INFO ON FOLATE, FOLIC ACID (FA), NEURAL TUBE DEFECTS AND MTHFR GENE VARIANTS

What is covered in this document:

My recommendations for prenatal vitamins:

- Nature Made Prenatal Multi + Dha, 200mg
    - ADD CHOLINE
- One A Day Prenatal Advanced Complete Multivitamin with Brain Support with Choline, Folic Acid, Omega-3 DHA & Iron
- Gummies
    - GUMMY PNV Typically don't have iron. Because of their design, they cannot carry enough of the ingredients necessary for supplmentation. THEY should be TAKEN AS a last resort.
    - Nature Made Prenatal Gummies with DHA & Folic Acid
        - add iron
    - Vitafusion
        - add choline & iron
    - One A Day Prenatal Multivitamin Gummies
        - ADD CHOLINE & IRON
- VEGETARIAN
    - Rainbow Light Prenatal One Daily Multivitamin, Non-GMO, Vegetarian & Gluten Free
        - ADD DHA
- VEGAN
    - Deva vegan prenatal
        - add DHA

Summary of vitamins and minerals needed in pregnancy

Check out ACOGs recommendations for key vitamins and minerals in pregnancy

Rules to follow for prenatal vitamins:

- A higher cost *does not mean* better quality or better for you!
- Avoid the designer subscription prenatal vitamins that influencers promote.
- "Natural" doesn't mean good for you. The supplement industry makes billions of dollars from supplements that have little research or data. Companies touting that their PNV contains "natural ingredients" are using that as a marketing ploy to charge a premium for their designer PNV.
- Take only one serving of your PNV each day. If you need an extra amount of a vitamin or mineral, take it as a separate supplement under the guidance of an

obstetrical care provider. Look at the nutrition and supplement facts label to see what the recommended serving size is.
- A healthy balanced diet is the best way to get all the vitamins and minerals you need in pregnancy. However, take a prenatal vitamin (PNV) with the key vitamins and minerals in the event that you don't get what you need daily in your diet alone. The purpose of a PNV is NOT to cover ALL the extra vitamin and mineral needs in pregnancy. That is why PNV will ONLY have a portion of any specific vitamin and mineral that is recommended.

<u>Iron, Iodine and Choline</u>

- RECOMMENDED DAILY AMOUNT for pregnancy:
    - IRON - 27 MILLIGRAMS (MG)
    - IODINE - 220 MICROGRAMS (MCG)
    - CHOLINE - 450 MG
- BEST FOOD SOURCES:
    - IRON - beans, lentils, enriched breakfast cereals, beef, turkey, liver, shrimp.
        - You should also eat foods that help your body absorb iron, including oranges, grapefruit, strawberries, broccoli, & peppers.
    - IODINE - Iodized table salt, dairy products, seafood, meat, some breads, eggs
    - CHOLINE - chicken, beef, eggs, milk, soy products, peanuts
        - Plant sources, such as navy beans, Brussels sprouts, broccoli, & spinach contain lower amounts of choline.
        - vegetarians & vegans should seek out supplemental sources of choline.
- TYPICAL AMOUNT IN A PNV:
    - IRON - 30-60 mg of elemental iron
        - adequate for non-anemic persons
    - IODINE - Many PNV contain no iodine since requirements are often met with dietary sources, such as iodized salt.
    - CHOLINE - not found in most PNV SO GET IT FROM YOUR DIET OR TAKE AN EXTRA SUPPLEMENT.
        - 200-300 MG of choline IN A daily supplement is recommended in pregnancy.
        - The forms of choline in dietary supplements include choline bitartrate, phosphatidylcholine, & lecithin.

<u>Omega 3 Fatty Acids (DHA)</u>

- RECOMMENDED DAILY AMOUNT for pregnancy:
- BEST FOOD SOURCES: Flaxseed (ground or as oil) & fish
    - Other sources include broccoli, cantaloupe, kidney beans, spinach, cauliflower, and walnuts.
- TYPICAL AMOUNT IN A PNV: DHA is not found in most PNV SO GET IT FROM YOUR DIET OR TAKE AN EXTRA SUPPLEMENT.

- Can add a supplement of 200-300 mg a day.
    - THERE ARE VEGAN FORMS OF DHA.
- Eat at least 2-3 servings of A VARIETY OF fish or shellfish per week before getting pregnant & while pregnant.
    - A serving of fish is 4 ounces (oz).
- Some types of fish have higher levels of mercury than others.
    - Do not eat bigeye tuna, king, MACKEREL, MARLIN, ORANGE ROUGHY, SHARK, SWORDFISH, OR TILEFISH.
    - Limit white (albacore) tuna to 6 oz A WEEK.
    - Check for advisories for fish CAUGHT WHERE NO ADVISORIES EXIST.
        - limit eating those fish to one SERVING A WEEK & DO NOT EAT OTHER FISH THAT WEEK.

<u>Calcium and Vitamin D</u>

- *RECOMMENDED DAILY AMOUNT For pregnancy*:
    - CALCIUM - 1000-1300 MILLIGRAMS (MG)
    - VIT D - 600 international units (IU)
- *BEST FOOD SOURCES*:
    - CALCIUM - Milk & other dairy products, such as cheese and yogurt, are the best sources of calcium.
        - you can ALSO get calcium from broccoli, fortified foods (cereals, breads, & juices), almonds & sesame seeds, sardines or anchovies with the bones, & dark green leafy vegetables.
    - VIT D - Sunlight, fortified milk & BREAKFAST CEREAL, fatty fish such as salmon, sardines & MACKEREL, fish liver oils, egg yolks
- *TYPICAL AMOUNT IN A PNV*:
    - CALCIUM - 200-300 mG
    - VIT D - 400 IU
        - Many commercial nonprescription products labeled "vitamin D" (multivitamin supplements, fortified milk, bread) contain D2.
        - D3 is more readily converted to active forms of vit D & is more effective at increasing serum 25-hydroxyvitamin D.
- *Many people do not get enough vit D*.
    - a test can be done to check the level in your blood if you are at risk for low levels.
    - If below normal, you may need EXTRA vit D supplementATION.
    - 1000–2000 iU per day of vit D is safe if vit d deficiency is diagnosed.

<u>Folic acid (Vitamin B9)</u>

- *RECOMMENDED DAILY AMOUNT For pregnancy*: 400 MICROGRAMS (MCG)
- *BEST FOOD SOURCES*: Fortified cereal, enriched bread and pasta, peanuts, dark green leafy vegetables, orange juice, beans
- *TYPICAL AMOUNT IN A PNV*: 400-800 MCG
- take a daily PNV with at least 400 mCG starting at least 1 month before pregnancy & during the first 12 weeks of pregnancy.

    - THE Nutrition Facts or Supplement Facts label SHOULD EXPLICITLY SAY "FOLIC ACID" WITH THE AMOUNT IN MCG LISTED.
- The terms “folate” and “folic acid” are often used interchangeably, even though they are different.
    - Folate is a general term to describe many different types of vit B9.
    - Types of folate include:
        - Folic acid -SYNTHETIC
        - Dihydrofolate (DHF)
        - Tetrahydrofolate (THF)
        - 5-methyltetrahydrofolate (5-Methyl-THF or *5-MTHF OR METHYLFOLATE*)
    - The other types of folate are different from the folate found IN FRUITS AND VEGETABLES, EVEN IF THE NUTRITION LABEL SAYS “NATURAL FOOD FOLATE.”
        - These types of folate ARE ALSO MAN-MADE.
- Folic acid has been shown to be effective in preventing neural TUBE DEFECTS IN RANDOMIZED CONTROL TRIALS & FOOD FORTIFICATION PROGRAMS.
    - The effectiveness of OTHER FORMS OF FOLATE in preventing NEURAL TUBE DEFECTS HAS NOT BEEN STUDIED.
- PERSONS WITH MTHFR GENE VARIANTS CAN TAKE FOLIC ACID.

<u>Vitamins A and C and other B vitamins</u>

- *RECOMMENDED DAILY AMOUNT For pregnancy*:
    - VIT B6 - 1.9 milligrams (MG)
    - VIT B12 - 2.6 micrograms (MCG)
    - VIT A - 770 MCG retinol equivalents
    - VIT C - 85 MG
- *BEST FOOD SOURCES*:
    - VIT B6 - Beef, liver, pork, ham, whole-grain cereals, bananas
    - VIT B12 - Meat, fish, poultry, milk
        - vegetarians should take aN EXTRA B12 supplement.
    - VIT A - Carrots, green leafy vegetables, sweet potatoes
    - VIT C - citrus fruits and juices, strawberries, broccoli, tomatoes
- *TYPICAL AMOUNT IN A PNV*:
    - VIT B6 - ANY amount is fine since the most pregnant persons consume an adequate amount of B6 through a combination of foods plus supplements.
    - VIT B12 - 6 Mcg
    - VIT A - 300-770 mcg
    - VIT C - Any amount is OK.
- VIT B1, B2 & B9 ARE ALSO IN A TYPICAL PNV.

Folate Metabolism

https://www.annualreviews.org/content/journals/10.1146/annurev-nutr-043020-091647

Folate is essential for cell growth as well as DNA synthesis and methylation, and, if depleted, results in double-stranded breaks in the DNA, triggering apoptosis (30). Folate is particularly important for preventing megaloblastic anemia due to the high folate requirements needed to maintain adequate blood counts. In addition to the causal link with NTDs, epidemiological studies have shown that low folate status is associated with heart diseases and an increased risk of most cancers (5).

Naturally occurring dietary folate exists in both monoglutamate and polyglutamate forms. To be absorbed through the intestinal mucosa, polyglutamate dietary folate must be converted into 5-methyltetrahydrofolate (5-MTHF), a monoglutamate form of folate that is the primary form taken up by cells in peripheral tissue (**5**). Once in the cells, 5-MTHF must be polyglutamated to be retained and to function as a one-carbon-cycle coenzyme. To accomplish this task, methionine synthase (MS) converts 5-MTHF to tetrahydrofolate (THF). Synthetic FA is a monoglutamate form of folate that is readily transported across the intestinal epithelium. The enzyme dihydrofolate reductase reduces FA into dihydrofolate and THF (**30**).
Once formed from either dietary folate or FA, THF can be converted to 5,10-methylene-THF by serine hydroxymethyltransferase, which is a vitamin B6–dependent reaction. Importantly for the one-carbon pathway, methylenetetrahydrofolate reductase (MTHFR) can catalyze the conversion of 5,10-methylene-THF to 5-MTHF, which serves as a carbon donor in the vB12-dependent conversion of homocysteine to methionine by MS. Methionine is an essential amino acid used for protein synthesis and as a substrate for *S*-adenosylmethionine (SAM), which is required for many methylation reactions for DNA, RNA, lipids, neurotransmitters, and hormones.

Are folate and folic acid the same thing?

The terms “folate” and “folic acid” are often used interchangeably, even though they are different. ‘Folate’ is essentially the umbrella term for all forms of vitamin B9 found in prenatal vitamins and supplements, including folic acid. It is a water-soluble vitamin B9 that naturally occurs in foods, so folate is also called “natural food folate” or “dietary folate”. Folic acid is a B vitamin that is the ‘core’ molecule in all folates but is not itself present in natural food. It is the synthetic, oxidized form of vitamin B9 used in a multivitamin, prenatal vitamin, or single supplements and fortified foods. Folic acid becomes biologically active after metabolic reduction in the liver. Recently, vitamin B9 has been used for ‘folate’ and sometimes as ‘vitamin B9 fortification’ when referring to folic acid fortification and as ‘vitamin B9 supplements’ when referring to folic acid supplements.

Types of folate found in supplements and prenatal vitamins include:
-Folinic acid: AKA calcium folinate, leucovorin

-Folic acid: The standard synthetic form used in most prenatals and fortified foods, proven to significantly lower neural tube defects.
-*5-methyltetrahydrofolate*–AKA 5-Methyl-THF, 5-MTHF
-Often labeled as L-methylfolate, methylfolate, or L-5-MTHF.
-*Levomefolic Acid or Metafolin*®–calcium salt of L-5-methyltetrahydrofolate acid or L-5-MTHF
-AKA L-methylfolate [L-MTHF], (6S)-5-methyltetrahydrofolate [(6S)-5-MTHF], L-Methylfolate Calcium, D-Methylfolate, D-5-Methylfolate, 5-Methylfolate, L-5-methyltetrahydrofolic, levomefolate, levomefolic acid
-*Quatrefolic*®–glucosamine salt of (6S)-5-methyltetrahydrofolate
-AKA [(6S)-5-methyltetrahydrofolate]

**Folic acid is the only form of folate found in prenatal vitamins and supplements that has been proven to help prevent neural tube defects in the fetus. The nutrition/ supplements facts label should specifically say "folic acid" followed by the amount in micrograms (mcg).*

Online Misinformation Fuels a Fight Over Folic Acid
Despite the scientific consensus, some nutritionists and dieticians — along with prominent complementary health practitioners including naturopaths, chiropractors, and functional medicine doctors — are causing many people, and not just those who are or may become pregnant, to question whether they should be consuming any folic acid at all. Many medical experts worry that these vocal individuals are urging people who could become pregnant to avoid vital folic acid supplementation, putting unborn babies at unnecessary risk for neural tube defects.

Addl Resources:
https://journals.sagepub.com/doi/10.1177/09691413221102321

Why is folate so important in pregnancy?

- In the United States, 3,000 pregnancies are affected by neural tube defects every year.
- Hispanic individuals have higher rates of neural tube defects than non-Hispanic individuals in the United States.
- Increasing folic acid intake among individuals of childbearing age can help prevent 150,000-210,000 of the more than 300,000 neural tube defects that occur each year in low- and middle-resource countries.
- Worldwide, approximately 300,000 infants are born annually with an NTD, and NTDs account for as many as 29% of neonatal deaths associated with congenital abnormalities in low-income settings

https://doi.org/10.1016/S2214-109X(22)00213-3

-Of the few known causes of spina bifida and anencephaly, the most predominant is insufficient concentration of folate in the mother before and during early pregnancy. Neural tube defects happen in the first few weeks of pregnancy, often before a woman finds out she's pregnant (5-6 weeks after LMP). Also, almost half of all pregnancies in the United States are unintended. This is why it is important for all individuals who could become pregnant get 400 mcg of folic acid every day, even if they are not actively planning a pregnancy. By the time a woman realizes she is pregnant, it might be too late to prevent these birth defects.

https://doi.org/10.1177/09691413221102321
-The defects cause miscarriages, stillbirths and neonatal deaths, and they often lead to a termination of pregnancy if an NTD is identified through antenatal screening. Individuals born with spina bifida have disabilities that include paraplegia, incontinence of urine or faeces or both, and hydrocephalus.

The two most common types of neural tube defects are anencephaly and spina bifida. Anencephaly is a serious birth defect in which parts of a baby's brain and skull do not form correctly. Almost all babies born with anencephaly will die shortly after birth. Spina bifida is a serious birth defect in which a baby's spine does not develop correctly and can result in some severe physical disabilities.

https://doi.org/10.1016/S2214-109X(22)00213-3
-Spina bifida and anencephaly are common neural tube defects. Anencephaly is fatal, and open spina bifida, also known as myelomeningocele, is treatable but not curable, and is associated with an increased risk of child mortality. Many individuals living with spina bifida suffer permanent disability and chronic physical and psychological health complications, requiring lifelong surgical and medical care. Surgical care for spina bifida is expensive, complex, and scarce in many countries due to a shortage of specialised surgeons and multidisciplinary care teams.
-An analysis published in 2018 estimated that at least 214 000–322 000 pregnancies worldwide are affected by spina bifida and anencephaly annually, at an average prevalence of about 20 cases per 10 000 births.1 The burden corresponds to one in every 500 births globally. In low-income and middle-income countries, the prevalence exceeds one in every 100 births. Annually, about 60 000 affected pregnancies are

electively terminated after prenatal diagnosis, and another 60 000 result in stillbirths.

