cases N (%) White non-Hispanic: 75(68) Black non-Hispanic: 16 (14) Hispanic: 16 (14) Asian non-Hispanic: 1 (0) Other: 3 (3) Controls N (%)

Appendix F Table 1. Study Characteristics of Included Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Authors, Year Study Name Design Risk of Bias Sample Size	Population	Inclusion Exclusion Criteria	Timing and Setting	Supplementation Groups	Age	% Non-White
Petersen et al, 2019[95] (continued)						White non-Hispanic: 752 (60) Black non-Hispanic: 179 (14) Hispanic: 254 (20) Asian non-Hispanic: 26 (2) Other: 29 (2) Unknown: 3 (0)
Gildestad et al, 2016[92] N=896,674 live- and stillborn infants Gildestad et al, 2020[93] N=894,927 live- and stillborn infants Cohort Medium (fair quality)	Live-birth and stillbirth gestations after 16 weeks from 1999–2002 Live and stillbirth gestations after 12 weeks from 2002–2013	Inclusion: Live births and stillbirths of gestations after 16 weeks from 1999 and live and stillbirth gestations after 12 weeks after 2002 within the Medical Birth Registry of Norway Exclusion: fetuses/infants with teratogenic syndromes, chromosomal abnormalities, genetic syndromes, and microdeletions	1999–2013 Medical Birth Registry of Norway	Use of only folic acid before pregnancy Use of only multivitamins before pregnancy Folic acid and/or multivitamins before pregnancy Folic acid and/or multivitamins during pregnancy only No use of folic acid or multivitamins before or during pregnancy	<20: N=19,701 20–24: N=129,518 25–29: N=284,902 30–34: N=291,329 34–39: N=131,341 >40: N=23,697	NR
Nishigori et al, 2019[94] Japan Environment and Children's Study	Pregnant women nationwide	Inclusion: NR Exclusion: Multiple pregnancies, withdrew agreement, incomplete enrollment	Recruitment occurred in 15 regional centers between January 2011 and March 2014	Adequate users: started 1 year before conception Inadequate users started after pregnancy or nonuse	Mean (SD)=31.2 (5.1)	NR

Abbreviations: N=number; NR=not reported; SD=standard deviation.

Appendix F Table 2. Results of Included Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Authors, Year Design Risk of Bias (Quality) Sample Size	Supplementation and Comparison	Timing of Measurement of Supplementation	Period of Supplementation	Outcome	Comparison	Odds Ratio (95% CI)	N	Adjustments
Petersen et al, 2019[95] Case-control Medium (fair quality) N=1,429	Folic acid supplementation vs. no folic acid supplementation	Pregnancies identified retrospectively, consenting mothers completed an interview within 6 months after delivery	Daily folic acid supplementation 28 days before and 28 days after last menstrual period	Anencephaly, spina bifida, encephalocele	No folic acid supplementation	Pregestational diabetes Daily folic acid (aOR1): 0.25 (0.04 to 1.05) Daily folic acid (aOR2): 0.37 (0.06 to 1.55)	Pregestational diabetes Daily folic acid cases: 2/28 (7.14%) Control: 26/28 (92.86%) None Cases: 10/43 (23.26%) Control: 33/43 (76.74%)	aOR1: Maternal age (<25, 25–34, 35+ years) and study center; aOR2: Planned pregnancy and study center
						Prepregnancy diabetes >1,000 µg of Daily folic acid (aOR1): 0.46 (0.07 to 2.08) >1,000 µg of Daily folic acid (aOR2): 0.73 (0.11 to 3.91)	Prepregnancy diabetes >1,000 µg Cases: 2/14 (14.29%) Control: 12/14 (85.71%) None Cases: 10/43 (23.25%) Control: 33/43 (76.74%)	
						Prepregnancy obesity <Daily folic acid (aOR1): 0.99 (0.50 to 1.81) <Daily folic acid (aOR2): 1.02 (0.51 to 1.87)	Prepregnancy obesity <Daily folic acid Cases: 12/135 (8.89%) Control: 123/135 (91.11%) None	

Appendix F Table 2. Results of Included Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Authors, Year Design Risk of Bias (Quality) Sample Size	Supplementation and Comparison	Timing of Measurement of Supplementation	Period of Supplementation	Outcome	Comparison	Odds Ratio (95% CI)	N	Adjustments
Petersen et al, 2019[95] (continued)						Prepregnancy obesity Daily folic acid (aOR1): 0.65 (0.04 to 1.04) Daily folic acid (aOR2): 0.69 (0.42 to 1.10) Prepregnancy obesity <400 µg of Daily folic acid (aOR1): 1.29 (0.40 to 3.37) <400 µg of Daily folic acid (aOR2): 1.37 (0.42 to 3.56) 400 µg to 1,000 µg of Daily folic acid (aOR1): 0.54 (0.29 to 0.95) 400 µg to 1,000 µg of Daily folic acid (aOR2): 0.57 (0.30 to 1.02) >1,000 µg of Daily folic acid (aOR1): 0.84 (0.38 to 1.68) >1,000 µg of Daily folic acid (aOR2): 0.89 (0.40 to 1.82)	Cases: 72/789 (9.13%) Control: 717/789 (90.87%) Prepregnancy obesity Daily folic acid Cases: 27/430 (6.28%) Control: 403/430 (93.72%) None Cases: 72/789 (9.13%) Control: 717/789 (90.87%) Prepregnancy obesity <400 µg of Daily folic acid Cases: 4/35 (11.43%) Control: 31/35 (88.57%) None Cases: 72/789 (9.13%) Control: 717/789 (90.87%) Prepregnancy obesity 400 µg to 1,000 µg	

Appendix F Table 2. Results of Included Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Authors, Year Design Risk of Bias (Quality) Sample Size	Supplementation and Comparison	Timing of Measurement of Supplementation	Period of Supplementation	Outcome	Comparison	Odds Ratio (95% CI)	N	Adjustments
Petersen et al, 2019[95] (continued)							Cases: 14/298 (4.70%) Control: 284/298 (95.30%) None Cases: 72/789 (9.13%) Control: 717/789 (90.87%) Prepregnancy obesity >1,000 µg Cases: 9/97 (9.28%) Control: 88/97 (90.72%) None Cases: 72/789 (9.13%) Control: 717/789 (90.87%)	
Gildestad et al, 2016[92] N=896,674 live- and stillborn infants Gildestad et al, 2020[93] N=894,927 live- and stillborn infants	Folic acid and/or multivitamin supplements, folic acid supplement only, and multivitamin supplement only vs. no use of vitamins	Supplementation data collected at birth and during the stay in the delivery unit	Before and/or during pregnancy	Anencephaly, spina bifida, encephalocele	No use	Anencephaly, spina bifida, or encephalocele Folic acid supplements and/or multivitamins, aRR (95%, CI) Before pregnancy (1999–2013): 0.76 (0.53 to 1.10) Before pregnancy	Anencephaly, spina bifida, or encephalocele Before pregnancy (1999–2013) Folic acid and/or multivitamins (1999–2013) Cases: 44/189217 (0.023%) No use of vitamins	Maternal age, marital status, parity, maternal smoking, pre-gestational diabetes and epilepsy