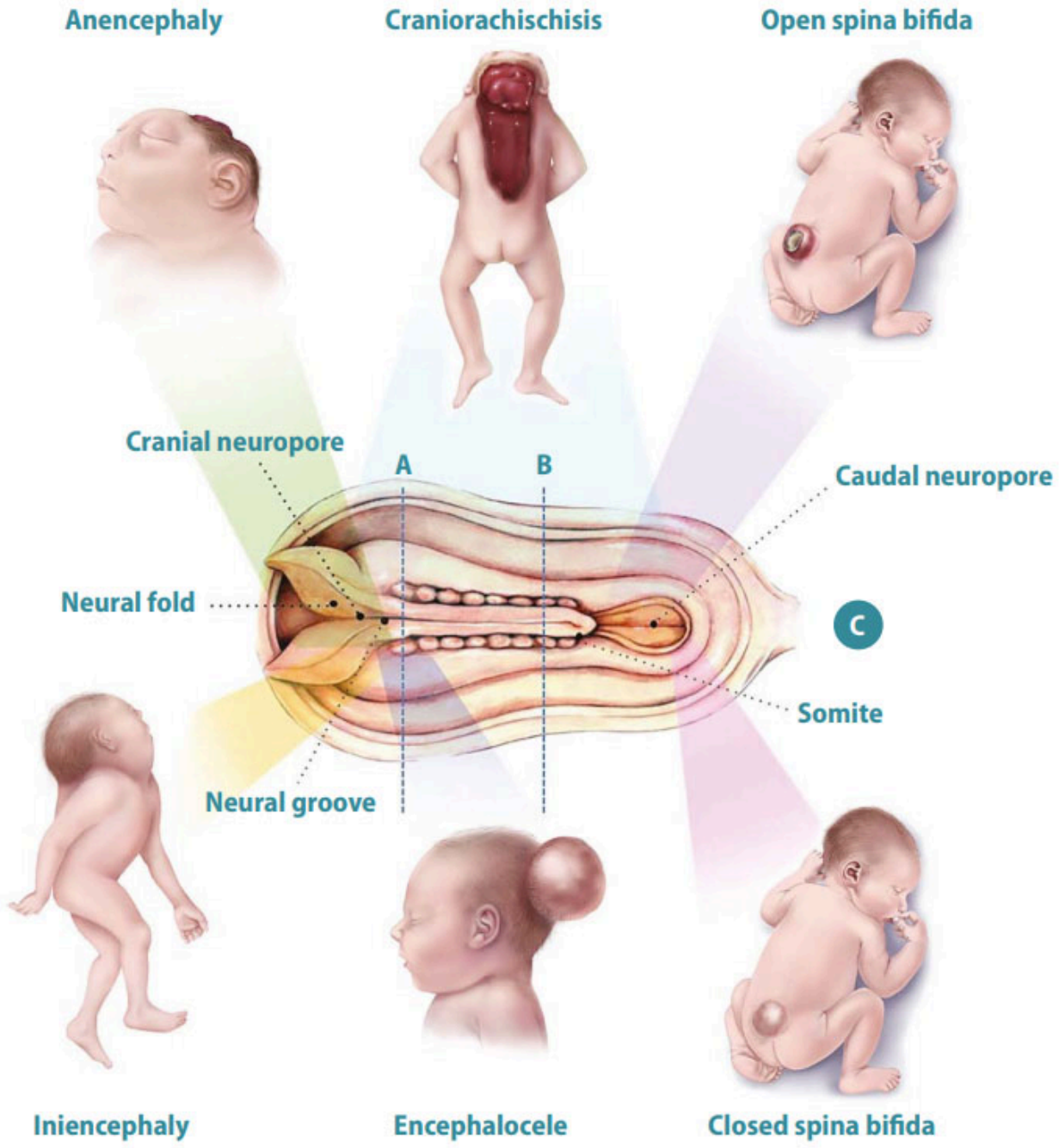

Table 1. Neural Tube Defect Pathophysiology ⇦

Neural Tube Defect	Malformation
Cranial	
Anencephaly	Failure of fusion of cephalic portion of neural folds; absence of all or part of brain, skull, and skin
Exencephaly	Failure of scalp and skull formation; exteriorization of abnormally formed brain
Encephalocele	Failure of complete skull formation; extrusion of brain tissue into membranous sac
Iniencephaly	Defect of cervical and upper thoracic vertebrae; abnormally formed brain tissue and extreme retroflexion of upper spine
Spinal	
Spina bifida	Failure of fusion of caudal portion of neural tube, usually of 3–5 contiguous vertebrae; spinal cord or meninges, or both, exposed to amniotic fluid
Meningocele	Failure of fusion of caudal portion of neural tube; meninges exposed
Myelomeningocele	Failure of fusion of caudal portion of neural tube; meninges and neural tissue exposed
Myeloschisis	Failure of fusion of caudal portion of neural tube; flattened mass of neural tissue exposed
Holorachischisis	Failure of fusion of vertebral arches; entire spinal cord exposed
Craniorachischisis	Coexisting anencephaly and open neural tube defect, often in the cervical–thoracic region

Historical significance of folic acid

More than four decades ago it was recognized that individuals with pregnancies complicated by fetal neural tube defects (NTDs) have lower plasma levels of B vitamins,

including folate, than individuals whose pregnancies were unaffected [44]. Neural tube defects are major birth defects of the baby's brain and spine.

The first trial (multicenter randomized trial) examining FA for the prevention of NTDs took place in 1991 among women with a previous NTD-affected pregnancy (*n* = 1,817) who were randomized to receive a 4,000 µg/day supplement containing only FA before pregnancy and through the 12th week of gestation; this study showed a 72% reduction (RR, 0.28; 95% CI, 0.12, 0.71) in subsequent pregnancies ([116]). In 1992, a second trial (a double-blind, placebo-controlled, randomized trial) was conducted among women without a history of an NTD-affected pregnancy (*n* = 4,753) who were randomized to receive a daily multivitamin supplement containing 800 µg/day FA. In this trial, there were six NTD-affected pregnancies, with no cases among women who received the FA-containing supplement (*p* = 0.029) ([32]). The results of the MRC Vitamin Study led to the recommendation that all individuals contemplating pregnancy should take 400 micrograms of folate daily, and individuals at high risk of pregnancy affected with NTD should take 4 mg (4,000 micrograms) daily [48]. This high-quality evidence set the stage for the March of Dimes to petition the FDA to mandate FA fortification of products labeled as enriched cereal grain products (authorized in 1996 and fully implemented in 1998) ([45]).

In 1998, the United States began mandatory fortification of wheat flour with folic acid; several other countries including Canada, South Africa, Australia, and countries in South America followed suit. In the United States, food fortification has been linked to a 19% decrease in all NTDs, with an 11% decrease in anencephaly and a 23% reduction in spina bifida **49**.

FA fortification has been heralded as one of the top 10 public health achievements in the USA during the first 10 years of the twenty-first century (**20**). More than 1,300 NTDs are prevented annually in the USA, and mandatory FA fortification is estimated to provide savings of more than $600 million in direct costs each year (**61**). Fortification of staple grains is one of the best ways to increase health equity by reaching at-risk populations with an intervention that requires no behavioral change and has nominal costs. It has been estimated that between 16% and 58% of NTDs could be prevented by folic acid supplementation **57**. A recent case–control study reported that prepregnancy folic acid supplementation resulted in a 79% reduction in risk of spina bifida and a 57% reduction in risk of anencephaly.

Despite years of ongoing research, the precise mechanism through which folic acid prevents NTDs has not been fully defined. Folic acid is involved in one-carbon metabolism, which includes synthesis of purines and pyrimidines for DNA replication and methyl group transfer to macromolecules. Many folate-dependent reactions are important for cell growth and proliferation, crucial processes during neural tube formation. Thus, it is biologically plausible that the interruption of folate pathways in the embryo could result in aberrant neural tube closure [2]. The important role of folate in neural tube closure is illustrated by the fact that certain medications that are associated

with increased risk of NTDs. Diphenylhydantoin, aminopterin, and carbamazepine interfere with folic acid metabolism **50**.

Addl Resources:

https://www.annualreviews.org/content/journals/10.1146/annurev-nutr-043020-091647

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Why aren't other forms of folate, like methylfolate, used in food fortification?

https://doi.org/10.1111/nyas.13499
-Folic acid is a synthetic and chemically stable form of folate and not the natural form of the vitamin found in whole foods. It is highly stable to oxidative degradation and therefore the preferred form of folate used in dietary supplements, ready-to-eat breakfast cereals, and fortified food.
-Physiological forms of folate that function as enzyme cofactors in metabolism include tetrahydrofolates and dihydrofolate. These forms of the vitamin are unstable and often undergo irreversible degradation during food preparation and cooking.29

Food fortification is the process by which vitamins and minerals are added to foods. Folic acid is more heat- and light-stable than natural food folate, which is broken down easily by heat and light; therefore, folic acid is better suited for food fortification because many fortified products, such as bread, are baked [8], and is not degraded in cooking. Cooking can decrease or degrade natural folate, but folic acid's structure makes it highly stable: It can even be boiled without changing shape.

Folic acid has been shown to be effective in preventing neural tube defects in randomized control trials and food fortification programs [2-6].

Folic acid is absorbed easily by the body, and studies have shown that it can increase blood folate concentrations (the amount in the blood) across populations (including those with the *MTHFR* TT genetic variant) [9, 10].

No scientific studies exist that show that supplements containing other forms of folate [such as 5-methyltetrahydrofolate (5-MTHF)] can prevent neural tube defects.

FA is about twice as bioavailable as food folate, especially when administered on an empty stomach.

Food folate is present in a polyglutamate form and must be digested to monoglutamates prior to absorption, resulting in ~50% bioavailability.

Micronutrient deficiencies (i.e., zinc), combinations of foods being consumed in the meal (i.e., alcohol or vitamin C-rich foods), and food preparation methods (i.e., raw versus cooked or processed) can also influence food folate digestion and absorption.

What does DV (daily value) and dietary folate equivalents (DFE) for folate mean?

Perspective: Time to Resolve Confusion on Folate Amounts, Units, and Forms in Prenatal Supplements

The % DV on Supplement and Nutrition Facts labels was developed by the FDA to help consumers determine how the levels of various nutrients in a standard serving of the product compare with their approximate requirement for them, based on a 2000-calorie diet. DVs are different than the RDAs and Adequate Intakes (AIs), but they are based on them. Specifically, the FDA selects the highest RDA (or AI) value within each of 4 established DV groups: *1*) adults and children aged ≥4 y, *2*) children aged 1–3 y, *3*) infants aged 1–12 mo, and *4*) pregnant and lactating people.

The original folate DV did not distinguish between food folate, folic acid, and other synthetic forms of the vitamin. However, in the new labeling regulations for food and dietary supplements issued in July of 2016, the updated folate DVs are expressed as µg DFE (dietary folate equivalents), conforming to the FNB's (Food and Nutrition Board of the National Academies of Science) concept of DFEs. DFE values differentiate between the naturally occurring folate in food and the more highly bioavailable added synthetic forms of the vitamin. The updated DV of 600 µg DFE (equivalent to 360 µg folic acid) for pregnant and lactating women is much lower than the old DV of 800 µg folic acid.

It is important to note that neither the updated DV nor the old DV is based on the recommendations from the CDC and the USPSTF for the prevention of neural tube defects in the fetus. To address concerns about consumer understanding, the FDA requires products containing folic acid to list the micrograms of folic acid in parenthesis following the declaration of folate in micrograms DFE. Figure 1 below contains 2 examples from the FDA on how to calculate and label products where 100% of the folate is from folic acid and where the product contains a combination of food folate, folic acid, and L-5-MTHF. Thus, to compare folic acid amounts in products with amounts recommended for NTD prevention, health care providers and women of childbearing age must focus on the amount of folic acid in parentheses, and not on the % DV.

Example A: A dietary supplement contains *only* folic acid (400 µg per serving)

Folate Conversion to µg DFE

Folate (µg DFE) = Folic acid (µg per serving) × 1.7 (conversion factor for folic acid)

Folate (µg DFE) = 400 µg x 1.7 = 680 µg DFE

% DV Calculation

% DV = [Folate (µg DFE) ÷ 2016 RDI for folate (µg DFE)] × 100

% DV = (680 µg DFE ÷ 400 µg DFE) × 100 = 170%

Supplement Facts	
	% Daily Value
Folate 680 mcg DFE (400 mcg folic acid)	170%

Example B: A dietary supplement contains naturally occurring folate (50 µg), folic acid (100 µg), and synthetic folate (as calcium L-5-MTHF, 150 µg) per serving

Folate Conversion to µg DFE

Folate (µg DFE) = [Naturally occurring folate (µg per serving) × 1 (conversion factor for naturally occurring folate)] + [folic acid (µg per serving) × 1.7 (conversion factor for folic acid)] + [synthetic folate (µg per serving) × 1.7 (conversion factor for synthetic folate7)]

Folate (µg DFE) = (50 µg × 1) + (100 µg × 1.7) + (150 µg × 1.7) = 475 µg DFE

% DV Calculation

% DV = [Folate (µg DFE) ÷ 2016 RDI for folate (µg DFE)] × 100

% DV = (475 µg DFE ÷ 400 µg DFE) × 100 = 119%

Supplement Facts	
	% Daily Value
Folate 475 mcg DFE (100 mcg folic acid)	119%

From CDC:

**Of note, micrograms, mcg and µg are all the same thing.*

The DVs are the amounts of nutrients people should consume as part of their daily diet. The % DV is how much a certain nutrient in a single serving of food contributes to the daily diet. The DVs are reference amounts (in grams, milligrams, or micrograms) of nutrients to consume or not to exceed each day.

The recommended amount of folate to consume each day (100% DV) is actually based on the amount needed to prevent anemia (low number of red blood cells) in adults. For folate, the % DV shows how much folate in a serving of a food product contributes to a total daily diet. The % DV for folate helps determine if a serving of food is high or low in folate. For example, a serving of food or a supplement that contains 25% DV of folate means that one serving contributes to 25%, or one-quarter, of the folate needed in the daily diet.

For most adults, 100% DV for folate is 400 micrograms dietary folate equivalents (DFE) or 400 mcg DFE. Women who are not pregnant or breastfeeding should consume 100%

DV (400 mcg DFE per day or 240 mcg folic acid) of folate to prevent anemia (low number of red blood cells). To prevent anemia, pregnant women and women who are breastfeeding should consume 600 mcg DFE and 500 mcg DFE, respectively, each day.

To help prevent NTDs, CDC recommends that all women of reproductive age take 400 micrograms (mcg) of folic acid each day, in addition to consuming food with folate from a varied diet. It is easier for the body to form healthy cells using folic acid than using natural food folate.

Folic acid can be measured in mcg DFE or in mcg (1.67 mcg DFE is the same as 1 mcg of folic acid). Because it takes more folic acid to help prevent NTDs than to prevent anemia (low number of red blood cells), 400 mcg folic acid is recommended to help prevent NTDs, which is equal to 667 mcg DFE (167% DV).

	Recommended mcg DFE	**Recommended mcg Folic Acid**	**% DV***
To Prevent Anemia	400 mcg DFE	240 mcg folic acid	100%
To Prevent NTDs	667 mcg DFE	400 mcg folic acid	167%

*% DV based on new Nutrition Facts and Supplement Facts labels starting **January 1, 2021**.

- **5% DV or less** of folate per serving means the product is low in folate.
- **20% DV or more** of folate per serving means the product is high in folate.

There has been a change in the %DV for folate on new Nutrition Facts and Supplement Facts labels. Prior to January 1, 2021, micrograms of folic acid (mcg folic acid) was the unit of measure on the old Nutrition Facts and Supplement Facts labels. The old labels showed the % DV of folic acid contained in each serving—100% DV of folic acid was equal to 400 mcg of folic acid. See below.

In 2016, DFEs were included in the Nutrition Facts and Supplement Facts labels to differentiate between the different forms of folate found in prenatal vitamins and supplements and their bioavailability. On the new Nutrition Facts and Supplement Facts labels, the unit of measure for folate is “mcg DFE,” or “micrograms of dietary folate equivalents.” The % DV of folate in the new labels is based on 400 mcg DFE, while the old label was based on 400 mcg of folic acid.

Folic acid is 1.67 times more bioavailable than dietary folates, thus for DFEs: total micrograms = micrograms food folate + [micrograms FA × 1.7]. The measure of mcg DFE is used because the body has an easier time absorbing folic acid than dietary

folate. In other words, folic acid can be measured in mcg DFE or in mcg (1.67 mcg DFE is the same as 1 mcg of folic acid).

https://doi.org/10.1111/nyas.13499
-Because of its increased chemical stability and lack of a conjugated polyglutamate peptide compared with natural food folate, folic acid is more bioavailable than natural folate contained in food. Whereas natural food folate is approximately 50% bioavailable, folic acid is 85% bioavailable and hence is ~1.7 times more bioavailable than food folate. For this reason, folate intake is expressed as dietary folate equivalents, where 1.7 μg of natural food folate equals 1.0 μg of folic acid.

How do I read the nutrition/supplements facts label?

In addition to the % DV, Nutrition Facts and Supplement Facts labels now show the amount of folate in mcg DFE. The form of folate in the prenatal vitamin or supplement may vary. If the form of folate is folic acid, the label will have the mcg folic acid per serving. The “mcg folic acid” will be listed in parentheses.

For example, in the “New Label” shown below, the total folate contained in one tablet of the supplement is listed as “667 mcg DFE (400 mcg folic acid),” which indicates that 400 mcg of folic acid is part of the total amount of folate (667 mcg DFE) in one serving.

Only folic acid is proven to help prevent NTDs. In order to help prevent NTDs, individuals who are pregnant or planning to become pregnant should check the Nutrition Facts and Supplement Facts labels on supplements and prenatal vitamins to make sure they are taking a supplement every day that contains 400 mcg of folic acid, in addition to eating foods with folate from a varied diet.

Old Label

Supplement Facts

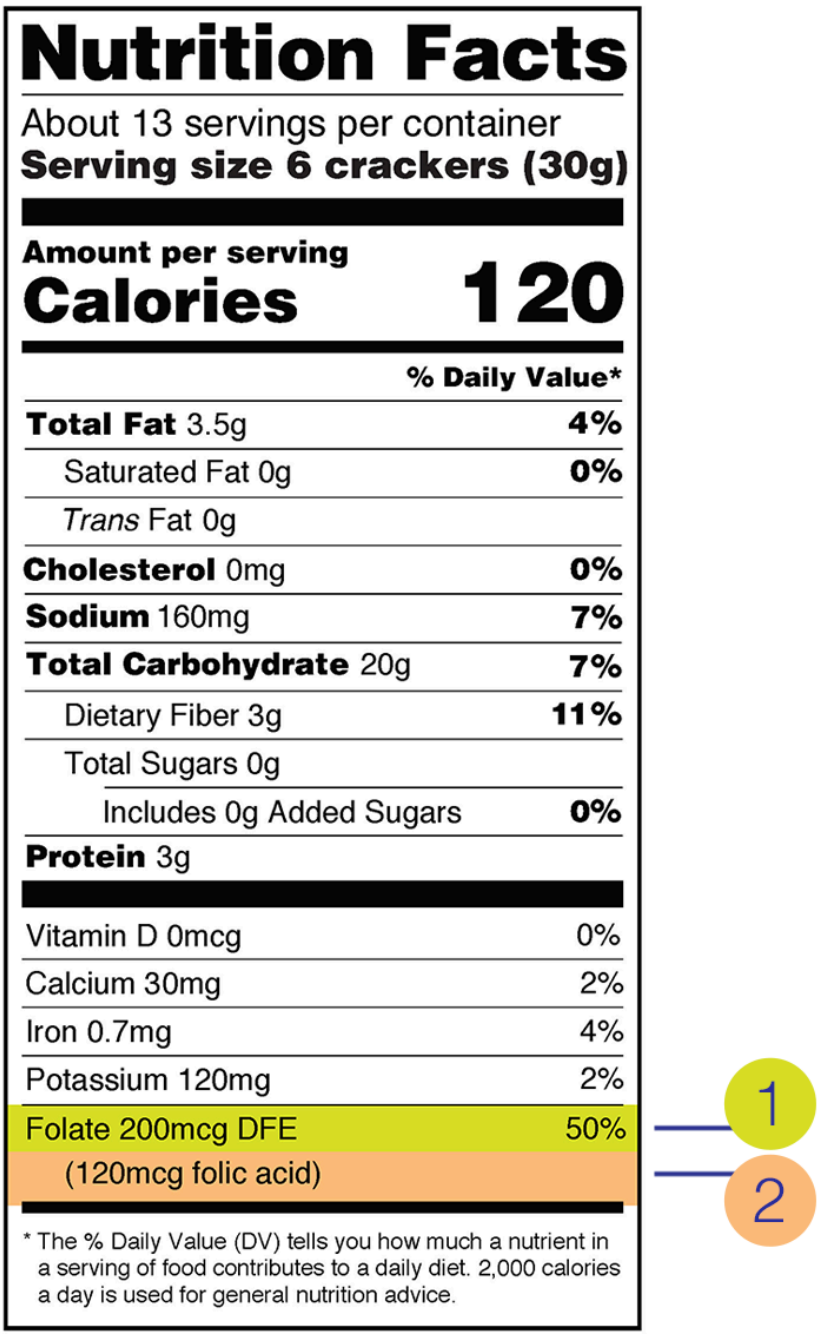
	Amount Per Serving	% Daily Value
Folic Acid	400 mcg	100%
Vitamin B12	6 mcg	100%
Pantothenic	5 mg	50%
Calcium	450 mg	45%
Iron	18 mg	100%
Magnesium	50 mg	12%
Zinc	15 mg	100%

New Label

Supplement Facts

Serving Size 1 Tablet

	Amount Per Serving	% Daily Value
Folate	667 mcg DFE (400 mcg folic acid)	167%
Vitamin B12	6 mcg	250%
Pantothenic Acid	10 mg	200%
Calcium	200 mg	15%
Iron	18 mg	100%
Magnesium	100 mg	24%
Zinc	11 mg	100%



1 – Folate is shown as mcg DFE and as a %DV

2 – Folic acid is shown in parenthesis and is important for individuals who could become pregnant

Tolerable upper intake level (UL) for folic acid

Resource: Perspective: Time to Resolve Confusion on Folate Amounts, Units, and Forms in Prenatal Supplements

The FNB established a Tolerable Upper Intake Level (UL) of 1000 µg for all adults that applies to “folate from fortified foods or supplements” (8). It did not establish a UL for naturally occurring folate (food folate) because high intakes had not been reported to cause adverse effects. Therefore, the UL is expressed in micrograms, not micrograms DFE, and appears to apply only to folic acid, as explained in a recent article (13), but not explicitly stated in the official report (8). The other synthetic salts of the vitamin (i.e., L-5-MTHF), were not addressed because they were first marketed after the FNB report was released (14). Notwithstanding the relatively high amounts of folic acid in most prenatal supplements, the probability of exceeding the UL amount is obviously greater if all forms of folate are considered. 92% of the prescription products and 7% of the nonprescription products would meet or exceed the 1000 µg folate from fortified foods or supplements UL amount if all forms of folate are included in the calculations. Thus, clarification is needed on the application of the UL. Does it apply only to folic acid or to all added forms of folate, which would include all forms in supplements. A literal reading of the recommendation in the FNB report would suggest the latter (8).

https://doi.org/10.1111/nyas.13499
-There is no UL for natural reduced folates found in foods.8 The UL for the provitamin folic acid was established to avoid a delayed diagnosis of vitamin B12 deficiency, as assessed by hematological indices, and thereby minimize the risk of neurological complications in vitamin B12-deficient individuals. The IOM stated, “The weight of the limited but suggestive evidence that excessive folate intake may precipitate or exacerbate neuropathy in vitamin B12-deficient individuals justifies the selection of this end point as the critical end point for the development of a UL for folate.”8 The IOM was careful to note that there was not sufficient evidence to establish a UL on the basis of a no-observed-adverse-effect level (NOAEL) but rather on the basis of a lowest-observed-adverse-effect level (LOAEL).8 The LOAEL was set at 5 mg/day on the basis of several case reports and small observational studies showing that, at doses of 5 mg/day folic acid and above, there were more than 100 reported cases (from more than 20 studies) of neurological progression in patients with pernicious anemia, compared with fewer than eight cases in studies administering less than 5 mg/day oral folic acid.8 As stated by the IOM, “The LOAEL of 5 mg/day of folate was divided by an uncertainty factor of 5 to obtain the UL for adults of 1 mg/day or 1000 µg/day of folate from supplements or fortified food. A UL of 1000 µg/day is set for all adults rather than just for the elderly because of (1) the devastating and irreversible nature of the neurological consequences of a delayed diagnosis and treatment of a vitamin B12 deficiency, (2) data suggesting that pernicious anemia may develop at a younger age in some racial or ethnic groups,20 and (3) uncertainty about the occurrence of vitamin B12 deficiency in younger age groups.” According to the IOM, “the prevalence of vitamin B12 deficiency in females in the childbearing years is very low and the consumption of supplemental folate at or above the UL in this subgroup is unlikely to produce adverse

effects,”8 although exceptions might include vegetarians, subsets of the population that have low dietary meat intake, and chronic users of proton pump inhibitors. Hematological indices are not commonly used to assess vitamin B12 deficiency, as they have been replaced with the use of serum biomarkers;21 hence, the basis for the UL for folic acid, which is based on hematological assessment of vitamin B12 deficiency, is less meaningful today relative to when it was established nearly 20 years ago.

According to the National Academy of Sciences Food and Nutrition Board, the tolerable upper intake level of folic acid in women 19 years and older is 1 mg/d (1,000 µg/d) from supplements or fortified food (excluding naturally occurring folate) and 0.8 mg/d (800 µg/d) for those aged 14 to 18 years.10 Fewer than 3% of girls and women aged 14 to 50 years receive more than 1 mg/d (1,000 µg/d) of folic acid from supplements or food.3,11,12

https://www.aafp.org/pubs/afp/collections/departments.u-s--preventive-services-task-force.html
The term, tolerable upper intake level (UL), is defined by the Institute of Medicine (IOM) as “the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population” [15]. The USDA defines the UL as “the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population”

In 1998, the IOM set the UL at 1,000 micrograms per day (mcg/day) of folic acid (coming from foods fortified with folic acid and from vitamin supplements).

Although the UL is used more broadly, it is only intended to prevent people who don’t know that they have a deficiency of vitamin B12 from consuming too much folic acid.

When taking supplements, more is not better. individuals who can get pregnant (whether planning to or not) need just 400 mcg/day of folic acid, and they can get this amount from vitamins or fortified foods. This is in addition to eating foods rich in folate.

People with a severe deficiency of another B vitamin – vitamin B12 – can develop a type of anemia called pernicious anemia, and eventually they might have nervous system damage. If people with vitamin B12 deficiency receive high doses of folic acid, the folic acid supplementation can correct the anemia; however, the nervous system damage can still occur because these people are still deficient in vitamin B12 [15]. This effect was seen among some patients who were given very high dosages [more than 5,000 micrograms per day (mcg/day)] of folic acid for many months [15]. Based on these data, the Institute of Medicine specified a dose of 5,000 mcg/day of folic acid as the Lowest Observed Adverse Effect Level (LOAEL). The tolerable upper intake level (UL) for folic acid was established at 1,000 mcg/day (one-fifth of the LOAEL) to prevent individuals from taking so much folic acid that they reach the LOAEL risk level of 5,000 mcg/day [15].

The Institute of Medicine has not established a separate tolerable upper intake level (UL) for individuals of reproductive age. It states that, in general, vitamin B12 deficiency among U.S. individuals of reproductive age is rare, and they are unlikely to have any adverse effects from consuming supplemental folic acid at or above the UL [15].

Theoretically, supplemental folic acid could mask the symptoms of pernicious anemia and, thus, delay treatment. However, pernicious anemia is an uncommon disorder in young individuals and there is no evidence that supports this or other concerns regarding potential folic acid toxicity.

Fortification isn't perfect. If someone is eating a lot of processed foods, and also taking a multivitamin, they could exceed the recommended upper level of 1,000 micrograms per day, which was set so there wouldn't be a risk of folic acid masking the symptoms of a severe vitamin B12 deficiency called pernicious anemia. An estimated 33 percent of pregnant people exceed this threshold, possibly because many prenatal vitamins contain 800 to 1,000 micrograms of folic acid.

Who recommends folic acid supplementation in pregnancy for neural tube defect prevention?

- ACOG: The American College of Obstetricians and Gynecologists
- SMFM: The Society of Maternal-Fetal Medicine
- USPSTF: US Preventive Services Task Force
- ACMG: American College of Medical Genetics
- CDC: Centers for Disease Control and Prevention
- FDA: Food and Drug Administration.
- AAFP: American Academy of Family Physicians
- AAP: American Academy of Pediatrics
- AAN: American Academy of Neurology
- SOGC: The Society of Obstetricians and Gynaecologists of Canada
- RCOG: The Royal College of Obstetricians and Gynaecologists
- The Academy of Nutrition and Dietetics (FORMERLY the American Dietetics Association)
- The Food and Nutrition Board (FNB) of the National Academies of Sciences, Engineering, and Medicine, Health, and Medicine Division

What is the recommended amount of folic acid to prevent neural tube defects in the fetus in the low risk pregnancy?

Because folic acid (FA) is the only folate form tested to protect against folate-sensitive neural tube defects (NTDs), there are specific recommendations for FA depending on the relative risks of having a child with NTDs.

As outlined by the Society of Obstetricians and Gynaecologists of Canada, a minimum of 400 µg/d in the form of FA from supplements and/or fortified foods is recommended for individuals in the low-risk category of having a child with NTDs and who are healthy and under good compliance.

CDC and ACOG urges all individuals of reproductive age to consume 400 mcg of folic acid each day, in addition to consuming food with folate from a varied diet, to help prevent neural tube defects.

The National Academy of Medicine's Recommended Dietary Allowance (RDA) of folates for individuals of childbearing age range from 400 to 600µg/d of dietary folate equivalents (DFEs), with the recommended daily allowance (RDA) during pregnancy set at 600 µg/d DFE.44,45

Because it takes more folic acid to help prevent NTDs than to prevent anemia (low number of red blood cells), 400 mcg folic acid is recommended to help prevent NTDs, which is equal to 667 mcg DFE (167% DV).

Recommended Dietary Allowance of folate and folic acid for individuals of childbearing age for the prevention of neural tube defects in the offspring

	Nonpregnant	**Pregnancy**	**Lactation**
Female age (y)a	FA dose in µg of DFEb (ULc)		
14–18	400 (800)	600 (1000)	500 (1000)
>19	400 (1000)		
Risk of NTDd		*FAe dose (µg)*	
Low		400	
Medium		1000–4000	
High		4000	

a
Recommendations by the Institute of Medicine.47

b
1 DFE = 1 mg of food folate = 0.6 mg of FA as food or supplement = 0.5 mg of supplement taken on an empty stomach.
c
Tolerable UL established for FA from supplements and/or fortified foods.
d
Risk category of having a child with an NTD. Risk category and weights differ between jurisdictions.
e
Recommendations by the Society of Obstetricians and Gynecologists of Canada for NTD reduction for individuals in different risk categories.45
Abbreviations: DFE, dietary folate equivalent; FA, folic acid; NTD, neural tube defect; UL, upper level.

Table 1. Folic Acid Supplementation for the Prevention of Neural Tube Defects: Clinical Summary of the USPSTF Recommendation

Population	Women who are planning or capable of pregnancy
Recommendation	Take a daily supplement containing 0.4 to 0.8 mg (400 to 800 µg) of folic acid. Grade: A
Risk assessment	All women of childbearing age are at risk of having a pregnancy affected by neural tube defects. Some factors increase this risk, including a personal or family history of neural tube defects, use of particular antiseizure medications, maternal diabetes, obesity, and mutations in folate-related enzymes.
Preventive medication	Folic acid is the synthetic form of folate, a water-soluble B vitamin. Folic acid is usually given as a multivitamin, prenatal vitamin, or single supplement, and is also used to fortify cereal grain products. Folate occurs naturally in foods such as dark green leafy vegetables, legumes, and oranges. However, most women do not receive the recommended daily intake of folate from diet alone.
Timing	The critical period for supplementation starts at least 1 month before conception and continues through the first 2 to 3 months of pregnancy.
Dosage	Supplementation with a multivitamin containing 0.4 to 0.8 mg (400 to 800 µg) of folic acid decreases the risk of neural tube defects.
Balance of benefits and harms	The USPSTF concludes with high certainty that the net benefit of daily folic acid supplementation to prevent neural tube defects in the developing fetus is substantial for women who are planning or capable of pregnancy.

NOTE: *For a summary of the evidence systematically reviewed in making this recommendation, the full recommendation statement, and supporting documents, go to http://www.uspreventiveservicestaskforce.org/.*

USPSTF = U.S. Preventive Services Task Force.

Resource: Perspective: Time to Resolve Confusion on Folate Amounts, Units, and Forms in Prenatal Supplements

Intake recommendations for folate from the National Academies of Sciences, Engineering, and Medicine, Food and Nutrition Board (FNB), the CDC, and the US Preventive Services Task Force (USPSTF):

The primary functional indicator of adequacy for folate used by the FNB to set the RDA for nonpregnant, pregnant, and lactating women [which ranges from 400 to 600 µg DFE (equivalent to 240–360 µg folic acid)] was the maintenance of RBC folate. In setting the folate RDA, the FNB did not consider NTD risk reduction, as NTD risk reduction during the periconceptional period was viewed as an inappropriate functional indicator for women of childbearing age who were not likely to, or who did not plan to, become pregnant. In contrast, the recommendations by the CDC (400 µg folic acid/d) and USPSTF (400–800 µg supplemental folic acid/d) differ from the RDA values because they focus only on reducing the risk of NTDs in the pre- and periconceptional period. They also include all women capable of becoming pregnant, and specifically state that the source of supplemental folate must be from folic acid.

Folate recommendations from governmental and other organizations1

Organization	Recommendation
National Academies of Science, Engineering, and Medicine's Food and Nutrition Board (FNB) 1998 Report (8)2	RDA, nonpregnant women ≥14 y: 400 µg DFE (equivalent to 240 µg folic acid). Note: The FNB also recommends that "women capable of becoming pregnant consume 400 µg of folate daily from supplements, fortified foods, or both in addition to consuming food folate from a varied diet." RDA, pregnant women 14–50 y: 600 µg DFE (equivalent to 360 µg folic acid) RDA, lactating women 14–50 y: 500 µg DFE (equivalent to 300 µg folic acid)
	Tolerable Upper Intake Level (UL),3 pregnant and lactating women 14–18 y: 800 µg folate from fortified foods or supplements (not stated as folic acid or in µg DFE)3

	UL, pregnant and lactating women 19–50 y: 1000 µg folate from fortified foods or supplements (not stated as folic acid or in µg DFE)3
CDC (2)	“All women of childbearing age in the United States who are capable of becoming pregnant should consume 0.4 mg of folic acid per day for the purpose of reducing their 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 B-12 deficiency, care should be taken to keep total folate consumption at less than 1 mg per day, except under the supervision of a physician. Women who have had a prior NTD-affected pregnancy are at high risk of having a subsequent affected pregnancy. When these women are planning to become pregnant, they should consult their physicians for advice.”
US Preventive Services Task Force (USPSTF) (3)	“The USPSTF recommends that all women who are planning or capable of pregnancy take a daily supplement containing 0.4 to 0.8 mg (400–800 µg) of folic acid.”
FDA	Pre–July 2016 DVs for pregnant and lactating women: 800 µg folic acid (9)
	Post–July 2016 DV for pregnant and lactating women: 600 µg DFE (equivalent to 360 µg folic acid) (6)
	Health claims permissible for folate and the prevention of neural tube defects on supplement labels:

	Health claim: “Women who are capable of becoming pregnant and who consume adequate amounts of folate daily during their childbearing years may reduce their risk of having a pregnancy affected by spina bifida or other neural tube defects.” Prenatal supplements labeled at 800 µg must include the safe upper limit of daily intake value of 1000 µg (1 mg) in the claim statement (10).
	Qualified health claim: “0.8 mg folic acid in a dietary supplement is more effective in reducing the risk of neural tube defects than a lower amount in foods in common form” to be accompanied by the appropriate disclaimer: “FDA does not endorse this claim. Public health authorities recommend that women consume 0.4 mg folic acid daily from fortified foods or dietary supplements or both to reduce the risk of neural tube defects” (11).