Appendix F Table 2. Results of Included Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Authors, Year Design Risk of Bias (Quality) Sample Size	Supplementation and Comparison	Timing of Measurement of Supplementation	Period of Supplementation	Outcome	Comparison	Odds Ratio (95% CI)	N	Adjustments
Gildestad et al, 2016[92] Gildestad et al, 2020[93] (continued) Cohort Medium (fair quality)						(1999–2005): 1.02 (0.63 to 1.65) Before pregnancy (2006–2013): 0.54 (0.31 to 0.91) During pregnancy only (1999–2013): 0.89 (0.67 to 1.19) During pregnancy only (1999–2005): 1.07 (0.74 to 1.56) During pregnancy only (2006–2013): 0.67 (0.43 to 1.04) Anencephaly, spina bifida, or encephalocele Folic acid supplements, aRR (95%, CI) Before pregnancy (1999–2013): 0.90 (0.54 to 1.48) Before pregnancy (1999–2005):	Cases: 141/380273 (0.037%) Before pregnancy (1999-2005) Folic acid and/or multivitamins Cases: 22/54,702 (0.04%) No use of vitamins Cases: 95/242,696 (0.04%) Before pregnancy (2006–2013) Folic acid and/or multivitamins Cases: 22/134,515 (0.02%) No use of vitamins Cases: 46/137,577 (0.03%) During pregnancy only (1999–2013) Folic acid and/or multivitamins (1999–2013)	

Appendix F Table 2. Results of Included Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Authors, Year Design Risk of Bias (Quality) Sample Size	Supplementation and Comparison	Timing of Measurement of Supplementation	Period of Supplementation	Outcome	Comparison	Odds Ratio (95% CI)	N	Adjustments
Gildestad et al, 2016[92] Gildestad et al, 2020[93] (continued)						1.80 (0.99 to 3.27) Before pregnancy (2006–2013): 0.37 (0.15 to 0.90) Anencephaly, spina bifida, or encephalocele Multivitamin (containing 0.2 of folic acid), aRR (95%, CI) Before pregnancy (2006–2013): 1.02 (0.31 to 3.31) NTDs (total birth defects) Folic acid supplements and/or multivitamins, aRR (95%, CI) Before and/or during pregnancy (1999–2013): 0.73 (0.50 to 1.06) Before and/or during pregnancy (1999–2005):	Cases: 85/311,078 (0.03%) No use of vitamins Cases: 141/380,273 (0.04%) During pregnancy only (1999–2005) Folic acid and/or multivitamins Cases: 42/101,977 (0.04%) No use of vitamins Cases: 95/242,696 (0.04%) During pregnancy only (2006–2013) Folic acid and/or multivitamins Cases: 43/209,101 (0.02%) No use of vitamins Cases: 46/137,577 (0.03%)	

Appendix F Table 2. Results of Included Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Authors, Year Design Risk of Bias (Quality) Sample Size	Supplementation and Comparison	Timing of Measurement of Supplementation	Period of Supplementation	Outcome	Comparison	Odds Ratio (95% CI)	N	Adjustments
Gildestad et al, 2016[92] Gildestad et al, 2020[93] (continued)						1.01 (0.63 to 1.62) Before and/or during pregnancy (2006–2013): 0.49 (0.29 to 0.83) During pregnancy only (1999–2013): 0.86 (0.63 to 1.17) During pregnancy only (1999–2005): 1.06 (0.72 to 1.54) During pregnancy only (2006–2013): 0.62 (0.39 to 0.97) NTDs (total birth defects in singleton births) Folic acid supplements and/or multivitamins, aRR (95%, CI) Before and/or during pregnancy (1999–2013): 0.71 (0.47 to 1.07)	Anencephaly, spina bifida, or encephalocele Before pregnancy (1999–2013) Folic acid supplements Cases: 19/71,615 (0.03%) No use of vitamins Cases: 141/380,273 (0.04%) Before pregnancy (1999–2005) Folic acid supplements Cases: 13/18,426 (0.07%) No use of vitamins Cases: 95/242,696 (0.04%) Before pregnancy (2006–2013) Folic acid supplements Cases: 6/53,189 (0.01%)	

Appendix F Table 2. Results of Included Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Authors, Year Design Risk of Bias (Quality) Sample Size	Supplementation and Comparison	Timing of Measurement of Supplementation	Period of Supplementation	Outcome	Comparison	Odds Ratio (95% CI)	N	Adjustments
Gildestad et al, 2016[92] Gildestad et al, 2020[93] (continued)						During pregnancy (1999–2013): 0.91 (0.66 to 1.27) NTDs (isolated birth defects) Folic Acid Supplements and/or multivitamins, aRR (95%, CI) Before and/or during pregnancy (1999–2013): 0.84 (0.56 to 1.26) During pregnancy only (1999–2013): 1.03 (0.74 to 1.44) NTDs (isolated birth defects in singleton births) Folic acid supplements and/or multivitamins, aRR (95%, CI) Before and/or during pregnancy (1999–2013): 0.80 (0.51 to 1.26)	No use of vitamins Cases: 46/137,577 (0.03%) Anencephaly, spina bifida, or encephalocele Before pregnancy (2006–2013) Multivitamins (0.2 folic acid) Cases: 3/8,880 (0.03%) No use of vitamins Cases: 46/137,577 (0.03%) NTDs (total birth defects) Before and/or during pregnancy (1999–2013): 0.73 (0.50 to 1.06) Folic acid and/or multivitamins Cases: 43/192951 (0.02%) No use of vitamins	

Appendix F Table 2. Results of Included Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Authors, Year Design Risk of Bias (Quality) Sample Size	Supplementation and Comparison	Timing of Measurement of Supplementation	Period of Supplementation	Outcome	Comparison	Odds Ratio (95% CI)	N	Adjustments
Gildestad et al, 2016[92] Gildestad et al, 2020[93] (continued)						During pregnancy only (1999–2013): 1.12 (0.78 to 1.59)	Cases: 142/386,012 (0.04%) Before and/or during pregnancy (1999–2005): 1.01 (0.63 to 1.62) Folic acid and/or multivitamins Cases: 22/55,954 (0.04%) No use of vitamins Cases: 95/246,499 (0.04%) Before and/or during pregnancy (2006–2013): 0.49 (0.29 to 0.83) Folic acid and/or multivitamins Cases: 21/139,513 (0.02%) No use of vitamins Cases: 47/136,997 (0.03%)	

Appendix F Table 2. Results of Included Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Authors, Year Design Risk of Bias (Quality) Sample Size	Supplementation and Comparison	Timing of Measurement of Supplementation	Period of Supplementation	Outcome	Comparison	Odds Ratio (95% CI)	N	Adjustments
Gildestad et al, 2016[92] Gildestad et al, 2020[93] (continued)							During pregnancy only (1999–2013): 0.86 (0.63 to 1.17) Folic acid and/or multivitamins Cases: 85/315,964 (0.03%) No use of vitamins Cases: 142/386,012 (0.04%) During pregnancy only (1999–2005): 1.06 (0.72 to 1.54) Folic acid and/or multivitamins Cases: 42/103,760 (0.04%) No use of vitamins Cases: 95/246,499 (0.04%) During pregnancy only (2006–2013): 0.62 (0.39 to 0.97) Folic acid and/or multivitamins	