1
DFE, dietary folate equivalents; DV, Daily Value; NTD, neural tube defect.
2
Sources: references 2, 3, 6, 8–10, and 11.
3
The UL is assumed to be established only for folic acid and does not include the naturally occurring or other synthetic forms of the vitamin, such as L-methylfolate (from reference 13) but this is not explicitly stated in the official FNB report.

To address NTD risk reduction, the FNB also recommends that nonpregnant women capable of becoming pregnant consume 400 µg folate daily from supplements, fortified foods, or both in addition to consuming food folate from a varied diet (8). Although not specifically stated in the FNB report, it must be assumed that the intended form of folate is folic acid because folic acid was the only synthetic form of folate used in supplements and fortified foods at the time the recommendations were established, and only folic acid was tested in clinical trials for preventing NTDs.

How can I get folic acid?

You can get 400 mcg of folic acid each day by taking a prenatal vitamin or supplement with folic acid in it, eating fortified foods, or a combination of the two, in addition to consuming a balanced diet rich in natural food folate.

Folate can be found in foods like beans, peas, and lentils; oranges and orange juice; asparagus and broccoli; and dark leafy green vegetables such as spinach and mustard greens. However, it can be hard for most individuals to get the recommended daily amount of folate through food alone.[1]

https://doi.org/10.1016/S2214-109X(22)00213-3

-Diet alone is insufficient to provide the recommended daily intake of folic acid, because a considerable amount of food folate is lost during food processing and cooking.3 In addition, food folate has poorer bioavailability than folic acid.4 Folic acid supplement pills are recommended along with a healthy diet. Folic acid is essential for the development of the neural tube in the first 4 weeks of pregnancy, but given that half of all pregnancies worldwide are unplanned,5 most women are unaware of their pregnancies during this critical window and might not take the recommended intake of folic acid.

An easy way to be sure you're getting enough folic acid is to take a daily vitamin with 400 mcg of folic acid in it (most vitamins contain the recommended daily amount of 400 mcg of folic acid individuals need). If taking a vitamin upsets your stomach, try taking it with food or just before bed. If you have trouble taking pills, try a chewable vitamin. Also, be sure to take it with a full glass of water. In the United States, supplements containing folic acid generally have 400 to 800 mcg of folic acid per dose, but doses up to 1,000 mcg are allowed without a prescription [14]

A dietary supplement can provide the full recommended amount of folic acid to a woman of reproductive age to help prevent her baby from having a neural tube defect. However, this approach requires remembering to take the supplement every day. Moreover, while they are relatively inexpensive, supplements can be costly for some individuals.

Because of its high bioavailability and stability, FA is the form of folate used in supplements and to fortify food (Greenberg et al., 2011). Folic acid is added to foods such as enriched breads, pastas, rice, cereals, and corn masa flour in food fortification programs. Corn masa flour is used to make foods such as corn chips, tortillas, tamales, and taco shells. Folic acid is the ideal form of folate to use for food fortification. It is more stable than types of natural food folate, which can easily be broken down by heat and light. Folic acid is better suited for food fortification because many fortified products, such as bread and pasta, are cooked.[6]

In the United States, folic acid can be found in foods with mandatory or voluntary fortification, or in supplements. All products labeled as "enriched" are required by the U.S. Food and Drug Administration to be fortified (mandatory fortification) with folic acid, in addition to other micronutrients. The dietary labels on these products must specify that folic acid is included as an ingredient [12].

Consuming folic acid from fortified foods and vitamin supplements has been proven to prevent neural tube defects, which can be fatal or cause varying degrees of disability. Since fortification started in the United States in 1998, and with the use of folic acid supplements, on average, 1,300 babies are born each year without a neural tube defect who might otherwise have had a neural tube defect. This means that the number of babies born in the United States each year with a neural tube defect has been reduced by about 35% [46].

Despite mandatory fortification, research shows that some U.S. individuals still do not get enough folic acid to prevent neural tube defects.[3]

Hispanic/Latina individuals

- Have lower levels of folate in their blood[6] compared to non-Hispanic white individuals
- Are less likely to know about the benefits of folic acid[8]
- Are less likely to get folic acid from fortified foods or take a multivitamin with folic acid in it,[8-14] particularly those individuals who
    - Primarily speak Spanish;
    - Were born outside of the United States; and
    - Have lived in the United States for a shorter period of time.[14]

https://www.aafp.org/pubs/afp/collections/departments.u-s--preventive-services-task-force.html

-Evidence shows that most women in the United States are not consuming fortified foods in a quantity needed to demonstrate optimal benefit. An analysis of NHANES data found that 48% of respondents of childbearing age consumed the recommended amount of folic acid from mandatorily fortified foods only.

Who needs a higher dose of folic acid?

Some risk factors for NTDs include:

- A previous pregnancy affected by an NTD or an NTD in either parent. A genetic contribution to NTDs also is reflected in the association between family history and increased NTD risk. The increased NTD risk for relatives of an affected individual has been well documented, and parents who have had one child with an NTD are at significantly increased risk of having another child with the same or a similar defect **27**. The risk of having a fetus with an NTD when there is an affected sibling, a second-degree relative, or a third-degree relative is 3.2%, 0.5%, and 0.17%, respectively. With two affected siblings, the risk is 10%
- Family history (first- or second-degree relative) of neural tube defects.
- The use of particular antiseizure medications (eg, valproic acid [an antiepileptic medication used also for treatment of bipolar and personality

disorders, has been associated with a 10-fold to 20-fold increased risk of NTDs] or carbamazepine); other antiseizure medications
    - Pregestational diabetes, other medical conditions associated with decreased FA absorption, and obesity. A meta-analysis of 12 studies published between 1980 and 2007 found a 1.7-fold increased risk of NTDs for obese individuals (11 studies), and a 3.1-fold increased risk for severely obese individuals (defined as a BMI greater than or equal to 38 or more than 243 pounds) (five studies) when compared with interest is the *methylenetetrahydrofolate reductase* (MTHFR) gene, which encodes a cytoplasmic enzyme involved in the conversion of homocysteine to methionine. Specific polymorphisms, C677T, in this gene have been associated with a higher frequency of NTDs in some populations, but not others **26**. Given these inconsistent associations, routine screening for MTHFR status is not recommended.

ACOG: individuals at high risk of NTDs should supplement with a higher dose of folic acid than 400 micrograms **48**. This group includes those with histories of previous pregnancies affected with NTDs, individuals who are affected with an NTD themselves, those who have a partner who is affected, or those with a partner with a previous affected child. Individuals at high risk of NTDs should take 4 mg (4,000 micrograms) of folic acid daily. The daily supplement should be initiated 3 months before pregnancy and continued until 12 weeks of gestational age **48** **53**. Following the recommended supplementation in this high-risk group may reduce risk by as much as 70% **58**. If you have risk factors for NTD (see above), consume 400 mcg/0.4 mg of FA supplementation each day, even when not planning to become pregnant.

Antiepileptic medication use during pregnancy, particularly valproate, also has been associated with folate-resistant NTDs. For these patients, the benefit of high-dose folic acid therapy has not been definitively proved **61**, and recent guidelines for individuals on antiepileptic medications do not recommend higher doses of prepregnancy folate supplementation. Similarly, prepregnancy folic acid intake in obese individuals may not decrease the risk of NTDs **60**.

CDC:
If you are planning to become pregnant, take 1000-4000mcg /1-4mg of FA supplementation each day, starting 1-3 months before becoming pregnant and continuing through the first 3 months of pregnancy, depending on the risk factor.
    - The dose can be reduced to 400 mcg/0.4 mg after the first trimester since the reason for therapy (prevention of NTDs) is no longer relevant.

SOGC:
A higher dose (1000 mcg/1 mg per day) of periconceptional/first-trimester folic acid supplementation should be considered for those with a history of one of the following malformations in themselves or their partner, a prior offspring, or a first- or second-degree relative:

- Cleft lip/palate, Congenital heart defects, Limb reduction defects, Urinary tract defects
- The benefit of this approach is based on low-quality data, but the birth defects are serious and the potential for harm from this dose of FA is low
    - The SOGC also recommends 1000 mcg/1 mg per day for these persons

Currently, high-dose FA supplements are prescribed to all at-risk persons, without assessment of their folate status.

- Studies that have investigated associations of NTD risk with RBC folate concentrations consistently show a substantially lower risk among those with concentrations of 906 nmol/L or greater
- It has been proposed that clinicians measure RBC folate concentrations as part of preconceptional care and prescribe the necessary level of FA supplementation according to a person's individual risk
- However, for the majority of pregnancies which are unplanned given the adequate levels of FA documented in the vast majority, the same 400–800 mcg/0.4-0.8 mg daily dose would seem sufficient even for recurrent risk cases.

Indication for supplementation	Dose (daily)	Start (minimum)	Duration*	Recommended by
High risk				
Prior open NTD in offspring of either parent or personal history of open NTD in either parent[1,2]	4 mg	3 months PTC	12 weeks	SOGC, ACOG
Moderate risk				
Personal or family history of folate-sensitive congenital anomaly other than NTD[2]	1 mg	3 months PTC	12 weeks	SOGCa as was
Family history of NTD (first- or second-degree relative)[2]	1 mg	3 months PTC	12 weeks	SOGC
Type I or II diabetes[2,4,5]	1 mg	3 months PTC	12 weeks	SOGC

	0.4 mg	1 month PTC	12 weeks	ADA, ACOG
Maternal gastrointestinal malabsorption[2]	1 mg	3 months PTC	12 weeks	SOGC
Medical conditions associated with risk (advanced liver disease, dialysis, alcohol overuse)[2]	1 mg	3 months PTC	12 weeks	SOGC
Low risk				
Pregnancy or potential for pregnancy[1,3,6]	0.4 mg	At least 1 month PTC	12 weeks	ACOG, CDC
	0.4 to 0.8 mg	1 month PTC	First 2 to 3 months of pregnancy	USPSTF

https://doi.org/10.1016/j.pmedr.2021.10161

-Maternal seizure disorder: There is a strong association (drug and dosage) ofanti-epileptic drugs (AEDs) with increased congenital anomalies (prevalence 2.5%) including neural tube defects. There has been no impact on the congenital anomaly prevalence with 'high dose' FA supplementation in epileptic pregnancy care as the AEDs teratogenic mechanism may have no FA component or association. High dose FA should no longer be recommended for congenital anomaly reduction for pregnant women with epilepsy.

-Benefit from FA supplementation use in epileptic pregnancy cohorts has been associated with neonatal neurodevelopmental benefits. A population-based biobank study (Norway) and the NEAD study (USA) have shown a decreased risk of autistic traits in children, exposed 'in utero' to AEDs, following periconceptional FA supplementation. Periconceptual FA supplementation in women with epilepsy is associated with better cognitive development in neonatal – childhood up to age 6. The critical period for FA supplementation exposure is during the first trimester as plasma folate levels later in the pregnancy were not associated with better cognitive outcomes although they were inversely associated with autistic traits. It is recommended that fertile epileptic women using AEDs should take FA supplements continuously with periconceptual FA supplementation, using a dose of at least 400 µg daily.

What if I have a baby with a neural tube defect?

When planning to become pregnant, individuals who have already had a pregnancy affected by a neural tube defect should consult with their healthcare provider. CDC recommends that these individuals consume **4,000 mcg** of folic acid each day one month before becoming pregnant and through the first 3 months of pregnancy. The recommendation to take a higher dose of folic acid is based on data from the most rigorous scientific study involving individuals who had previous NTD-affected pregnancies. Dosages lower than 4,000 mcg have not been studied in individuals who have had a previous NTD-affected pregnancy.

Can I take another form of folate to prevent neural tube defects?

https://www.annualreviews.org/content/journals/10.1146/annurev-nutr-043020-091647

Folate occurs in various forms, known as vitamers. All folate vitamers consist of a common biochemical structure but differ in the number of glutamic acids, oxidation state, and presence of one-carbon groups (e.g., FA, 5-MTHF, THF, 5,10-methylene-THF, dihydrofolate). Although most FA is converted to 5-MTHF during intestinal absorption and first-pass metabolism in the liver, concerns about low levels of circulating unmetabolized FA have led to discussions about the potential advantages of using 5-MTHF instead of FA for food fortification and periconceptional supplementation for reducing NTD risk. This concept is problematic for several reasons. No randomized controlled trials (RCTs), or even less-rigorous studies, have examined any other folate form for the prevention of NTDs. Arguments in favor of 5-MTHF supplements in the form of L-MTHF or (6S)-5-methyltetrahydro-FA claim that 5-MTHF is more bioavailable and more "natural" than FA and not as affected by polymorphisms in the *MTHFR* gene (**47**, **144**). Studies have found that supplementation with L-MTHF or (6S)-5-methyltetrahydro-FA increases red blood cell (RBC) and serum folate concentrations (**6**, **68**, **85**). However, RCTs would have to be conducted to determine effectiveness, timing, dosage, stability, and safety in order for 5-MTHF (or a synthetic equivalent) to be recommended. Given its position in the folate metabolism pathway, it is unlikely that 5-MTHF in the presence of lower concentrations of vB12 would be as effective as FA. Studies have found that to reach ideal RBC folate concentrations, much higher serum/plasma folate concentrations (25.6 versus 34.6 nmol) are necessary in the presence of vB12 deficiency (**22**). Lastly, despite years of study, the unmetabolized FA that is in circulation has had no confirmed adverse effects, thus it is not necessary to replace a known effective intervention with one that does not have established effectiveness.

Supplements containing forms of folate other than folic acid (such as methylfolate or 5-MTHF) should not be confused with the natural food folate found in fruits and vegetables. The effectiveness of these supplements in preventing neural tube defects has not been studied. The other types of folate found in some vitamins or supplements (such as 5-MTHF) are different from the folate found in fruits and vegetables, even if the nutrition label claims it is “natural food folate.” These types of folate, just like folic acid, are not made from food but are man-made.

L-methylfolate is more difficult, and therefore more costly, to make into a supplement and is less stable than folic acid. And because it is less stable, it can be hard to know exactly how much someone is getting if it's been sitting on the shelf, since over-the-counter supplements are largely unregulated by the FDA and are exempt from requirements demonstrating that their ingredients are made carefully — or that they even work. Folic acid is more stable than natural folate and if added to food is not destroyed in cooking or heating as is the case with natural folate.

UKTIS: USE OF METHYLFOLATE IN PREGNANCY, Date of issue: January 2023, Version: 2.0

SUMMARY: Methylfolate (L-methylfolate) is the biologically active form of folate which, unlike folic acid, does not require enzymatic reduction by methylenetetrahydrofolate reductase (MTHFR). It is not a licensed medication and there is limited evidence available relating to efficacy or safety.

UKTIS has received a number of enquiries about whether routine use of methylfolate offers a superior alternative to use of folic acid in pregnancy, on the basis that conversion in the body to the biologically active form is not required. However, to date, no studies have addressed rates of neural tube and other birth defects in infants born to women taking methylfolate in pregnancy, while the population-wide data on the beneficial effects of folic acid are unequivocal.

Routine use of prenatal methylfolate in preference to folic acid cannot currently be recommended. In the UK, current guidelines recommend routine use of 400mcg/day of folic acid preconceptually and until at least 12 weeks of gestation. In women considered to be at increased risk of folate deficiency (family history of neural tube defect, concurrent treatment with drugs which interfere with folate metabolism, or maternal obesity), higher dose folic acid (5mg/day) is recommended. Methylfolate supplementation may, however, be advised in individual cases e.g., women with specific metabolic disorders, where benefit of use is likely to outweigh any theoretical or known risks to mother or fetus.

Online Misinformation Fuels a Fight Over Folic Acid

Well-made L-methylfolate may be adequate for preventing neural tube defects, Shane says, though he doesn't recommend it. But folic acid is unlikely to be unseated as the recommended form of folate, because experts point out that it would be unethical to test a different form of folate through a randomized trial — considered the gold standard for

medical research — which would require that some participants not get any folic acid and could put babies at risk. And scientists caution that neural tube closure is still something of a mystery.