Appendix F Table 2. Results of Included Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Authors, Year Design Risk of Bias (Quality) Sample Size	Supplementation and Comparison	Timing of Measurement of Supplementation	Period of Supplementation	Outcome	Comparison	Odds Ratio (95% CI)	N	Adjustments
Gildestad et al, 2016[92] Gildestad et al, 2020[93] (continued)							Cases: 43/212,204 (0.02%) No use of vitamins Cases: 47/136,997 (0.03%) NTDs (total birth defects in singleton births) Before and/or during pregnancy (1999–2013) Folic acid and/or multivitamins Cases: 36/184,789 (0.03%) No use of vitamins Cases: 123/373,012 (0.03%) During pregnancy only (1999–2013) Folic acid and/or multivitamins Cases: 80/305,199 (0.03%) No use of vitamins	

Appendix F Table 2. Results of Included Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Authors, Year Design Risk of Bias (Quality) Sample Size	Supplementation and Comparison	Timing of Measurement of Supplementation	Period of Supplementation	Outcome	Comparison	Odds Ratio (95% CI)	N	Adjustments
Gildestad et al, 2016[92] Gildestad et al, 2020[93] (continued)							Cases: 123/373,012 (0.03%) NTDs (isolated birth defects) Before and/or during pregnancy (1999–2013) Folic acid and/or multivitamins Cases: 36/192,951 (0.02%) No use of vitamins Cases: 108/386,012 (0.03%) During pregnancy only (1999–2013) Folic acid and/or multivitamins Cases: 74/315,964 (0.02%) No use of vitamins Cases: 108/386,012 (0.03%) NTDs (isolated birth defects in singleton births)	

Appendix F Table 2. Results of Included Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Authors, Year Design Risk of Bias (Quality) Sample Size	Supplementation and Comparison	Timing of Measurement of Supplementation	Period of Supplementation	Outcome	Comparison	Odds Ratio (95% CI)	N	Adjustments
Gildestad et al, 2016[92] Gildestad et al, 2020[93] (continued)							Before and/or during pregnancy (1999–2013) Folic acid and/or multivitamins Cases: 30/184,789 (0.02%) No use of vitamins Cases: 93/373,012 (0.02%) During pregnancy only (1999–2013) Folic acid and/or multivitamins Cases: 71/305,199 (0.02%) No use of vitamins Cases: 93/373,012 (0.02%) NTD Total Before and/or during pregnancy (1999–2013) Folic acid and/or multivitamins	

Appendix F Table 2. Results of Included Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Authors, Year Design Risk of Bias (Quality) Sample Size	Supplementation and Comparison	Timing of Measurement of Supplementation	Period of Supplementation	Outcome	Comparison	Odds Ratio (95% CI)	N	Adjustments
Gildestad et al, 2016[92] Gildestad et al, 2020[93] (continued)							Cases: 457/897,062 (0.05%) NTD isolated before and/or during pregnancy (1999–2013) Folic acid and/or multivitamins Cases: 821/897,062 (0.09%) Live births NTD total before and/or during pregnancy (1999–2013) Folic acid and/or multivitamins Cases: 229/888,294 (0.03%) Live births NTD isolated before and/or during pregnancy (1999–2013) Folic acid and/or multivitamins Cases: 189/888,294 (0.02%)	

Appendix F Table 2. Results of Included Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Authors, Year Design Risk of Bias (Quality) Sample Size	Supplementation and Comparison	Timing of Measurement of Supplementation	Period of Supplementation	Outcome	Comparison	Odds Ratio (95% CI)	N	Adjustments
Gildestad et al, 2016[92] Gildestad et al, 2020[93] (continued)							Stillbirths NTD total before and/or during pregnancy (1999–2013) Folic acid and/or multivitamins Cases: 41/6,633 (0.62%) Stillbirths NTD isolated before and/or during pregnancy (1999–2013) Folic acid and/or multivitamins Cases: 29/6,633 (0.44%) TOPFA NTD total before and/or during pregnancy (1999–2013) Folic acid and/or multivitamins Cases: 551/2,135 (25.81%) TOPFA NTD isolated before and/or during	

Appendix F Table 2. Results of Included Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Authors, Year Design Risk of Bias (Quality) Sample Size	Supplementation and Comparison	Timing of Measurement of Supplementation	Period of Supplementation	Outcome	Comparison	Odds Ratio (95% CI)	N	Adjustments
Gildestad et al, 2016[92] Gildestad et al, 2020[93] (continued)							pregnancy (1999–2013) Folic acid and/or multivitamins Cases: 457/2,135 (21.41%)	
Nishigori et al, 2019[94] Cohort Medium (fair quality) N=92,269 singleton pregnancies	Adequate (started before conception) vs. inadequate use (started after pregnancy recognition or nonuse of folic acid supplements) of folic acid supplements	NR	1 year before pregnancy confirmation and for 12 weeks after pregnancy confirmation	Spina bifida, anencephaly, encephalocele	Use	All NTDs Adequate use: aOR=0.62 (0.23 to 1.71), p=0.36 Spina Bifida Adequate use: aOR=0.36 (0.05 to 2.66), p=0.32 Anencephaly Adequate use: aOR=1.45 (0.43 to 4.90), p=0.55 Encephalocele Adequate use: NR	All NTDs: 74 (1 case of anencephaly and encephalocele) Adequate use: N = 4/7634 (0.05%) Inadequate use: N=70/84,635 (0.08%) Spina Bifida: N=32 Adequate use: N=1/7634 (0.01%) Inadequate use: N=31/84,635 (0.04%) Anencephaly: N=24 Adequate use: N=3/7634 (0.02%) Inadequate use: N=21/84,635 (0.04%) Encephalocele: N=19	Age, smoking habits, BMI, history or complication of diabetes and gestational diabetes mellitus, valproic acid and other AEDs

Appendix F Table 2. Results of Included Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Authors, Year Design Risk of Bias (Quality) Sample Size	Supplementation and Comparison	Timing of Measurement of Supplementation	Period of Supplementation	Outcome	Comparison	Odds Ratio (95% CI)	N	Adjustments
Nishigori et al, 2019[94] (continued)							Adequate use: N=0/7634 (0.00%) Inadequate use: N=19/84,635 (0.02%)	

Abbreviations: AED=antiepileptic drug; aOR= adjusted odds ratio; aRR=adjusted relative risk; BMI=body mass index; CI=confidence interval; N=number; NR=not reported; NTD=neural tube defect; TOPFA=termination of pregnancy due to fetal anomaly; vs=versus.

Appendix F Table 3. Variations in Effect of Folic Acid Supplementation on Neural Tube Defects by Dosage

First Author, Year Design Risk of Bias (Quality) Sample Size	Subgroup	N	Results
Petersen et al, 2019[95] Case-control Medium (fair quality) N=1,429	Prepregnancy diabetes Prepregnancy obesity	Prepregnancy diabetes >1,000 µg Cases: 2/14 (14.29%) Control: 12/14 (85.71%) None Cases: 10/43 (23.25%) Control: 33/43 (76.74%) Prepregnancy obesity <400 µg of daily folic acid Cases: 4/35 (11.43%) Control: 31/35 (88.57%) None Cases: 72/789 (9.13%) Control: 717/789 (90.87%) 400 µg to 1,000 µg Cases: 14/298 (4.70%) Control: 284/298 (95.30%) None Cases: 72/789 (9.13%) Control: 717/789 (90.87%) >1,000 µg Cases: 9/97 (9.28%) Control: 88/97 (90.72%) None Cases: 72/789 (9.13%) Control: 717/789 (90.87%)	Prepregnancy diabetes >1,000 µg of daily folic acid (aOR1): 0.46 (0.07 to 2.08) >1,000 µg of daily folic acid (aOR2): 0.73 (0.11 to 3.91) Prepregnancy obesity <400 µg of daily folic acid (aOR1): 1.29 (0.40 to 3.37) <400 µg of daily folic acid (aOR2): 1.37 (0.42 to 3.56) 400 µg to 1,000 µg of daily folic acid (aOR1): 0.54 (0.29 to 0.95) 400 µg to 1,000 µg of daily folic acid (aOR2): 0.57 (0.30 to 1.02 >1,000 µg of daily folic acid (aOR1): 0.84 (0.38 to 1.68) >1,000 µg of daily folic acid (aOR2): 0.89 (0.40 to 1.82)

Abbreviations: aOR=adjusted odds ratio.

Appendix F Table 4. Variations in Effect of Folic Acid Supplementation on Neural Tube Defects by Timing

First Author, Year Design Risk of Bias (Quality) Sample Size	Subgroup	N	Results
Gildestad et al, 2016[92]	Anencephaly, spina bifida, or encephalocele	Anencephaly, spina bifida, or encephalocele	Anencephaly, spina bifida, or encephalocele Folic acid and/or multivitamin: ARR (95% CI)
N=896,674 live- and stillborn infants		Before pregnancy (1999–2013)	Before pregnancy (1999–2013)
	NTDs (total birth defects)	Folic acid and/or multivitamin	0.76 (0.53 to 1.10)
		Cases: 44/189,217 (0.023%)	Before pregnancy (1999–2005)
	NTDs (total birth defects in singleton births)	No use of vitamins	1.02 (0.63 to 1.65)
Gildestad et al, 2020[93]		Cases: 141/380,273 (0.037%)	Before pregnancy (2006–2013 0.54 (0.31 to 0.91)
N=894,927 live- and stillborn infants	NTDs (isolated birth defects)	Before pregnancy (1999–2005)	During pregnancy only (1999–2013)
		Folic acid and/or multivitamins	0.89 (0.67 to 1.19)
		Cases: 22/54,702 (0.04%)	During pregnancy only (1999–2005)
	NTDs (isolated birth defects in singleton births)	No use of vitamins	1.07 (0.74 to 1.56)
Cohort		Cases: 95/242,696 (0.04%)	During pregnancy only (2006–2013) 0.67 (0.43 to 1.04)
Medium (fair quality)		Before pregnancy (2006–2013)	
		Folic acid and/or multivitamins	Anencephaly, spina bifida, or encephalocele
		Cases: 22/134,515 (0.02%)	Folic acid only: ARR (95% CI)
		No use of vitamins	Before pregnancy (1999–2013)
		Cases: 46/137,577 (0.03%)	0.90 (0.54 to 1.48)
			Before pregnancy (1999–2005)
		During pregnancy only (1999–2013)	1.80 (0.99 to 3.27)
		Folic acid and/or multivitamins	Before pregnancy (2006–2013
		Cases: 85/311,078 (0.03%)	0.37 (0.15 to 0.90)
		No use of vitamins	
		Cases: 141/380,273 (0.04%)	Anencephaly, spina bifida, or encephalocele Multivitamins only: ARR (95% CI)
		During pregnancy only (1999–2005)	Before pregnancy (1999–2013)
		Folic acid and/or multivitamins	0.77 (0.31 to 1.88
		Cases: 42/101,977 (0.04%)	Before pregnancy (1999–2005)
		No use of vitamins	0.52 (0.12 to 2.12)
		Cases: 95/242,696 (0.04%)	Before pregnancy (2006–2013 1.02 (0.31 to 3.31)
		During pregnancy only (2006–2013)	
		Folic acid and/or multivitamins	
		Cases: 43/209,101 (0.02%)	
		No use of vitamins	
		Cases: 46/137,577 (0.03%)	
		Anencephaly, spina bifida, or encephalocele	
		Before pregnancy (1999–2013)	

Appendix F Table 4. Variations in Effect of Folic Acid Supplementation on Neural Tube Defects by Timing

First Author, Year Design Risk of Bias (Quality) Sample Size	Subgroup	N	Results
Gildestad et al, 2020[93] (continued)		Folic acid supplements Cases: 19/71,615 (0.03%) No use of vitamins Cases: 141/380,273 (0.04%) Before pregnancy (1999–2005) Folic acid supplements Cases: 13/18,426 (0.07%) No use of vitamins Cases: 95/242,696 (0.04%) Before pregnancy (2006–2013) Folic acid supplements Cases: 6/53,189 (0.01%) No use of vitamins Cases: 46/137,577 (0.03%) Anencephaly, spina bifida, or encephalocele Before pregnancy (2006–2013) Multivitamins (0.2 folic acid) Cases: 3/8,880 (0.03%) No use of vitamins Cases: 46/137,577 (0.03%) Anencephaly, spina bifida, or encephalocele Before pregnancy (2006–2013) Multivitamins (0.2 folic acid) Cases: 3/8,880 (0.03%) No use of vitamins Cases: 46/137,577 (0.03%) NTDs (total birth defects) Before and/or during pregnancy (1999–2013): 0.73 (0.50 to 1.06) Folic acid and/or multivitamins Cases: 43/192951 (0.02%) No use of vitamins Cases: 142/386,012 (0.04%) Before and/or during pregnancy (1999–2005): 1.01 (0.63 to 1.62)	

Appendix F Table 4. Variations in Effect of Folic Acid Supplementation on Neural Tube Defects by Timing

First Author, Year Design Risk of Bias (Quality) Sample Size	Subgroup	N	Results
Gildestad et al, 2020[93] (continued)		Folic acid and/or multivitamins Cases: 22/55,954 (0.04%) No use of vitamins Cases: 95/246,499 (0.04%) Before and/or during pregnancy (2006–2013): 0.49 (0.29 to 0.83) Folic acid and/or multivitamins Cases: 21/139,513 (0.02%) No use of vitamins Cases: 47/136,997 (0.03%) During pregnancy only (1999–2013): 0.86 (0.63 to 1.17) Folic acid and/or multivitamins Cases: 85/315,964 (0.03%) No use of vitamins Cases: 142/386,012 (0.04%) During pregnancy only (1999–2005): 1.06 (0.72 to 1.54) Folic acid and/or multivitamins Cases: 42/103,760 (0.04%) No use of vitamins Cases: 95/246,499 (0.04%) During pregnancy only (2006–2013): 0.62 (0.39 to 0.97) Folic acid and/or multivitamins Cases: 43/212,204 (0.02%) No use of vitamins Cases: 47/136,997 (0.03%) NTDs (total birth defects in singleton births) Before and/or during pregnancy (1999–2013) Folic acid and/or multivitamins Cases: 36/184,789 (0.03%) No use of vitamins Cases: 123/373,012 (0.03%) During pregnancy only (1999–2013)	