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-Recognition of possible unintended and adverse effects of supplemental FA has resulted in the encouraged use of alternative folate forms. Several supplements consumed by individuals now contain the salts of the biologically active form, 5-MTHF, because it is thought to be a safer alternative to FA. Although various formulations exist, the stabilized crystalline form of the calcium salt of 5-MTHF, patented as Metafolin by Merck Eprova, has gained attention, with >20% of nonprescription supplements now containing this form[12] and its incorporation into baby formula being discussed.[65]
-MTHF is generally recognized as safe in the United States, is registered as a new dietary ingredient in the Natural Health Product Ingredient Database in Canada, and is permitted as a form of folate for food fortification in Austria and New Zealand.[65] Notably, MTHF does not have a UL because it is not predicted to mask clinical symptoms of vitamin B_{12} deficiency. However, it also does not have an established conversion factor to express micrograms of MTHF in DFEs. Manufacturers are permitted to use their own conversion factor provided it does not surpass the 1.7-fold bioavailability of FA.
-Despite MTHF being marketed as a preferred alternative to FA, it also contributes to prenatal supplements meeting or exceeding the UL. When accounting for all supplemental folate forms, >90% of prescription and 7% of nonprescription supplements were identified as exceeding the UL.[12] Although no adverse effects of MTHF have been reported to date, it is noteworthy that no clinical trial has completed investigation of its effects when consumed during pregnancy.[66] Better understanding of the potential differences in metabolism and use of FA compared with other folate forms during pregnancy is required to inform public health policies.

Can my baby still have a neural tube defect even if I take folic acid?

ACOG:
Folate-resistant NTDs include those associated with poor glucose control in the first trimester, hyperthermia, maternal obesity, and aneuploidy or genetic disorders. Although folic acid supplementation in diabetic patients may decrease the risk of NTDs, the risk is not eliminated, which emphasizes the importance of prepregnancy glycemic control **59**.

At least 30% of NTDs are not prevented by folic acid supplementation **51**, which underscores the multifactorial etiology of NTDs. Current areas of research, many involving the use of murine models, are exploring other pathways or mechanisms, including neuronal migration pathways, cell signaling, mitochondrial folate metabolism, and inositol pathways **52**. Although consuming 400 mcg of folic acid each day can prevent the majority of neural tube defects, some neural tube defects have other causes

unrelated to folic acid intake (for example, chromosomal abnormalities or medical conditions, such as diabetes).

Some causes of neural tube defects are not known.

Should FA be continued after the first trimester of pregnancy?

https://pmc.ncbi.nlm.nih.gov/articles/PMC11574634/pdf/MCN-20-e13668.pdf

The common practice of continuing FA supplementation beyond the first trimester, especially in countries with staple foods FA fortification, is concerning due to increasing reports suggesting excessive FA intakes in late pregnancy may be associated with adverse maternal and child health outcomes, including an increased risk of gestational diabetes (Karaçil Ermumcu & Acar Tek, 2023; Kintaka et al., 2020; Li et al., 2019), allergic disease (McGowan et al., 2020; Ogawa et al., 2018; Roy et al., 2018), and obesity and metabolic dysfunction in the child later on (Yajnik et al., 2008). Although findings from observational studies have been inconsistent, evidence from randomized controlled trials (RCTs) is lacking. Thesuggestion of risk necessitates further exploration of excessive FA intake beyond the first trimester.

Is folic acid safe to take in pregnancy?

In 2017, the USPSTF reviewed the effectiveness of FA supplementation in persons of childbearing age for the prevention of NTDs and assessed new evidence on the benefits and harms of FA supplementation

- The USPSTF found no new substantial evidence on the benefits and harms of FA supplementation that would lead to a change in its recommendation from 2009
- The USPSTF reaffirmed its 2009 recommendation that all persons who are planning or capable of pregnancy take a daily supplement containing 400-800 mcg/0.4 to 0.8 mg of FA

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There is strong evidence of the safety of providing folic acid through the fortification of staple foods. Contrary to concerns raised in the 1970s, folic acid does not mask anaemia caused by vitamin B12 deficiency.9 Evidence of potential adverse effects of circulating unmetabolised folic acid from supplemental folic acid is inconclusive, with no clear evidence pointing to adverse metabolic and clinical effects of high intake. Similarly, claims of potential risks related to cancer, cognitive impairment, diabetes-related disorders, thyroid disease, and hypersensitivity-related outcomes have not been

validated. By contrast, food fortification with folic acid is highly effective in safely preventing spina bifida and anencephaly, as proven by multiple studies from different countries.

Online Misinformation Fuels a Fight Over Folic Acid
When fortification was introduced, concerns were raised about possible side effects in the general population: Could doses above the recommended levels of folic acid promote autism or cancer? These worries proved to be unfounded, according to Field, who co-authored a research review on the safety of folic acid, summarizing evidence from studies that investigated these issues. Interestingly, she says some studies show that the risk of autism, and some cancers, may even be reduced by taking the recommended amount of folic acid.

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-There is a large body of literature demonstrating the efficacy of maternal folic acid intake in preventing birth defects, as well as investigations into potential adverse consequences of consuming folic acid above the UL. Recently, two separate authoritative bodies convened expert panels to assess the risks from high intakes of folic acid. In May 2015, the U.S. National Toxicology Program (NTP) and the Office of Dietary Supplements convened an expert panel and performed systematic reviews of existing literature regarding the safe use of high folic acid intakes and was charged with developing future research priorities.45 The NTP panel was tasked with (1) identifying the areas of consistency and uncertainty in current science, (2) identifying research needs given the state of the science, and (3) proposing approaches to address knowledge gaps.45 This group was divided into several subpanels, with each subpanel addressing an area of concern, including cancer, cognition (especially as related to interactions with vitamin B12 deficiency), hypersensitivity-related outcomes, and thyroid- and diabetes-related disorders. These areas of concern were based on previous studies reporting adverse effects in these health effect categories with intakes of greater than 400 μg/day folic acid, serum folates greater than 10 nM/L, or red blood cell folate greater than 340 nM/L.46 The overall conclusion of the NTP report was that, for the outcomes considered, there was no conclusive evidence for adverse effects because of folic acid, but, in each case, a research agenda was proposed to address current knowledge gaps.

Does folic acid help prevent any other congenital (birth) defects?

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In addition to NTDs, there might be an association between FA fortification and reduction of orofacial clefts (OFCs). A meta-analysis of studies comparing the prevalence of OFCs before and after mandatory FA fortification in multiple countries,

including Argentina, Brazil, Chile, and the USA, demonstrated that the prevalence of nonsyndromic cleft lip and palate significantly decreased (RR, 0.88; 95% CI, 0.81, 0.96) following fortification, but there were no significant effects of fortification on the prevalence of total OFCs or cleft palate only (105). These results are similar to those from another meta-analysis, which demonstrated a nonstatistically significant decrease in both cleft lip with or without palate (RR, 0.87; 95% CI, 0.76, 1.00) and cleft palate only (RR, 0.90; 95% CI, 0.71, 1.15) (184).

Certain types of congenital heart defects (CHDs) might also be prevented through FA fortification. A Canadian time-trend analysis that compared the prevalence of severe CHDs prefortification (1990–1998) with the prevalence postfortification (1998–2005) demonstrated a decrease from 1.64 cases (95% CI, 1.55, 1.73) per 1,000 births prefortification to 1.47 (95% CI, 1.37, 1.58) per 1,000 live births postfortification, or a 6% decrease per year in the 7 years following the initiation of mandatory FA fortification (74). Another Canadian population-based study of all live births and stillbirths from 1990 to 2011 demonstrated lower rates of conotruncal defects (RR, 0.73; 95% CI, 0.62, 0.85), coarctation of the aorta (RR, 0.77; 95% CI, 0.61, 0.96), ventricular septal defects (RR, 0.85; 95% CI, 0.75, 0.96), and atrial septal defects (RR, 0.82; 95% CI, 0.69, 0.95) following FA fortification (93). Although FA fortification was associated with an overall 11% decrease in the prevalence of CHDs, it was not associated with a significant reduction in severe nonconotruncal heart defects, suggesting a differential effect across subtypes of CHDs (93).

https://doi.org/10.1186/s12937-022-00772-2
We have conducted a meta-analysis of the recent 21 studies concerning maternal FA supplementation and CHD. Although the data from our analysis implicate that maternal FA supplementation is associated with the reduced risk of CHD, the heterogeneity of this association is high.

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Birth defects related to folate deficiency account for 2%–3% of prenatal or neonatal major anomalies and 4%–5% of total structural malformations or developmental conditions identified after birth. Folate-sensitive birth defects include neural tube defects, certain congenital heart and urinary tract defects, oral facial clefts, and limb-reduction anomalies (high).

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There is good evidence that folate supplementation may have a protective effect against severe types of CHD while the impact on CHD prevalence, could be greater than for NTD.

Unmetabolized folic acid

https://www.sciencedirect.com/science/article/pii/S0022316623005369

Observational studies conducted in countries with either mandatory or voluntary FA food fortification have found detectable amounts of unmetabolized FA in the circulation in considerable proportions of older adults (5, 7, 8, 9), younger adults (9, 10, 11, 12), pregnant women (11, 12, 13, 14), lactating women (12, 15), children (9), and newborns (11, 13, 14, 15, 16). Although it remains to be proven whether there are adverse effects associated with unmetabolized FA in the circulation, it may be of particular interest to further consider pregnancy in this context as a vulnerable time of the life cycle. Moreover, the usage of FA supplements during pregnancy is widespread because FA is recommended worldwide from preconception until the end of the first trimester for protection against neural tube defects, and in late pregnancy, it is prescribed for the treatment and prevention of folate deficiency anemia. Obeid et al. (13) investigated various folate forms in maternal and corresponding umbilical cord samples at birth in relation to reported FA usage during pregnancy, and although unmetabolized FA was detected in 50% of cord blood samples, the concentrations were 5 times lower than in maternal blood, whereas the natural folate forms showed a reverse pattern with higher concentrations in cord relative to maternal blood. However, the interpretation of these results is limited by the fact that the study was not a randomized trial of FA supplementation but rather an observation relying on self-reported FA supplement usage. Furthermore, the contribution of FA from fortified foods in the diet was not taken into consideration.

Our results showed that continuing FA supplementation at a dose of 400 μg/d throughout the second and third trimesters led to an increased maternal and neonatal folate status but did not cause higher concentrations of unmetabolized circulating FA. Although a significantly higher proportion of FA-supplemented women compared with placebo had detectable FA in plasma (42% compared with 16%), this did not correspond to a significant increase in concentrations of unmetabolized FA in the maternal circulation. An increase of total folate and 5-MTHF in the cord blood occurred in response to FA supplementation during pregnancy, but unmetabolized FA was quantifiable only in a negligible number of samples and represented only a small proportion (i.e., <10%) of that in the maternal compartment irrespective of treatment group.

It is unknown whether exposure to higher FA intake or related unmetabolized FA in plasma causes any adverse health effects in the fetus. In any case, our analysis failed to detect significant differences between neonates from the FA and placebo groups, either in unmetabolized FA concentrations or in general neonatal characteristics such as birth weight, head circumference, or Apgar score. Mechanistic studies showed that FA has a very high affinity for folate binding protein in the placenta (29); thus, theoretically, exposure to high FA may interfere with the transport of reduced folates across the placenta. Although no such effects arising from 400 μg supplemental FA/d are evident in the current study, the effects of higher concentrations of unmetabolized FA on metabolic pathways in the fetus are unknown.

In conclusion, a supplemental dose of 400 µg FA/d throughout pregnancy, over and above FA intakes through fortified foods, appears to have no significant effect on unmetabolized FA concentrations in maternal or cord blood despite improving folate status of mothers and neonates. In the event that adverse effects of unmetabolized FA are ever proven, this trial indicates that the exposure of pregnant women to 400 µg FA/d will have little impact.

https://pmc.ncbi.nlm.nih.gov/articles/PMC11574634/pdf/MCN-20-e13668.pdf

When consumed, FA is reduced and methylated to 5-methyltetrahydrofolate (5-MTHF) in the enterocyte or liver. At higher intakes, the enzymes required to convert FA to 5-MTHF are saturated, and the excess FA circulates in its unmetabolized form (UMFA) (Kelly et al., 1997). UMFA has been proposed as a potential biomarker of excessive FA intake (Kelly et al., 1997). Concerns have been raised over whether high concentrations of circulating UMFA may adversely affect the developing fetus (Smith et al., 2008). In acute dosing studies in nonpregnant individuals, UMFA rises rapidly after FA ingestion and falls over the following hours (Kelly et al., 1997; Sweeney et al., 2007). The greater the dose of FA, the higher the UMFA concentration and the longer it is detected in serum. The effect of chronic excessive intake of FA on UMFA concentrations is less clear.

Unmetabolized FA has been detected in maternal blood samples in several population studies (Best et al., 2020; Obeid et al., 2010; Plumptre et al., 2015; West et al., 2012) and one RCT in a country without mandatory fortification (Pentieva et al., 2016). However, there are no published RCTs investigating the effect of prolonged intake of commonly used higher-dose prenatal FA-containing supplements combined with background intakes from mandatory fortification of staple foods on UMFA concentration.

We investigated the effect of removing FA from prenatal supplements after 12 weeks gestation on maternal UMFA concentrations in late pregnancy in a country with mandatory FA fortification of staple foods. UMFA concentrations were higher in those women randomized to the 800 µg FA/day supplement group compared to women in the 0 µg FA/day supplement group. UMFA concentrations were below the level of detection in only a quarter of women (11/44) in the 800 µg FA/day supplement group compared to half (23/46) of women in the 0 µg FA/day supplement group. Our results are dissimilar to the findings of the only other published RCT investigating the effect of prenatal FA supplementation on maternal UMFA concentration. Pentieva et al. (2016) reported that women randomized to FA supplements were more likely to have detectable plasma UMFA at 36 weeks gestation than women randomized to placebo (42% vs. 16%) but found no significant difference in the mean ± SD concentration of UMFA between groups (0.13 ± 0.49 vs. 0.44 ± 0.80, interaction p-value = 0.38) (Pentieva et al., 2016).

Our mean UMFA concentration was similar in the 0 μg/day FA-supplemented group in the Pentieva et al. study but higher in those receiving 800 μg/day FA supplementation. The dose of FA used by Pentieva et al. (2016) was lower than that found in common prenatal multivitamin and mineral supplements in Australia and many other countries, which range from 500 μg to 1000 μg/day (Parr et al., 2017; Plumptre et al., 2015). Furthermore, the Pentieva et al. study was conducted in Northern Ireland, which only had voluntary (rather than mandatory) FA fortification of food (Pentieva et al., 2016). The prevalence of detectable UMFA in our trial participant population (62%) is lower than observational studies in pregnant women in Australia (93%, >0.03 to 244.7 nmol/L) (Best et al., 2020), USA (81%, 0.23–1.47 nmol/L) (West et al., 2012) and Canada (97%, 0.00–0.91 nmol/L) (Plumptre et al., 2015). However, UMFA concentrations differ substantially between studies and appear to be influenced by recent FA intakes (including ingestion of an FA containing supplement), which may explain the variability. Pfeiffer et al. reported detectable levels of UMFA in nearly all National Health and Nutrition Examination Survey (NHANES) participants (>95%, range >0.3–397 nmol/L) (Pfeiffer et al., 2015). NHANES is a representative sample of the US population, including men, women, and children. Although 38% of NHANES survey participants were fasting >8 h, Pfeiffer et al. reported that the detection of UMFA was evident regardless of fasting status, yet concentrations differed significantly by length of fasting (Pfeiffer et al., 2015).

In conclusion, our trial showed that removing FA from prenatal multivitamin and mineral supplements reduced the serum UMFA concentration at 36 weeks gestation; however, UMFA concentrations were low in both groups. UMFA, even when measured under standardized conditions, has a high within-subject variation. Moreover, there is no cutoff concentration based on clinical outcomes for UMFA, above which there is increased risk of poor maternal and child outcomes (Gibson, 2023). Thus, UMFA may not be the best biomarker for chronic excessive FA ingestion. Our findings do not prove that excessive maternal FA supplementation or UMFA does not cause harm. There is no question that FA supplementation is essential before and in early pregnancy, but investigating excess intake, especially in countries with mandatory fortification, is warranted. High-quality randomized controlled trials powered with clinical endpoints are needed to resolve concerns regarding the potential adverse effects of excessive FA intakes in late pregnancy on maternal and child health.

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Some vitamins (such as vitamin D and vitamin A) can collect in fat tissues in the body, so they can be toxic if someone consumes too much. Folic acid does not collect in fat, but instead dissolves in water.

-Unmetabolized folic acid is any amount of folic acid that is found in the blood because it has not been converted into other forms of folate or removed from the body through urination.

Folic acid is absorbed by the intestines into the bloodstream, and then converted to other forms of folate by the liver. The liver is capable of processing only a certain amount of folic acid at one time. Any amount of folic acid that is not used by the body (also called “unmetabolized folic acid”) goes through the kidneys, into the urine, and out of the body [27].
-Studies dating back to the late 1990s have shown that people taking a single dose of folic acid of more than 200 micrograms (mcg) can have some unmetabolized folic acid circulating in their blood [28, 29]. Research also has shown that individuals consuming folic acid from fortified foods, ready-to-eat cereals, or vitamin supplements, or any combination thereof, have varying amounts of unmetabolized folic acid in their blood [28, 30-32]. Since the beginning of mandatory folic acid fortification, most people have had some unmetabolized folic acid circulating in their blood [32].

Although some people have been concerned about unmetabolized folic acid in the blood, no confirmed health risks have been found [20, 33, 34]. A recent review found no evidence of harmful effects of unmetabolized folic acid in the blood of infants [35].

When FA supplementation is excessive, unmetabolized FA can accumulate in the serum. The exact dose at which this happens is not known and may differ between individuals. Studies in both nonpregnant and pregnant persons show that FA doses greater than ~800–1,000 mcg/day result in detectable levels of unmetabolized FA in both maternal and fetal blood samples. The risks of higher levels of FA supplementation are believed to be minimal because unused FA in the blood goes to the kidneys and leaves the body in urine.