Appendix F Table 4. Variations in Effect of Folic Acid Supplementation on Neural Tube Defects by Timing

First Author, Year Design Risk of Bias (Quality) Sample Size	Subgroup	N	Results
Gildestad et al, 2020[93] (continued)		Folic acid and/or multivitamins Cases: 80/305,199 (0.03%) No use of vitamins Cases: 123/373,012 (0.03%) NTDs (isolated birth defects) Before and/or during pregnancy (1999–2013) Folic acid and/or multivitamins Cases: 36/192,951 (0.02%) No use of vitamins Cases: 108/386,012 (0.03%) During pregnancy only (1999–2013) Folic acid and/or multivitamins Cases: 74/315,964 (0.02%) No use of vitamins Cases: 108/386,012 (0.03%) NTDs (isolated birth defects in singleton births) Before and/or during pregnancy (1999–2013 Folic acid and/or multivitamins Cases: 30/184,789 (0.02%) No use of vitamins Cases: 93/373,012 (0.02%) During pregnancy only (1999–2013) Folic acid and/or multivitamins Cases: 71/305,199 (0.02%) No use of vitamins Cases: 93/373,012 (0.02%) NTD total Before and/or during pregnancy (full study period) Folic acid and/or multivitamins Cases: 457/897,062 (0.05%) NTD isolated Before and/or during pregnancy (full study period)	

Appendix F Table 4. Variations in Effect of Folic Acid Supplementation on Neural Tube Defects by Timing

First Author, Year Design Risk of Bias (Quality) Sample Size	Subgroup	N	Results
Gildestad et al, 2020[93] (continued)		Folic acid and/or multivitamins Cases: 821/897,062 (0.09%) Live births NTD total Before and/or during pregnancy (full study period) Folic Acid and/or multivitamins Cases: 229/888,294 (0.03%) Live births NTD isolated Before and/or during pregnancy (full study period) Folic acid and/or multivitamins Cases: 189/888,294 (0.02%) Stillbirths NTD total Before and/or during pregnancy (full study period) Folic acid and/or multivitamins Cases: 41/6,633 (0.62%) Stillbirths NTD isolated Before and/or during pregnancy (full study period) Folic acid and/or multivitamins Cases: 29/6,633 (0.44%) TOPFA NTD total Before and/or during pregnancy (full study period) Folic acid and/or multivitamins Cases: 551/2,135 (25.81%) TOPFA NTD isolated before and/or during pregnancy (full study period) Folic acid and/or multivitamins Cases: 457/2,135 (21.41%)	

Abbreviations: N=number; NTD=neural tube defect; TOPFA= termination of pregnancy due to fetal anomaly.

Appendix F Table 5. Variations in Effect of Folic Acid Supplementation on Twin Deliveries by Dose

First Author, Year Design Risk of Bias (Quality) Sample Size	Supplementation	Period of Supplementation	Timing of Measurement of Supplementation	N	Outcome	Comparison	Overall Risk Ratio (95% CI)	Adjustments
Bortolus et al, 2021[96] RCT Low (good quality) N=431	Folic acid supplements	Before and during 12 weeks of gestation pregnancy	Prospective	4.0-mg folic acid use before and during 12 weeks pregnancy: N=227 0.4-mg folic acid use before and during 12 weeks pregnancy: N=204	Twin delivery in 4.0-mg arm: N=3 (1.3%)	Twin delivery in 0.4-mg arm: N=6 (2.9%)	0.45 (0.11 to 1.77)	None

Abbreviations: CI=confidence interval; N=number.

Appendix F Table 6. Harms of Folic Acid Supplementation: Study Characteristics of Included Autism Spectrum Disorder Studies

First Author, Year Study Name Design Risk of Bias Sample Size	Population	Inclusion Exclusion Criteria	Timing and Setting	Supplementation Groups	Age	% Non-White
Sharman Moser et al, 2019[104] Case-control Medium (fair quality) N=21,895 (2009 cases; 19,886 controls)	Mothers of children with and without autism spectrum disorder	Inclusion: Singleton births in the Maccabi Healthcare Services from 2000 to 2013 (inclusive), whose mothers had continuous healthcare plan enrollment for at least 12 months before the index date Exclusion: Multiple births, children with siblings with ASD	2000–2013 Maccabi Healthcare Services database	Unsupplemented or very low supplemented (median daily dispensed dose <0.2 mg/day) Low supplemented (median daily dispensed dose 0.2–<0.4 mg/day) Typically supplemented (median daily dispensed dose 0.4–<1 mg/day) High supplemented (median daily dispensed dose 1–<3 mg/day) Very high supplemented median daily dispensed dose (>3 mg/day)	Cases: Mean (SD) 31.65 (4.9) Controls: Mean (SD) 31.75 (4.9)	NR
Levine et al, 2018[103] Cohort Medium (fair quality) N=45,300	Children with information in the Meuhedet healthcare organization registry	Cases: children with ASD Controls: Random sample of children born alive between January 1, 2003, and December 31, 2007	2003–2015 Healthcare registers from the Meuhedet healthcare organization	Before pregnancy (540–271 days before childbirth) During pregnancy (270 days before childbirth up to the date of childbirth) Unexposed	Mothers' age at birth Cases: <35: 454 (79.37%) >35: 118 (20.63%) Control: <35: 35,753 (79.93%) >35: 8975 (20.07%) Mean age of children: (SD): 10.0 (1.4)	NR

Appendix F Table 6. Harms of Folic Acid Supplementation: Study Characteristics of Included Autism Spectrum Disorder Studies

First Author, Year Study Name Design Risk of Bias Sample Size	Population	Inclusion Exclusion Criteria	Timing and Setting	Supplementation Groups	Age	% Non-White
DeVilbiss et al, 2017[101] Stockholm Youth Cohort Cohort Medium (fair quality) N=98,864	Children with and without ASD	Inclusion: children born in Sweden between 1996 and 2007 and living in Stockholm county for at least four years between 2011 and 2011 Exclusion: children not in the medical birth register, not linked to birth mother, adopted, or with missing data on family disposable income or maternal age	1996–2011 Medical Birth Register, computerized registers covering all pathways of ASD diagnosis and care in Stockholm county, integrated database for labor market research, national patient register, Stockholm count adult outpatient psychiatric register, prescription drug register	Folic acid supplement use at first antenatal visit No multivitamin, iron, or folic acid supplement use at first antenatal visit	Folic acid supplement use at first antenatal visit: 31.4 (5.0) No folic acid supplement use at first antenatal visit: 30.9 (5.0)	NR
Virk et al, 2016[99] Cohort Medium (fair quality) N=35,059	Pregnant women in Denmark and their offspring recruited during 1996–2002	Inclusion: Pregnant women in Denmark and their offspring recruited during 1996–2002 Exclusion: Women with unsuccessful pregnancies, nonsingleton births, pregnancies where mothers emigrated, mothers who died, and unknown birth outcomes; women with missing values for weekly supplement use	2000–2002 Danish National Birth Cohort, recruited from primary care. Information on autism collected from National Hospital Register and the Central Psychiatric Register	Use of any supplement containing folic acid from −4 to −1; 1 to 4; or 5 to 8 weeks No supplement use during the −4 to 8 week period	Supplement users <24: 407 (5.7%) 25–29: 2,690 (37.8%) 30–34: 2,807 (39.5%) >35: 1,205 (17%) Nonsupplement users <24: 1,606 (13.5%) 25–29: 4,304 (36.2%) 30–34: 4,046 (34.0%) >35: 1,940 (16.3%) Mean age of children: 9.6 years (8.1–11.4)	NR