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Folate occurs in various forms, known as vitamers. All folate vitamers consist of a common biochemical structure but differ in the number of glutamic acids, oxidation state, and presence of one-carbon groups (e.g., FA, 5-MTHF, THF, 5,10-methylene-THF, dihydrofolate). Although most FA is converted to 5-MTHF during intestinal absorption and first-pass metabolism in the liver, concerns about low levels of circulating unmetabolized FA have led to discussions about the potential advantages of using 5-MTHF instead of FA for food fortification and periconceptional supplementation for reducing NTD risk. This concept is problematic for several reasons. No randomized controlled trials (RCTs), or even less-rigorous studies, have examined any other folate form for the prevention of NTDs. Arguments in favor of 5-MTHF supplements in the form of L-MTHF or (6S)-5-methyltetrahydro-FA claim that 5-MTHF is more bioavailable and more “natural” than FA and not as affected by polymorphisms in the *MTHFR* gene (**47**, **144**). Studies have found that supplementation with L-MTHF or (6S)-5-methyltetrahydro-FA increases red blood cell (RBC) and serum folate concentrations (**6**, **68**, **85**). However, RCTs would have to be conducted to determine effectiveness, timing, dosage, stability, and safety in order for 5-MTHF (or a synthetic equivalent) to be recommended. Given its position in the folate metabolism pathway, it is unlikely that 5-MTHF in the presence of lower concentrations of vB12 would be as

effective as FA. Studies have found that to reach ideal RBC folate concentrations, much higher serum/plasma folate concentrations (25.6 versus 34.6 nmol) are necessary in the presence of vB12 deficiency (**22**). Lastly, despite years of study, the unmetabolized FA that is in circulation has had no confirmed adverse effects, thus it is not necessary to replace a known effective intervention with one that does not have established effectiveness.

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-Concern has been raised over unmetabolized FA in the maternal circulation, due to perinatal folate fortification and supplementation. Various folate forms have been investigated in maternal and corresponding neonatal umbilical cord samples based on maternal reported perinatal FA intake with no dietary data. While unmetabolized FA identified in umbilical cord samples (50%), the concentration was 5X lower than the maternal blood while the natural folate forms showed a reverse pattern with higher cord concentrations than maternal blood samples (Obeid et al., 2010).

-A secondary analysis of stored blood, from the 2006–2007 RCT Folic Acid Supplementation in the Second and Third Trimesters (FASSTT) pregnancy cohort (McNulty et al., 2013, Pentieva et al., 2016) (RCT: all women in the first trimester were given 400 μg FA per day and then they were randomized in the second and third trimester to continuing the 400 μg FA per day or a placebo) measured unmetabolized folic acid in maternal and cord blood. Plasma concentration of unmetabolized FA from supplementation and fortified FA food intake, was low or undetectable in mothers and newborns (Pentieva et al., 2016).

-From a prospective study, the maternal and cord blood concentrations of folate and UMFA was determined in a cohort of pregnant women and their newborns examining the effect of maternal intake of FA and fetal genetic variants in folate metabolism on folate status. During early pregnancy, maternal plasma UMFA was detectable (≥0.2 nmol/L) in 97% of women (range: undetectable to 244 nmol/L). Plasma UMFA was detectable in 93% of cord blood samples (range: undetectable to 15 nmol/L). Cord plasma UMFA concentrations were 72% lower than maternal plasma UMFA concentrations during early pregnancy ($P < 0.0001$). The proportion of plasma UMFA that made up total serum folate was greater for maternal blood than for cord blood ($P < 0.0001$). Consistent with a previous study (Obeid et al., 2010), the lower concentration and percentage of plasma UMFA that contributed to total cord blood folate and a weak or no correlation between plasma UMFA and serum and RBC folate in cord blood, suggested that UMFA does not accumulate in the fetus even with a high folate status and detectable UMFA in mothers. Unlike adults, the fetus has limited folate storage in the liver and must use folic acid immediately available via the placenta. Therefore, the UMFA that reaches the fetus is likely metabolized to active folate forms in a more-efficient manner (Plumptre et al., 2015).

-A SR for adverse maternal health outcomes associated with high serum or red blood cell folate concentrations, demonstrated no consistent relationship between increasing folate concentrations and any of the adverse health outcomes examined (Colapinto et al., 2016).

-An evaluation of micronutrients, on placental function, found low maternal micronutrient status (vitamin D and A and B12, iron, folate) was associated with a range of pregnancy

pathologies involving placental dysfunction (fetal growth restriction (FGR), small for gestational age (SGA), pre-eclampsia (PE), preterm birth (PTB)). The beneficial effects of micronutrients on fetal/neonatal outcomes indicates a reduction of low birth weight (LBW) (RR 0.88; 0.85–0.91) and SGA (RR 0.92, 0.86–0.98). (Baker et al., 2018).

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-Several studies have also found that plasma levels of unmetabolized FA (UMFA) have been detected in nearly every serum, umbilical cord blood, and breast milk sample collected from pregnant and/or postpartum individuals.[57,59–62] UMFA may account for up to 60% of total serum folates >78.5 nmol/L[61] and up to 50% of total milk folates in individuals taking supplements.[62] UMFA is also widely detected in nonsupplement users,[60] which suggests FA intake from fortified foods alone may exceed the body's capacity to metabolize FA into its bioactive form.
-Although the biological consequences of high FA intake and UMFA levels are largely unknown, concerns of potential adverse effects on fetal and later-life health have been raised.[10,11,45] These concerns include increased risk of cancer development and progression, childhood asthma, autism, immune dysfunction, adverse birth outcomes, and metabolic health consequences such as diabetes and obesity. However, research findings supporting adverse effects remain inconclusive and unclear,[10] particularly given the arbitrary definition of *high* and supplement-reporting bias in clinical studies.[10,58] Nevertheless, implementation of the precautionary principle, which acknowledges scientific uncertainty about the unintended negative health consequences of public health interventions, has been suggested,[9,45] and continued research is urged to fill in the uncertainty gaps.

https://www.sciencedirect.com/science/article/pii/S0022316622002036?via%3Dihub

Higher intakes of folic acid can saturate the capacity of DHFR, leading to the presence of unmetabolized folic acid (UMFA) in the circulation (12., 13., 14., 15.). Circulating UMFA has been detected in pregnant women and in cord blood (16., 17., 18., 19.). There is speculation that UMFA in the circulation is a biomarker of excessive folic acid intake and may be causing harm through epigenetic changes to fetal gene expression, with subsequent increased disease risk (20, 21).

Animal models have shown that pregnant mice fed diets high in folic acid exhibit altered expression of immune genes through changes in DNA methylation in the offspring. Such changes have been associated with enhanced severity of allergic airway disease (22). During pregnancy, the human fetus begins to develop and adapt its immune system to maternal diet, lifestyle, and environmental exposures (23., 24., 25.). The measurement of these in utero exposures, especially in the latter stages of pregnancy, of known epigenetic modifiable factors like folic acid is critical.

Several observational cohort studies have reported inconsistent associations between higher prenatal folic acid or folate intakes and risk of allergic disease in the offspring (26, 27., 28., 29., 30., 31., 32., 33., 34., 35., 36., 37., 39.); however, many rely on dietary assessment or recall of supplement use to measure exposure. Of the studies that

examined biomarkers to determine exposure, only one differentiated between specific forms of folate (34). This nested case-control study from the United States, reported that UMFA concentrations in cord blood were associated with an increased risk of food allergy, but not food allergen sensitization; however, other allergic disease outcomes were not reported (34).

This is the first prospective cohort study to examine the association between maternal late pregnancy UMFA status and multiple allergic disease outcomes in a “high risk” infant population. We found no evidence of associations between maternal UMFA or folate concentrations and any infant allergic disease outcomes.

In conclusion, we found no associations between maternal late-gestation serum UMFA or folate concentrations and risk of infant allergic disease at 1 y of age in a population with high hereditary risk of atopy. Further work, including randomized controlled trials with objective biomarkers, is needed to confirm that high folic acid exposure during late pregnancy, largely driven by the combination of food fortification and prenatal folic acid supplement use, does not increase the risk of childhood allergic disease in the general population.

Blood folate concentration

Blood folate concentration is the amount of folate that can be measured in the blood (many forms of folate are included in the measure). When a woman gets folic acid through fortified foods or supplements, her blood folate concentration increases. Having enough folate in the blood can reduce her risk of having a baby affected by a neural tube defect [11].

Once a woman starts consuming 400 micrograms (mcg) of folic acid every day, it can take several months for her to have a blood folate concentration that is high enough to help prevent neural tube defects.

The two most important factors that determine whether a woman has a blood folate concentration that is high enough to help prevent neural tube defects are the amount of folic acid consumed each day and [11] the length of time it is consumed before pregnancy. When taking supplements, more is not necessarily better.

Serum/plasma and red cell folate levels can be monitored, but the utility of this practice is unknown, and serum/plasma levels do not reflect total body saturation levels. No prospective studies have evaluated whether routinely monitoring levels during pregnancy improves outcomes. Some suggest that persons with an identified folate deficiency due to a known comorbidity (such as inflammatory bowel disease or bariatric surgery), rather than low dietary intake, should be monitored with monthly serum assessments to ensure adequate supplementation, but there is insufficient evidence to recommend for or against this practice.

https://doi-org.libux.utmb.edu/10.1093/nutrit/nuac025
-High FA intake is also reflected in folate status,[8,45,56,57] albeit high cutoffs are not well defined.[57] For example, data from the Canadian Health Measures Survey (2007–2009) on median red blood cell (RBC) folate concentration (an indicator of long-term folate status) showed that >40% of the general population exceeds the high cutoff concentration of >1380 nmol/L required for optimal NTD prevention, and folate deficiency (<305 nmol/L) is virtually nonexistent. According to more recent findings from the Folic Acid Clinical Trial, median RBC folate concentrations of pregnant individuals were 2701 nmol/L, with >30% of individuals in this study having RBC folate levels that were higher than the UL of the assay.[58]

- individuals of normal BMI 18.
- Environmental exposure and increased risk of NTDs was documented in individuals living on the Texas–Mexico border who were exposed to the fungal toxin fumonisin
- Maternal hyperthermia, including fever and heat exposure (such as a hot tub or sauna), also has been associated as a risk factor for NTDs. Maternal febrile illness during the first trimester may increase the risk of NTDs by as much as threefold. Similarly, a National Birth Defects Prevention Study demonstrated a 1.7-fold increased risk of anencephaly for individuals with history of hot tub use during early pregnancy
- Several studies have reported that in the U.S. population, the risk of NTDs is highest in the Hispanic population 19.
- Chromosomal abnormalities, including trisomy 13, trisomy 18, and triploidy are associated with NTDs; certain chromosomal deletions and duplications also have been associated with NTDs 22 23. In addition, some genetic syndromes associated with single gene disorders or chromosomal microdeletions, such as 22q11.2 deletion syndrome and Waardenburg syndrome, present an increased risk of NTDs 24
- The relationship between folic acid and NTDs has prompted interest in genes involved in the folate pathway and in the associations between gene alterations and increased risk of NTD.

Folic acid and epigenetic alterations

As FA plays a role in DNA methylation, there is a theoretical concern that maternal FA supplementation could lead to fetal epigenetic changes leading to long-term adverse effects

- One particular area of concern has centered on immune phenotypes that may alter childhood risk of atopic disease and reactive airway disease
- However, epidemiologic studies have reported inconsistent findings on the association of maternal FA exposure or folate levels and increased incidence or severity of childhood respiratory outcomes and atopic disease

Folic acid and neurocognitive development

One review suggested that excessive maternal FA intake may be associated with adverse neurologic effects in offspring unrelated to the neural tube

- Studies on the effect of periconceptional/early pregnancy use of high-dose FA (≥1 mg per day) and neurocognitive development in offspring are conflicting
- Given the uncertainties, only those at highest risk of NTDs should receive >1000 mcg (1 mg) per day of FA

Prenatal folic acid supplementation and autism spectrum disorder in 3-year-old offspring: the Japan environment and children's study:
We evaluated the relationship between prenatal folic acid supplementation and autism spectrum disorder (ASD) in 3-year-old offspring. Maternal use of folic acid supplements from the pre- or post-conception period was not significantly associated with ASD in 3-year-old offspring in Japan. Evaluation of the dietary folate intake from preconception also showed no significant association.

https://doi.org/10.1177/09691413221102321
-Studies have evaluated the fetal exposure to FA and subsequent brain development (DNA methylation; hypomethylation; imprinting; epigenetics) (Lassi et al., 2013, Obeid et al., 2010, McNulty et al., 2013). The Folic Acid Supplementation in the Second and Third Trimester (FASSTT) RCT (2005–2006) evaluated the effect of continuing FA supplementation after the first trimester of pregnancy on maternal and homocysteine responses and related effects of the newborn. The study conclusion was that continuing FA supplementation after the first trimester of pregnancy can prevent the decline in both serum folate and red blood cell folate concentrations and increase in plasma homocysteine concentrations that otherwise occur by the later stages of pregnancy (McNulty et al., 2013).
-The additional follow-up evaluations from the FASSTT RCT cohort have reported on the psychological developmental benefits for children (Caffrey et al., 2018), gene-specific DNA methylation in newborns (McNulty et al., 2019), the cognitive performance in the children (FASSTT Offspring Trial) (Caffrey et al., 2021), and the neurocognitive development in the children, eleven years after the RCT folic acid exposure (Schrott and Murphy, 2018).
-The continued intake of FA in the second and third trimester of pregnancy has identified important folate-mediated epigenetic changes in genes related to brain development and function, with limited evaluations (Caffrey et al., 2019, Irwin et al., 2016, Liu et al., 2020, Liu et al., 2021). The clinical message for continued FA exposure throughout pregnancy may be most important for countries without FA food fortification (Irwin et al., 2016).
-FA supplementation during early pregnancy is associated with a lower risk of offspring's autism spectrum disorders (ASD) (OR 0.57, 95% CI 0.41–0.78). The maternal daily

intake of at least 400ug FA (diet and supplements) was associated with reduced ASD risk in offspring (OR 0.55, 95% CI 0.36–0.83) (Roffman, 2018).
-Preconception management for timing and dosing of FA prior to conception is required (Liu et al., 2021, Roffman, 2018). Pre-conceptional supplements may provide the sufficient folate reserves against both, NTDs and neuropsychiatric risk (Murray et al., 2018).
-While the limited human data is encouraging, the data from animal studies with excess FA intake suggest there are behavioral, morphologic, and molecular changes in the brain of offspring (Molloy and Mills, 2018).

CLAIM: Excessive maternal folic acid intake may be associated with adverse neurologic effects in offspring unrelated to the neural tube.
•Conference abstract at the 2016 Johns Hopkins Bloomberg School of Public Health found that if a new mother has a very high level of folate right after giving birth – more than four times what is considered adequate – the risk that her child will develop an autism spectrum disorder doubles.

- Analyzed data from 1,391 mother-child pairs in the Boston Birth Cohort, a predominantly low-income minority population.
- Very high vitamin B12 levels in new moms are also potentially harmful, tripling the risk that her offspring will develop an autism spectrum disorder.
    - If both levels are extremely high, the risk that a child develops the disorder increases 17.6 times.
- Adequate supplementation is protective, but excessive amounts may also cause harm.
    - Must aim for optimal levels of this important nutrient.
- Researchers say they don't know exactly why some of the women had such high levels in their blood.
    - Consumed too many folic acid-fortified foods or took too many supplements.
    - Some women are genetically predisposed to absorbing greater quantities of folate or metabolizing it slower, leading to the excess.
    - Or it could be a combination of the two.

Here's how the whole thing came about: researchers at Johns Hopkins Bloomberg School of Public Health shared some interesting, yet unpublished and non peer-reviewed research at the International Meeting for Autism Research. It was in the form of a conference abstract, which means their peers had not yet had the chance to vet the full data set, the study design, or other factors. This is a vital step in the academic and scientific community; many findings don't ever make it to publication because they don't pass muster in the review process. That said, even the limited data presented at the conference showed that women who took a multivitamin 3-5/week had a **lower** chance of having a child with autism spectrum disorder. The misleading headlines arose from a secondary finding related not to maternal folic acid supplementation during pregnancy, but to a women's blood levels at or near the time of delivery. This is a crucial distinction: how much folate a woman gets from food or supplements is a very different notion than how much folate is circulating in a woman's blood at any given time. The latter refers to how a body, with its unique genetic makeup, processes folate.

Folic Acid Supplementation to Prevent Neural Tube Defects Updated Evidence Report and Systematic Review for the US Preventive Services Task Force
New evidence from observational studies provided additional evidence of the benefit of folic acid supplementation for preventing neural tube defects and no evidence of harms related to multiple gestation, autism, or maternal cancer. The new evidence was consistent with previously reviewed evidence on benefits and harms.

Association of Maternal Use of Folic Acid and Multivitamin Supplements in the Periods Before and During Pregnancy With the Risk of Autism Spectrum Disorder in Offspring
Question Does maternal folic acid and/or multivitamin supplement use before and/or during pregnancy increase the risk of autism spectrum disorder in offspring?
Findings In this case-control cohort study of 45 300 offspring, statistically significant associations between maternal vitamin supplement use before and/or during pregnancy and reduced risk of autism spectrum disorder in offspring were observed.
Meaning A reduced risk of autism spectrum disorder in children born to women who used the specified vitamin supplements before and during pregnancy has important public health implications; possible mechanisms include epigenetic modifications.