Appendix F Table 6. Harms of Folic Acid Supplementation: Study Characteristics of Included Autism Spectrum Disorder Studies

First Author, Year Study Name Design Risk of Bias Sample Size	Population	Inclusion Exclusion Criteria	Timing and Setting	Supplementation Groups	Age	% Non-White
Strom et al, 2018[98] Danish National Birth Cohort (DNBC) Cohort Medium (fair quality) N=87,210	Women consulted at their first antenatal visit in Denmark	Inclusion: singleton, liveborn children Exclusion: children with birthweights<2500g or gestational age <32 weeks, or missing information on supplement use	1996–2002 Danish National Birth Cohort, recruited from primary care	Use of any supplement containing folic acid from −4 to −1; 1 to 4; or 5 to 8 weeks No supplement use during the −4- to −1-week period No supplement use during the 1- to 4-week period No supplement use during the 5- to 8-week period <0.4 mg supplement use at mid-pregnancy >0.4 mg supplement use at mid-pregnancy No supplement use at mid-pregnancy	Exposure ≤20: 36.9% >20–25: 52.1% >25-35: 61.9% >35–40: 60.3% ≥40: 57.3% Control <20: 63.1% >20–25: 47.9% >25–35: 38.1% >35–40: 39.7% >40: 42.7%	NR
Suren et al, 2013[100] Norwegian Mother and Child Cohort Study (MoBa); Autism Birth Cohort (ABC) Cohort Medium (fair quality) N=85,176	Mothers of children with and without ASD	Inclusion: NR Exclusion: mothers who did not receive questionnaire screening for ASD and did not receive food frequency questionnaire, mothers with no information on supplement use, children too young for	2002–2008 Norwegian Patient Registry, Medical Birth Registry of Norway, Autism Birth Cohort (substudy of MoBa)	Folic acid supplement use during the entire or parts of the −4 to 8 weeks; no folic acid supplement Folic acid supplement initiation from −4 to −1; 1 to 4; 5 to 8; and 9 to 16 weeks; no folic acid supplement use 0.001 to 0.399 mg	NR	NR

Appendix F Table 6. Harms of Folic Acid Supplementation: Study Characteristics of Included Autism Spectrum Disorder Studies

First Author, Year Study Name Design Risk of Bias Sample Size	Population	Inclusion Exclusion Criteria	Timing and Setting	Supplementation Groups	Age	% Non-White
Suren et al, 2013[100] (continued)		ASD diagnosis, birth weight less than <2,500 g, gestational age <32 weeks, multiple births		folic acid use in week 22; 0.4 mg or more in week 22; no folic acid use in week 22		
Nilsen et al, 2013[102] Norwegian Mother and Child Cohort Study (MoBa); Autism Birth Cohort (ABC) Cohort, Medical Birth Registry of Norway (MBRN) Medium (fair quality) N=89,836 MoBa N=507,856 MBRN	Mothers of children with and without ASD	Inclusion: children born in 1999–2007 who were living in Norway past age 3 Exclusion: NR	1999–2007 Norwegian Patient Registry, Medical Birth Registry of Norway (MBRN), Autism Birth Cohort (substudy of MoBa)	Folic acid use before and/or during pregnancy No folic acid use before and/or during pregnancy	NR	NR

Abbreviations: ABC=Autism Birth Cohort; ASD=autism spectrum disorder; DNBC=Danish National Birth Cohort; MBRN=Medical Birth Registry of Norway; MoBa=Mother and Child Cohort Study; N=number; NR=not reported; SD=standard deviation.

Appendix F Table 7. Results of Included Studies on Association Between Folic Acid Supplementation and Autism Spectrum Disorders

First Author, Year Design Risk of Bias (Quality) Sample Size	Supplement-ation	Timing of Measurement of Supplementation	Period of Supplement-ation	Outcome	Comparison	Odds Ratio (95% CI)	N	Adjustments
Sharman Moser et al, 2019[104] Case-control Medium (fair quality) N=21,895 (2009 cases; 19,886 controls)	Folic acid supplements	NR	During the 12 months preceding the index date (birth date of the child)	ASD	Unsupplemented or very low supplemented (median daily dose dispensed <0.2 mg)	Odds ratio from multivariate conditional logistic regression (95% CI) Low supplemented (0.2-<0.4 mg/day): 1.15 (1.00, 1.32) Typically supplemented (0.4–<1 mg/day): 1.10 (0.98, 1.24) High supplemented (1-<3 mg/day): 1.14 (0.98, 1.34) Very high supplemented (> 3 mg/day): 1.01 (0.60, 1.70)	NR	Maternal age, subfertility, number of physician or obstetrics visits in the 15 months before index date, birth order, and number of children in the family
Levine et al, 2018[103] Cohort Medium (fair quality)	Folic acid supplements	NR	before pregnancy (540–271 days before childbirth) and during pregnancy (270	ASD	No folic acid use	Relative risks (95% CI) Before pregnancy:	N (%) Before pregnancy Folic acid use: 48/7,227 (0.665)	Sex, birth year, socioeconomic status (high vs. low), a maternal and paternal psychiatric diagnosis by childbirth

Appendix F Table 7. Results of Included Studies on Association Between Folic Acid Supplementation and Autism Spectrum Disorders

First Author, Year Design Risk of Bias (Quality) Sample Size	Supplement-ation	Timing of Measurement of Supplementation	Period of Supplement-ation	Outcome	Comparison	Odds Ratio (95% CI)	N	Adjustments
Levine et al, 2018[103] (continued) N=45,300			days before childbirth up to the date of childbirth)			0.56 (0.42 to 0.74) During pregnancy: 0.32 (0.26 to 0.41)	No folic acid use: 524/38,073 (1.38%) During pregnancy Folic acid use: 84/15883 (0.53%) No folic acid use: 488/29,417 (1.66%)	(present or absent), maternal and paternal age at childbirth, and parity
DeVilbiss et al, 2017[101] Stockholm Youth Cohort Cohort Medium (fair quality) N=94,864	Folic acid supplements	First antenatal visit (median = 10.7 weeks, interquartile range 9.0–12.7 weeks)	Use of any supplement containing folic acid in at least 2 weeks in the period that began 4 weeks prior to the last menstrual period and continued 8 weeks after the last menstrual period	ASD with or without intellectual disability	No folic acid supplement use at first antenatal visit	ASD with intellectual disability aOR (95% CI) Folic acid supplement use at first antenatal visit: 1.20 (0.71 to 2.01) ASD without intellectual disability aOR (95% CI) Folic acid supplement use at first antenatal visit: 1.29 (0.99 to 1.67) Any ASD	ASD with intellectual disability Folic acid use: 15/2,789 (0.5%) No folic acid use: 430/91,895 (0.5%) ASD without intellectual disability Folic acid use: 63/2,789 (2.3%) No folic acid use: 1,615/91,895 (1.8%) Any ASD Folic acid use: 78/2,789(2.8%) No folic acid use:	Child characteristics (sex, birth year, and years resided in Stockholm County), socioeconomic indicators (education, family income, and maternal birth country), maternal characteristics (age, body mass index, parity, smoking status), medication use during pregnancy (antidepressants or antiepileptics), and maternal neuropsychiatric conditions (anxiety disorders, autism, bipolar disorder, depression, epilepsy, intellectual disability, nonaffective psychotic disorders, and stress disorders)