Prenatal Folic Acid Supplements and Offspring's Autism Spectrum Disorder: A Meta-analysis and Meta-regression
We systematically reviewed the evidence on the association between maternal folic acid supplementation and the risk of offspring's autism spectrum disorders (ASD). A total of 10 studies with 23 sub-studies (9795 ASD cases) were included. Folic acid supplementation during early pregnancy was associated with a lower risk of offspring's ASD [OR 0.57, 95% CI 0.41–0.78]. The consumption of a daily amount of at least 400 μg folic acid from dietary sources and supplements, was associated with a reduced risk of offspring ASD [OR 0.55, 95% CI 0.36–0.83]. Critical effective maternal folic acid supplementation strategies, such as intake timing and intake dosage, may aid the reduction in the risk of offspring ASD. This meta-analysis provided new insights for the prevention of offspring's ASD.

Neurodevelopmental effects of maternal folic acid supplementation: a systematic review and meta-analysis
This study provided relatively comprehensive evidence for the impact of maternal intake of FA on neu rodevelopmental outcomes of offspring. Our meta-analysis indicated that appropriate maternal FA supplementation may have positive effects on offspring's neurodevelopmental out comes, including improved intellectual development and reduced risk of autism traits, ADHD, behavioral, and lan guage problems. We also discussed that FA over-supplementation was not associated with an improvement in offspring's brain development, and may have a negative impact on offspring's neurodevelopmental outcomes. Therefore, we suggested that appropriate maternal FA sup plementation, not over-supplementation, could benefit th neurodevelopmental outcomes of offspring. However, furthe high-quality studies on this topic are needed to confirm th optimal dosage and the right time of FA supplementatio and to investigate the underlying mechanisms.

Folic acid and pediatric respiratory and allergic diseases

https://doi.org/10.1177/09691413221102321
-Childhood respiratory illnesses associated with perinatal use of folic acid, have no consistent evidence of an increased risk from FA use during the perinatal period (Crider et al., 2013, Roy et al., 2018, Trivedi et al., 2018, Vereen et al., 2019, den Dekker et al., 2018, Veeranki et al., 2015, Chen et al., 2021).
-A systemic review/meta-analysis has suggested that pregnancy related FA intake could be a risk factor for allergic diseases especially respiratory tract allergies (RR = 1.050, 95% CI = 1.027–1.073) (Levy and Blickstein, 2006). The stratified analyses revealed the association was significant only for respiratory allergy, only for pregnant women taking oral supplements, and only for countries without FA food fortification while the meta-regression analysis found the risk effect decreased with increasing FA exposure. These outcome results create doubt on the conclusion of a risk association.

https://doi.org/10.1111/nyas.13499
-As noted in the NTP report, observational studies reporting hypersensitivity-related outcomes, such as childhood asthma and allergy, have emerged over the past 20 years.46 Concern for adverse effects of maternal folic acid intake are again related to the ability of folate and/or methyl donors to program fetal gene expression.67-70 The NTP panel concluded that, with respect to sensitization to asthma, data on the effects of high folic acid intakes were limited.45 The panel also emphasized the need to understand whether folic acid functions in biological pathways leading to asthma sensitization and to perform rigorous controlled human studies in pregnant women and in children to better assess the mechanisms and risk.45 In terms of risk for hypersensitivity outcomes, such as eczema and respiratory infections, the panel concluded that this is not a priority research area owing to lack of available data.45

Folic acid and and rate of twinning

No high-quality studies have demonstrated an association between folic acid supplementation and increased rates of twinning **55**.

Does folic acid mask vitamin b12 deficiency?

https://www.annualreviews.org/content/journals/10.1146/annurev-nutr-043020-091647

Maternal vB12 deficiency increases susceptibility to NTDs in offspring (110, 118, 126, 148). vB12 is a key micronutrient that is directly involved as a coenzyme in folate metabolism. Low vB12 status is correlated with lower serum or RBC folate concentrations (22, 118). Additionally, an association between low maternal vB12 levels and NTD risk has been established independently of folate levels (126), but additional studies are needed to elucidate these interactions. FA supplementation prevents NTDs in populations with substantial vB12 deficiency (12, 64, 65).

Vitamin B_{12} deficiency can cause macrocytic anemias and neurological complications, which, if left untreated, can be irreversible. Neurologic complications can be present with or without a macrocytic anemia. However, early case reports describing the occurrence or progression of neurological complications among vB12-deficient patients treated with FA led to concerns that increased FA exposure through fortification might lead to the masking of vB12 deficiency by correcting macrocytic anemia while allowing neurologic complications to progress. This hypothetical situation is particularly concerning for older adults, who are more likely to have vB12 deficiency (**73**).

Standard clinical practice includes testing any individual with unexplained neurological complications for vB12 deficiency regardless of anemia status. A Canadian study reported that the prevalence of serum vB12 insufficiency (<150 pmol/L) with high serum folate status (>45 nmol/L) among older Canadian women increased postfortification compared with prefortification (0.09% versus 0.61%) (**136**). Although this study did not examine folate and vB12 in regard to hematological indicators, results raised concerns about masking of vB12 deficiency by FA. Therefore, some public health and nutrition experts have suggested adding vB12 and FA to supplements and fortified foods, respectively, to reduce this risk.

If increased FA intake truly masks anemia in individuals with vB12 deficiency, then we would expect a drop in vB12 deficiency anemia following FA fortification. Several studies have compared the prevalence of vB12 deficiency without anemia before and after FA fortification (**94**, **104**, **108**, **132**); only one study from an academic hospital reported an increase in low serum vB12 without macrocytosis among US adults (≥19 years) after fortification (**176**). Because macrocytosis is not a specific indicator of vB12 status and could also be a result of folate deficiency, an overall improvement in population folate status may explain the lower prevalence of macrocytosis postfortification. As a whole, the available epidemiological evidence suggests that there is little risk of FA fortification masking vB12 deficiency macrocytosis and adversely affecting the clinical presentation of vB12 deficiency, in particular among older adults. This view agrees with assessments conducted by multiple governmental and independent advisory agencies that support the safety of mandatory fortification with FA (**43**, **45**, **50**–**52**, **67**, **145**).

As noted in a recent review (**9**), blood assays for folate and vB12 had not yet been developed in early case reports of vB12-deficient patients being treated with folic acid (**37**). Routine diagnostic tests used at the time (e.g., hemoglobin, hematocrit, stained blood smears) were not specific for vB12 deficiency and thus would not have been able to distinguish among macrocytic anemias from vB12, folate, or some other nutritional deficiency. Therefore, neurological complications from early reports likely arose from

incorrect treatment of vB12 deficiency anemia with FA and should not be used as evidence of FA's toxicity (**9**, **37**). Now that clinical and laboratory tools for diagnosing vB12 deficiency are readily available, incorrect treatment of vB12 deficiency with FA is a medical error.

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-Folate deficiency and vitamin B12 deficiency both cause a macrocytic anaemia. Folic acid can, in large enough doses, correct the anaemia of vitamin B12 deficiency without having any effect on the neurological damage that can be caused by vitamin B12 deficiency. This effect of folic acid has misleadingly been referred to as “masking” vitamin B12 deficiency. If this were true, it might cause the delay or missing of a diagnosis of vitamin B12 deficiency with the possible consequence of irreversible neurological damage. But there is no evidence that this is true. This concern was considered in 1998 by the US Institute of Medicine (IOM)21 (see Box 2) but not judged harmful, presumably because the IOM recognised that anaemia was not needed to make a diagnosis of B12 deficiency.

-During the second half of the twentieth century analytical tests for blood folate and vitamin B12 were introduced, and synthetic vitamin B12 became readily available. It then became clear which patient with a macrocytic anaemia needed treatment with folic acid and which with vitamin B12. The introduction of specific vitamin B12 assays together with improvements in clinical care meant that the presence or absence of an anaemia was not necessary in making a diagnosis of vitamin B12 deficiency; an estimated 28% of patients with a vitamin B12-deficient neuropathy present without anaemia.22 Standard of care requires that the investigation of patients with suggestive neurological symptoms should include a serum vitamin B12 assay, whatever the haematological findings. Reliance on haematological screening for neuropathic vitamin B12 deficiency is no longer acceptable medical practice and poses a risk of medical negligence claims.

Stated simply, the concept of ‘masking’ is historical and has no place in current medical practice.23,24

-The IOM, however, did reach a conclusion that turned out to be incorrect.23,24 They made an error that incorrectly indicated that there was evidence suggesting that folic acid could exacerbate the neuropathy of vitamin B12 deficiency and recommended a safe upper limit of 1mg folic acid intake daily. The IOM analysed data from the 23 small-scale studies referred to in Box 2, conducted 70–80 years ago (see Box 3).

-The error of falsely attributing neurological toxicity to folic acid has been an obstacle to instituting folic acid fortification in many countries. Retaining this 1mg per day upper limit creates a conflict of policies – folic acid fortification will shift the distribution of folate (including folic acid) intake to a higher level so that some people will exceed the supposed 1mg per day upper limit. Trying to increase average folic acid intake without increasing the number of people consuming more than 1mg per day severely constrains the fortification level, resulting in inadequate fortification and a smaller NTD preventive effect; a much larger effect would be achieved without this unwarranted and disproven upper limit.8,10,11

New laboratory tests can readily measure a person's vitamin B12 status. This means that vitamin B12 deficiency can be detected and diagnosed quite easily. It also means that not being able to identify the anemia caused by vitamin B12 deficiency, as described in early case reports, is unlikely to remain an issue. A recent population-based study examined anemia among people with vitamin B12 deficiency before and after folic acid fortification and found that folic acid did not delay the identification of vitamin B12 deficiency after fortification began [16].

A concern exists about excessive folate and the potential to mask and exacerbate neuropathy in those with vitamin B12 deficiency (more so in those over age 65).

- In those with megaloblastic anemia (due to folate or vitamin B12 deficiency), it is important to rule out vitamin B12 deficiency before administering FA because treatment with FA may delay diagnosis of vitamin B12 deficiency and allow progression of neurologic abnormalities and some hematologic abnormalities
- A patient with B12 deficiency may superficially appear to be treated successfully with FA because the macrocytic anaemia can resolve, but not the neurological disease
- However, taking FA as part of a multivitamin or prenatal vitamin with vitamin B12 reduces any potential risk
  - *In addition, the likelihood of masking an incorrect diagnosis disappeared during the latter half of the last century, with the introduction of specific assays for folate and B12 deficiency, and with the ready availability and common use of B12 therapy*
- But based on this potential risk, the UL of folate is 1000 mcg/1 mg from supplements/fortified foods for persons 19 years and older; the UL is 800 mcg/0.8 mg perday for persons 14–18 years old
  - Revising the UL has been proposed

In addition, the likelihood of masking an incorrect diagnosis disappeared during the latter half of the last century, with the introduction of specific assays for folate and B12 deficiency, and with the ready availability and common use of B12 therapy.

- But based on this potential risk, the upper intake level (UL) of folate in the form of folic acid is 1000 mcg/1 mg from supplements/fortified foods for persons 19 years and older; the UL is 800 mcg/0.8 mg per day for persons 14–18 years old

DEFICIENCIES OF BOTH FOLATE (VITAMIN B9) AND COBALAMIN (VITAMIN B12) CAN CAUSE ANEMIA. SEVERE B12 DEFICIENCY CAN CAUSE PERNICIOUS ANEMIA AND EVENTUALLY NERVOUS SYSTEM DAMAGE IF PEOPLE WITH B12 DEFICIENCY RECEIVE HIGH DOSES OF FA, THE FA SUPPLEMENTATION CAN CORRECT THE ANEMIA CAUSED BY B12 DEFICIENCY; HOWEVER, THE NERVOUS SYSTEM DAMAGE CAN STILL OCCUR BECAUSE THEY ARE STILL DEFICIENT IN VITAMIN B12 THIS EFFECT WAS SEEN AMONG SOME PATIENTS WHO WERE GIVEN VERY HIGH DOSAGES [MORE THAN 5,000 MCG PER DAY OF FA FOR MANY MONTHS].

THE INSTITUTE OF MEDICINE SPECIFIED A DOSE OF 5,000 MCG/DAY OF FA AS THE LOWEST OBSERVED ADVERSE EFFECT LEVEL (LOAEL)
THE TOLERABLE UL FOR FA WAS ESTABLISHED AT 1,000 MCG/DAY (ONE-FIFTH OF THE LOAEL) TO PREVENT INDIVIDUALS FROM TAKING SO MUCH FA THAT THEY REACH THE LOAEL RISK LEVEL OF 5,000 MCG/DAY
PATIENTS WITH A HISTORY OF NTD IN A PRIOR PREGNANCY, OR OTHER RISK FACTORS FOR NTD, ARE ADVISED TO TAKE 4000 MCG/DAY OF FA
FOR THOSE PERSONS REQUIRING HIGHER DOSES OF FA, NEW LABORATORY TESTS CAN READILY MEASURE THEIR VITAMIN B12 STATUS
VITAMIN B12 DEFICIENCY AMONG U.S. PERSONS OF REPRODUCTIVE AGE IS RARE, AND THEY ARE UNLIKELY TO HAVE ANY ADVERSE EFFECTS FROM CONSUMING SUPPLEMENTAL FA OR ABOVE THE UL

MTHFR gene variants

The MTHFR gene provides instructions for your body to make the MTHFR protein, which helps your body process folate. Your body needs folate to make DNA and modify proteins. MTHFR (methyltetrahydrofolate reductase) is an enzyme that plays a role in how all people process folate.

The 5,10-methylenetetrahydrofolate reductase (MTHFR) enzyme catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (5-MTHF)--the primary circulatory form of folate.

Over half of Americans have variations in a gene known as methylenetetrahydrofolate reductase (MTHFR).

Each person has two copies of the *MTHFR* gene: one from his or her mother and one from his or her father.

There are two common variants in the gene encoding for the MTHFR enzyme:

- The “thermolabile” variant, more commonly referred to as C677T. Enzyme less active at higher temperatures.
- The possible genotypes at the 677 position of the MTHFR gene are:
- MTHFR 677 CC (two copies of C, one copy from each parent)
- MTHFR 677 CT (one copy of C from one parent, one copy of T from the other parent)
- MTHFR 677 TT (two copies of T, one copy from each parent)
- The number of people who have each of these genotypes will vary from population to population. When consuming the same amount of folic acid, people with the MTHFR 677 TT genotype have an average blood folate concentration

(amount of folate in their blood) that is only slightly lower (about 16% lower) than people with the MTHFR 677 CC genotype.[4]
- C677T variant is common
- 10– 15% of North American Caucasians are homozygous
- > 25% of Hispanics are homozygous with the highest allele frequencies
- 6% in individuals of African descent are homozygous
- 20 to 40% of Caucasians or Hispanics in the US are heterozygotes (one allele)
- You may have seen the *MTHFR* C677T variant referred to as a "gene mutation;" however, the word, "mutation," usually refers to a change in the gene that is much less common. It is more accurate to refer to *MTHFR* C677T as a "gene variant."

- The A1298C variant also common.
- This gene variant occurs at the 1298 position in the *MTHFR* gene. This means at the 1298 position in the *MTHFR* gene, the expected DNA base "A", is replaced by "C", the gene variant.[5] There is not enough evidence to show that the *MTHFR* A1298C variant *alone* significantly affects how the body processes folate.
- Combined heterozygosity of A1298C and C677T results in an outcome like C677T homozygous individual

In the U.S., about 1 in 3 people have at least one MTHFR variant, and 1 in 10 people have variants in both copies of the MTHFR gene variant.

Having a common variant in the MTHFR gene does NOT change your medical care. Gene variants are common and normal. In fact, there are more people in the United States who have one or two copies of the *MTHFR* C677T variant than people who do not have it.[7] Variants in genes are what make us unique. They cause differences, such as eye color, hair color, and blood type.

A gene variant is a change in a DNA sequence that is different from the expected DNA sequence. The most common variant in the *MTHFR* gene is *MTHFR* C677T.[2] This variant may also be referred to as *MTHFR* 677 C>T **or** *MTHFR* 677 C→T. This means at the 677 position in the *MTHFR* gene, "C" is the expected DNA base and "T" is the gene variant.[3] A common genetic variant of the MTHFR enzyme determines how rapidly some people can process folate. Even though individuals with the *MTHFR TT* or *CT* genotypes process folate more slowly, they can increase their blood folate concentrations enough to help prevent neural tube defects—some serious birth defects of the brain and spine—by consuming the recommended 400 mcg/day of folic acid [11].