Appendix F Table 7. Results of Included Studies on Association Between Folic Acid Supplementation and Autism Spectrum Disorders

First Author, Year Design Risk of Bias (Quality) Sample Size	Supplement-ation	Timing of Measurement of Supplementation	Period of Supplement-ation	Outcome	Comparison	Odds Ratio (95% CI)	N	Adjustments
DeVilbiss et al, 2017[101] (continued)						aOR (95% CI), multivariate generalized estimating equations Folic acid supplement use at first antenatal visit: 1.27 (1.01 to 1.60) aOR (95% CI), matched cohort of siblings Folic acid supplement use at first antenatal visit: 1.48 (0.87 to 2.51) aOR (95% CI), propensity score analysis Folic acid supplement use at first antenatal visit: 1.17 (0.89 to 1.51)	2,045/91,895 (2.2%)	
Virk et al, 2016[99] Cohort	Folic acid supplements	Mean of 11.5 weeks gestation	4 weeks before pregnancy to 8 weeks after pregnancy	ASD (autism, Asperger's	No folic acid use	−4- to 8-week period Relative risks (95% CI)	N reported as expected and unexpected cases but not	Maternal age, household socioeconomic status, maternal smoking, alcohol

Appendix F Table 7. Results of Included Studies on Association Between Folic Acid Supplementation and Autism Spectrum Disorders

First Author, Year Design Risk of Bias (Quality) Sample Size	Supplement-ation	Timing of Measurement of Supplementation	Period of Supplement-ation	Outcome	Comparison	Odds Ratio (95% CI)	N	Adjustments
Medium (fair quality) N=35,059				syndrome, PDD-NOS)		Asperger's syndrome: 0.85 (0.46 to 1.53) ASD: 1.06 (0.82 to 1.36) No exposure: reference PDD-NOS: 1.07 (0.75 to 1.54) Autism: 1.18 (0.76 to 1.84)	defined so cannot be interpreted	consumption during pregnancy
Strom et al, 2018[98] Cohort Medium (fair quality) N=87,210	Folic acid supplements	First antenatal visit (6 to 10 weeks' gestation)	Four weeks before gestation until 8 weeks gestation	ASD, childhood autism	No folic acid use	Hazard ratio (95% CI) ASD Exposed −4 to 8: 1.06 (0.94 to 1.19) Childhood autism: Exposed −4 to 8: 1.09 (0.87 to 1.38)	N (%) ASD Folic acid −4 to 8: 749/52,822 (1.4%) No folic acid −4 to 8: 485/34,388 (1.4%) Childhood autism: Folic acid −4 to 8: 193/52,822 (0.4%) No folic acid −4 to 8: 119/34,388 (0.4%)	Maternal age, paternal age, parity, maternal smoking during pregnancy, maternal education, family socioeconomic status, whether the pregnancy was planned, maternal prepregnancy body mass index (BMI) and sex of the child

Appendix F Table 7. Results of Included Studies on Association Between Folic Acid Supplementation and Autism Spectrum Disorders

First Author, Year Design Risk of Bias (Quality) Sample Size	Supplement-ation	Timing of Measurement of Supplementation	Period of Supplement-ation	Outcome	Comparison	Odds Ratio (95% CI)	N	Adjustments
Strom et al, 2018[98] (continued)								
Suren et al, 2013[100] Cohort Medium (fair quality) N=85,176 (270 with ASD)	Folic acid supplements	Approximately 18 weeks' gestation	Before pregnancy (first day of the last menstrual period before conception) During pregnancy (at week 22)	ASD	No folic acid use	Autism Exposed: 0.61 (0.41 to 0.90) Asperger's syndrome Exposed: 0.65 (0.36 to −1.16) PDD-NOS Exposed: 1.04 (0.66 to 1.63)	Folic acid use: 64/61,042 (0.10%) No folic acid use: 50/24,134 (0.21%)	Year of birth, maternal education level, parity
Nilsen et al, 2013[102] Medium (fair quality) N=89,836 (234 with ASD) MoBa N=507,856 (2,034 with ASD) MBRN	Folic acid supplements	Approximately 18 weeks' gestation	Before and/or during pregnancy	ASD	No folic acid use	MBRN: 0.86 (0.78 to 0.95) MOBA: 0.85 (0.65 to 1.11)	NR	Year of birth, maternal age, paternal age, marital status, parity, hospital size

Abbreviations: aOR=adjusted odds ratio; ASD=autism spectrum disorder; BMI=body mass index; CI=confidence interval; MBRN=Medical Birth Registry of Norway; MoBa=Norwegian Mother and Child Cohort Study; NR=not reported; PDD-NOS=pervasive developmental disorder not otherwise specified; vs.=versus.

Appendix F Table 8. Harms of Folic Acid Supplementation: Study Characteristics of Included Cancer Studies

First Author, Year Study Name Design Risk of Bias Sample Size	Population	Inclusion Exclusion Criteria	Timing and Setting	Supplementation Groups	Age	% Non-White
Mortensen et al, 2015[97] Cohort Medium (fair quality) N=429,004	Women who did and did not receive a cancer diagnosis after pregnancy	Inclusion: Women living in Norway and giving birth in the period January 1, 1999, to December 31, 2010 Exclusion: Induced abortions, pregnancies to women who emigrated before birth, women diagnosed with cancer before delivery	1999–2010 Norwegian Central Population Registry; Medical Birth Registry of Norway; Cancer Registry of Norway; Norwegian Labour and Welfare Administration; Norwegian National Education Database	No folic acid use Folic acid use before pregnancy Folic acid use during pregnancy Folic acid before and during pregnancy	NR Maternal year of birth N (%) 1949–1959: 3,158 (1%) 1960–1969: 102,284 (31%) 1970–1979: 227,841 (55%) 1980–1989: 92,535 (12%) 1990–1996: 3,186 (1%)	NR

Abbreviations: N=number; NR=not reported.

Appendix F Table 9. Results of Included Studies on Association Between Folic Acid Supplementation and Cancer

First Author, Year Design Risk of Bias (Quality) Sample Size	Supplementation	Period of Supplementation	Timing of Measurement of Supplementation	N	Outcome	Comparison	Hazard Ratio (95% CI)	Adjustments
Mortensen et al, 2015[97] Cohort Medium (fair quality) N=429,004	Folic acid supplements	Before and/or during pregnancy	NR in Mortensen et al, 2015;[97] other MBRN studies data were collected at the time of birth and during the following stay at the delivery unit[92]	Folic acid use before pregnancy: N=5,082 Folic acid use during pregnancy: N=112,874 Folic acid use before and during pregnancy: N=58,428 No folic acid use: N=252, 620	Cancer diagnosis: N=3,781 No cancer diagnosis: N=425,223	No folic acid use	1.06 (0.91 to 1.22)	Maternal age, maternal age at first childbirth, maternal year of birth, parity, marital status, education, occupation, multivitamin use, smoking

Abbreviations: CI=confidence interval; MBRN=Medical Birth Registry of Norway; N=number; NR=not reported.

Appendix F Table 10. Variation in Harms of Folic Acid Supplementation by Dose

First Author, Year Design Risk of Bias (Quality) Sample Size	Subgroup	N (%)	Results
Suren et al, 2013[100] Cohort Medium (fair quality) N=85,176	0.001 to 0.399 mg folic acid use in week 22 0.4 mg or more in week 22 No folic acid use in week 22	0.001 to 0.399 mg: 26/20,872 (0.12) ≥0.4 mg: 31/26,467 (0.12) None: 42/32,064 (0.13)	aOR (95% CI) Controls: 1 (reference) Exposed 0.001 to 0.339 mg: 1.02 (0.62 to 1.67) Controls: 1 (reference) Exposure ≥0.4 mg: 0.96 (0.60 to 1.55)
Strom et al, 2018[98] Cohort Medium (fair quality)	<0.4 mg supplement use at mid-pregnancy >0.4 mg supplement use at mid-pregnancy No supplement use at mid-pregnancy	N (%) ASD <0.4 mg folic acid use: 237/17,444 (1.4%) ≥0.4 mg folic acid use: 358/26,092 (1.4%) No folic acid use: 60/4,482 (1.3%) Childhood autism: <0.4 mg folic acid use: 64/17,444 (0.4%) ≥0.4 mg folic acid use: 98/26,092 (0.4%) No folic acid use: 16/4,482 (0.4%)	HR (95% CI) ASD <0.4 mg folic acid use: 1.01 (0.76 to 1.34) No folic acid use: reference ≥0.4 mg folic acid use: 0.98 (0.75 to 1.29) No folic acid use: reference Childhood autism: <0.4 mg folic acid use: 1.03 (0.60 to 1.79) No folic acid use: reference ≥0.4 mg folic acid use: 1.06 (0.62 to 1.80) No folic acid use: reference

Abbreviations: aOR=adjusted odds ratio; ASD=autism spectrum disorder; CI=confidence interval; HR=hazard ratio; N=number.

Appendix F Table 11. Variation in Harms of Folic Acid Supplementation by Timing

First Author, Year Design Risk of Bias (Quality) Sample Size	Subgroup	N (%)	Results
Suren et al, 2013[100] Cohort Medium (fair quality) N=85,176	Folic acid supplement initiation from −4 to −1 weeks Folic acid supplement initiation from 1 to 4 weeks Folic acid supplement initiation from 5 to 8 weeks Folic acid supplement initiation from 9 to 16 weeks No folic acid supplement use	−4 to −1: 32/28,061 (0.11) 1 to 4: 18/16,797 (0.11) 5 to 8: 14/16,184 (0.09) 9 to 16: 18/9,395 (0.19) No folic acid: 32/14,721 (0.22)	aOR (95% CI) Exposed −4 to 1: 0.67 (0.40 to 1.14) Exposed 1 to 4: 0.58 (0.32 to 1.05) Exposed 5 to 8: 0.44 (0.23 to 0.83) Exposed 9 to 16: 0.87 (0.49 to 1.57)
Virk et al, 2016[99] Cohort Medium (fair quality) N=35,059	Use of any supplement containing folic acid from −4 to −1 week Use of any supplement containing folic acid from 1 to 4 Use of any supplement containing folic acid from 5 to 8 weeks No supplement use during the −4- to 8-week period	N (%) ASD −4 to −1: 52/3,330 (1.56%) 1 to 4: 69/4,328 (1.59%) 5 to 8: 87/5,793 (1.50%) No folic acid: 193/11,916 (1.62%) Autism −4 to −1: 19/3,330 (0.57%) 1 to 4: 25/4,328 (0.58%) 5 to 8: 28/5,793 (0.48%) No folic acid: 63/11,916 (0.52%) Asperger's syndrome −4 to −1: 7/3,330 (0.21%) 1 to 4: 10/4,328 (0.23%) 5 to 8: 15/5,793 (0.26%) No folic acid: 39/11,916 (0.33%) PDD-NOS −4 to −1: 26/3,330 (0.78%) 1 to 4: 34/4328 (0.79%) 5 to 8: 44/5,793 (0.76%) No folic acid: 91/11,916 (0.76%)	Relative risks (95% CI) ASD Exposed −4 to −1: 1.14 (0.82 to 1.58) Exposed 1 to 4: 1.12 (0.83 to 1.50) Exposed 5 to 8: 1.05 (0.80 to 1.37) Autism Exposed −4 to −1: 1.39 (0.79 to 2.43) Exposed 1 to 4: 1.36 (0.82 to 2.26) Exposed 5 to 8: 1.12 (0.70 to 1.81) Asperger's Syndrome Exposed -4 to -1: Not calculated Exposed 1 to 4: Not calculated Exposed 5 to 8: 0.85 (0.45 to 1.59) PDD-NOS Exposed −4 to −1: 1.15 (0.72 to 1.83) Exposed 1 to 4: 1.11 (0.73 to 1.69) Exposed 5 to 8: 1.09 (0.75 to 1.60)

Appendix F Table 11. Variation in Harms of Folic Acid Supplementation by Timing

First Author, Year Design Risk of Bias (Quality) Sample Size	Subgroup	N (%)	Results
Strom et al, 2018[98]		N (%)	Hazard ratio (95% CI)
		ASD	ASD
Cohort		Folic acid −4 to −1: 414/28895 (1.4%)	Exposed −4 to −1: 1.05 (0.93 to 1.18)
Medium (fair quality)		No folic acid −4 to −1: 820/58315 (1.4%)	Unexposed −4 to 1: reference
			Exposed 1 to 4: 1.04 (0.93 to 1.17)
		Folic acid 1 to 4: 545/38326 (1.4%)	Unexposed 1 to 4: reference
		No folic acid 1 to 4: 689/48884 (1.4%)	
			Exposed 5 to 8: 1.06 (0.94 to 1.18)
		Folic acid 5 to 8: 732/51559 (1.4%)	Unexposed 5 to 8: reference
		No folic acid 5 to 8: 502/35651 (1.4%)	
			Childhood autism
		Childhood autism	Exposed −4 to −1: 1.11 (0.88 to 1.41)
		Folic acid −4 to −1: 108/28895 (0.4%)	Unexposed −4 to 1: reference
		No folic acid −4 to −1: 204/58315 (0.4%)	Exposed 1 to 4: 1.17 (0.93 to 1.41)
			Unexposed 1 to 4: reference
		Folic acid 1 to 4: 145/38326 (0.4%)	Exposed 5 to 8: 1.09 (0.86 to 1.37)
			Unexposed 5 to 8: reference
		No folic acid 1 to 4: 167/48884 (0.3%)	
		Folic acid 5 to 8: 188/51559 (0.4%)	
		No folic acid 5 to 8: 124/35651 (0.4%)	

Abbreviations: aOR=adjusted odds ratio; ASD=autism spectrum disorder; CI=confidence interval; N=number; PDD-NOS=pervasive developmental disorder not otherwise specified.