Online Misinformation Fuels a Fight Over Folic Acid
-In 2017, the direct-to-consumer genetic testing service 23andMe wrote in a blog post that MTHFR was the most asked-about gene among its customers. People have even created cookbooks targeted toward those who have MTHFR variants, as well as shirts that play on the acronym's resemblance to a vulgar term. The gene has also been the subject of more than 3,000 research articles, some of which study whether the

common variations may be linked to over 600 disorders, including autism, anxiety, infertility, as well as neural tube defects.
-But the links between MTHFR and the majority of the disorders are weak, according to Barry Shane, a nutrition researcher and professor emeritus at the University of California, Berkeley, who has been studying folate metabolism for over 45 years. MTHFR variations can make someone more likely to have low folate, which does carry risks, especially for having a baby with a neural tube defect. However, folic acid is a safe and proven way to raise folate levels, even in people with MTHFR variants, says Shapira.
-Nonetheless, a search for MTHFR on YouTube, podcasts, search engines, and other social media channels turns up a host of articles, videos, and interviews warning, without solid evidence, that folic acid is poison for those with MTHFR variations (which they often call "mutations") or that the conditions associated with MTHFR variations are actually triggered or made worse by folic acid. They often recommend changing one's diet to include more folate-rich foods, avoiding foods fortified with folic acid, and replacing folic acid supplements with different, costlier ones in order to alleviate MTHFR-ascribed symptoms.
-These warnings about folic acid appear widely on sites that feature heavy doses of science skepticism or dubious claims, among them that MTHFR variations justify medical exemptions from vaccines. Typically, those sites also sell supplements, genetic testing, and analysis, or related services including nutritional coaching sessions, books, and online courses centered on MTHFR.
-Around the turn of the millennium, when it got easier to read DNA, scientists were enthusiastic that research would reveal genetic risk factors in complex diseases, and were hopeful that this could advance medical care. In many studies beginning around then, MTHFR variations popped up because they are so common in the population. But most of the correlations between MTHFR and the hundreds of diseases and conditions it became associated with were weak. So while scientists became less enthusiastic that these genetic studies were providing meaningful information, "in the general public, especially on the internet, it just took off in the other direction.
-We now know that there are few health risks that come from having common MTHFR variations, and there is no reason for these common variations to lead to any changes in medical care. Having MTHFR variations can contribute to low folate, which can have negative health effects, but these issues are uncommon due to food fortification.
-Hickey says people contact him "pretty regularly" to have MTHFR genes analyzed for the common variants. But he only orders gene testing for patients in cases where he suspects extremely uncommon MTHFR variants. The symptoms of these very rare variants are severe — intellectual disability, low muscle tone, concerning blood test results, and seizures. In his 11 years of clinical practice, Hickey says he has come across only one confirmed case that he knows of: two siblings who were diagnosed at the clinic where he did his residency. He estimates that these siblings represent 2 of about 100 to 200 people in the entire country that have the rare MTHFR variants causing these severe symptoms.

Anne Parle McDermott Nutritition and Genomics group at DCU. Experts in genetics, folate metabolism and its influence on human health.

MTHFR gene plays a role in the metabolism of folate. There are gene variants in MTHFR in the form of mutations or polymorphisms. Mutations are rare (<1% of population), but can have a sig impact on function of cells. Polymorphisms are common and have a small effect on cells. 10-14% of population can have the 677TT variation/polymorphism and manifestation of disease is multifactorial. The 677TT variation has shown to cause a slightly increased risk of having a baby with a NTD.

MTHFR gene variants and neural tube defects

https://www.annualreviews.org/content/journals/10.1146/annurev-nutr-043020-091647

MTHFR C677T polymorphism reduces the activity of the MTHFR enzyme, resulting in a decrease in plasma/serum and RBC folate concentrations across studies using an appropriate folate assay (microbiologic assay for RBC folate concentrations, where CC>CT>TT) for CC versus TT [plasma/serum, 13%; 95% creditable interval (CrI), 7%, 18%; RBC, 16%; 95% CrI, 12%, 20%] (164). Both maternal and fetal *MTHFR C677T* polymorphisms have been implicated as significant risk factors for NTDs (164). Depending on the genotype (homozygous versus heterozygous) for *MTHFR C677T*, the odds of an NTD increase by 30% to 80% (OR, 1.3, 1.8) (157, 181). In an analysis of the impact of *MTHFR C677T* polymorphism on RBC folate concentrations, predicted models were elevated for the *MTHFR TT* genotype compared with the *CC* genotype [RR, 1.49; 95% uncertainty interval (UI), 1.33, 1.70] and for the *TT* genotype compared with the *CT* genotype (RR, 1.28; 95% UI, 1.17, 1.39) (27). These data are very similar to those from a meta-analysis of NTD risk from *MTHFR* genotypes, suggesting that the impact of *MTHFR C677T* on NTDs arises mainly from its impact on folate concentrations at lower folate intakes as NTD risk increases dramatically (27).

Can people with MTHFR gene variants take folic acid?

The *MTHFR* C677T variant affects how your body processes folate. You might have read or heard that folic acid is not safe if you have one or two copies of the *MTHFR* C677T variant. This is not true. Even if you have one or two copies of the *MTHFR* C677T variant, your body can safely and effectively process all different types of folate, including folic acid.

You may have heard or read that if you have an *MTHFR* C677T variant, you should take other types of folate (such as 5-MTHF), but this is not true. Folic acid is the only type of folate shown to help prevent neural tube defects (severe birth defects of the brain or spine).[1-4,6]

Research studies have shown that among populations in which more people have the MTHFR TT or CT genotypes, getting 400 mcg/day of folic acid before and during early pregnancy can reduce by 85% the risk of having a baby with a neural tube defect [5].

Many studies have shown that consuming folic acid increases blood folate concentrations [26]. For example, a research study among a population at high risk for neural tube defects showed that, after consuming 400 mcg/day of folic acid for three months, average blood folate concentrations increased to levels that would prevent neural tube defects among individuals with all MTHFR genotypes, including the MTFHR TT genotype [9].

Testing for MTHFR polymorphisms is not recommended as routine FA supplementation at 400mcg/0.4 mg per day will adequately increase red cell and serum folate concentrations whether or not the person has a polymorphism.

Folic acid is one of a few forms of folate that can come from diet. The body uses folate to transport carbon for numerous crucial processes in cells. The gene MTHFR codes for an enzyme that helps to get the carbon into forms necessary for these processes. (In the process MTHFR generates L-methylfolate, another kind of folate, which other enzymes further modify to make things that cells need.)
These processes can be less efficient in people with the common MTHFR variations. This can lead to lower blood folate levels, but folic acid can boost folate levels even in people with variations.

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FA has a very similar dose–response increase in RBC folate concentrations across *MTHFR* genotypes when supplementing with 400 μg/day (**27**, **31**). A meta-analysis of FA supplementation trials has shown that FA increases RBC folate concentrations by 178% and serum/plasma folate concentrations by 200% at the recommended dosage of 350–500 μg/day (**28**). Another meta-analysis showed only moderate absolute differences across *MTHFR* genotypes in folate concentrations, with an 18% reduction in CC versus TT (**164**). This finding suggests that people with *MTHFR* variants increase their folate status adequately to prevent NTDs with the recommended FA intake. Supporting evidence comes from studies in areas with very high rates of *MTHFR C677T* variants, such as northern China (**12**, **31**) and Mexico (**100**), where FA reduces NTDs even when the variant is the major allele. Although efforts have been made to modify 5-MTHF to increase stability for commercial production (**6**, **68**, **85**), few studies have compared FA with these 5-MTHF isomers, and the impact of these modifications on birth defects has not been investigated.

Study of C677T Methylene Tetrahydrofolate Reductase Gene Polymorphism as a Risk Factor for Neural Tube Defects

Neural tube defects (NTDs) are group of severe congenital malformations that occur as a result of failure of closure of embryonic neural tube properly during early development. The identification of the causative factors is confounded by the complex interplay of the genetic, metabolic, and environmental components. Genetic abnormalities in folate-related enzymes could probably explain the role of folate in preventing NTDs. One of the critical genes to play significant role in folate metabolism is methylene tetrahydrofolate reductase (MTHFR). The hypothesis that an underlying genetic susceptibility interacts with folate-sensitive metabolic processes at the time of neural tube closure is very pertinent in context to the development of NTDs.[1] The risk that parents with known MTHFR mutations will have a baby with a NTD is extremely low and far less than 1% as the genetic variant alone may not be the causative factor in the development of NTDs.[2] An increased risk for NTDs has been predicted if the infant or the mother has homozygous MTHFR TT genotype. In many studies, it has been found that there is a significant association between MTHFR 677C>T and increased risk of NTDs.[3,4,5] Studies by De de Franchis *et al*. and Shields *et al*. supported the association between NTDs and MTHFR 677TT,[6,7] while Behunova *et al*., Félix *et al*., and Perez *et al*. did not find any such association.[8,9,10] To date, there are neither large-scale, well-designed epidemiological studies that explicitly prove that either of these MTHFR variants cause speculated health effects nor clear evidence establishing the clinical utility of genotyping for MTHFR in guiding drug therapy for any indication. Despite an exhaustive scientific literature on the effect of common MTHFR variants, there is apparently no convincing evidence linking MTHFR association to most of the health conditions.

With the above background, the present study was undertaken to investigate the levels of Vitamin B12 and folate in serum samples of patients with NTDs compared with healthy controls, to investigate genetic polymorphism of MTHFR in NTDs and to evaluate the correlation between Vitamin B12, folate levels, and pattern of gene polymorphism in NTDs in patients attending Post Graduate Institute of Medical Sciences (PGIMS) Hospital, Rohtak, Haryana.

We did not find evidence of the association between MTHFR C677T polymorphism and NTDs. Hence, we conclude that MTHFR variant may not be a risk factor for the selected population. However, our study does not rule out the impact of MTHFR gene mutation on folate metabolism. In our study, the frequency of prevalence of the homozygous 677CC genotype in cases and control was 80% and 87.5%, respectively, whereas that of heterozygous 677CT genotype was 20% in cases and 12.5% in controls. No TT genotype was found and no association between MTHFR 677C>T gene polymorphism and NTD was observed in our study. Larger and comprehensive multicentric but feasible studies involving proper subjects and appropriate and adequate controls from

several hospitals may provide more meaningful data which can help in resolving some of the unresolved controversies of the relationship between MTHFR gene polymorphism and NTDs.

Bringing clarity to the role of MTHFR variants in neural tube defect prevention

Because MTHFR functions to generate 5-methyltetrahydrofolate, and knowledge that the 677C>T *MTHFR*polymorphism can decrease MTHFR functional capacity in generating 5-methyltetrahydrofolate, it has been suggested in both the popular media and the scientific literature that dietary folic acid should be replaced by the more "natural" 5-methyltetrahydrofolate for NTD prevention. These suggestions should be interpreted with caution because there is currently no evidence that 5-methyltetrahydrofolate can prevent NTDs, whereas it has been shown conclusively that folic acid supplementation reduces the risk of NTDs across diverse populations (8). Importantly, folic acid supplementation in women with the *TT MTHFR*genotype has been shown to increase folate status above that which is considered maximally protective for NTDs (9). Furthermore, women with vitamin B-12 deficiency have diminished ability to metabolize 5-methyltetrahydrofolate, which may make it less effective in preventing NTDs than folic acid.

MTHFR 677C->T genotype is associated with folate and homocysteine concentrations in a large, population-based, double-blind trial of folic acid supplementation

Background: The *methylenetetrahydrofolate reductase* (*MTHFR*) genotype is associated with modification of disease and risk of neural tube defects. Plasma and red blood cell(RBC) folate and plasma homocysteine concentrations change in response to daily intakes of folic acid supplements, but no large-scale or population-based randomized trials have examined whether the *MTHFR* genotype modifies the observed response.Objective: We sought to determine whether the *MTHFR* 677C→T genotype modifies the response to folic acid supplementation during and 3 mo after discontinuation of supplementation.Design: Northern Chinese women of childbearing age were enrolled in a 6-mo supplementation trial of different folic acid doses: 100, 400, and 4000 μg/d and 4000 μg/wk. Plasma and RBC folate and plasma homocysteine concentrations were measured at baseline; after 1, 3, and 6 mo of supplementation; and 3 mo after discontinuation of supplementation. *MTHFR* genotyping was performed to identify a C→T mutation at position 677 (*n* = 932).Results: Plasma and RBC folate and homocysteine concentrations were associated with *MTHFR* genotype throughout the supplementation trial, regardless of folic acid dose. *MTHFR TT* was associated with lower folate concentrations, and the trend of *TT* < *CC* was maintained at even the highest doses. Folic acid doses of 100 μg/d or 4000 μg/wk did not reduce high homocysteine concentrations in those with the *MTHFR TT* genotype.Conclusion:*MTHFR* genotype was an independent predictor of plasma and RBC folate and plasma homocysteine concentrations and did not have a significant

interaction with folic acid dose during supplementation. This trial was registered at clinicaltrials.gov as NCT00207558.

The methylenetetrahydrofolate reductase (MTHFR) enzyme is responsible for the synthesis of 5-methylTHF—the coenzyme required for homocysteine remethylation to methionine, the precursor of *S*-adenosylmethionine (the body's primary methylating agent) and for endogenous production of THF and subsequent DNA synthesis. The *MTHFR C* allele has been shown in vitro to have higher enzyme activity than the *T* allele (7). Subjects with the *T* allele are also thought to have a higher folate requirement (8, 9). The *MTHFR*677C→T variant has been reported to be associated with elevated homocysteine concentrations and with variation in the risk of many disorders, including birth defects, cancer, and psychiatric conditions (10–12). The prevalence of the *MTHFR* 677C→T genotype varies among populations (11). Daly et al (13) described the relation between plasma and red blood cell (RBC) folate concentrations and the prevalence of NTD-affected pregnancies, but the plasma and RBC folate concentrations associated with optimal prevention of folic acid–sensitive NTDs is unknown. Periconceptional consumption of 400 μg folic acid/d in a community intervention trial in China achieved an 85% reduction in NTD-affected pregnancies among women with high pill-taking compliance (1). It is not known whether the reduction in risk of an NTD-affected pregnancy, in response to folic acid intake, is modified by *MTHFR* 677 genotype.

In this population of women, *MTHFR* 677 genotypes were associated with baseline plasma and RBC folate and plasma homocysteine concentrations. Throughout the intervention, the *MTHFR* 677 *TT* genotype was associated with lower plasma and RBC folate concentrations and with higher plasma homocysteine concentrations than was the *CT* or *CC* genotype. The *MTHFR* 677 genotype was an independent predictor of response to folic acid supplementation, but did not interact with folic acid dose.

NTD risk has been shown to be significantly elevated in women with impaired folate status (23), the *MTHFR TT* genotype (24), or an elevated homocysteine concentration (25, 26). It has been shown that NTD rates are higher in populations with a higher prevalence of the *MTHFR* 677 *T* allele, such as Hispanics (27, 28) and the northern Chinese (29) and suggested that the greatest potential effect of folic acid for the prevention of NTDs could be in populations with high NTD rates and high *MTHFR TT* frequencies (1, 28). The *MTHFR T* allele frequency in this northern Chinese population was consistent with previous reports (29) and was very high compared with other populations (11); the variant is the major allele.

Previous studies in this same northern Chinese population have shown the high NTD rates can be decreased by up to 85% with 400 μg periconceptional folic supplementation/d (1). In the current study, we found that RBC folate concentrations increased by 17–23% in those taking 400 μg/d (*see* supplemental Table 2 under "Supplemental data" in the online issue) after 1 mo of supplementation, regardless of genotype. RBC folate concentrations in women with the *MTHFR TT* genotype increased to the baseline concentration of the *CT* group after 1 mo of supplementation and to the baseline concentration of the *CC* group after 3 mo of supplementation. Because this is

the same population that participated in the community intervention trial (1), it might be that the plasma and RBC folate concentrations observed in the 400-μg/d dose group approximate those necessary to reduce NTD occurrence in this population.

In this study, we found that both folic acid dose and *MTHFR* genotype affected plasma and RBC folate and plasma homocysteine concentrations. However they did not significantly interact, such that changes in folic acid dose did not significantly change the effects of *MTHFR* genotype. Interestingly, in those who consumed 100 μg folic acid/d, plasma homocysteine concentrations decreased only minimally (nonsignificant). The decrease was also nonsignificant in those with the *MTHFR* 677 *CC* genotype who received 400 μg/d. Plasma homocysteine concentrations in women with the *MTHFR* 677 *CC*genotype were lower than those observed in US populations [6.1 μmol/L in this study compared with 8.5 μmol/L in non-Hispanic whites and 7.7 μmol/L in Mexican Americans (21)], even though this northern Chinese population was not folate replete (18).

A recent report suggests that a folic acid dose of <200 μg/d may be effective at lowering high homocysteine concentrations when taken for 26 wk (30). However, our data suggest that the 100-μg/d and 4000-μg/wk doses for 6 mo were insufficient to significantly reduce high concentrations of plasma homocysteine in those with the *MTHFR TT*genotype.

The 4000-μg/wk dose was included in the design of this study because researchers have shown that high-dose folic acid supplements administered weekly can be effective at preventing NTD (31, 32). Although the group who received 4000 μg/wk dose (≈571 μg/d) consumed a larger total dose than did the group who received 400 μg/d, the former dose was not as effective at lowering high homocysteine concentrations or increasing plasma or RBC folate concentrations as was the 400-μg/d dose.

The plasma folate concentrations increased dramatically with supplementation and began to decrease after 3 mo, as discussed previously (18). In this study, this effect was not dependent on *MTHFR* genotype or dose, but was dependent on the timing of supplement consumption. Plasma folate reached a plateau after 3 mo of supplementation and began to fall in the 4000-μg/d dose group, regardless of the plasma and RBC folate concentration achieved.

Despite 6 mo of supplementation with 4000 μg folic acid/d, women with the *MTHFR TT*genotype achieved lower plasma and RBC folate concentrations and higher plasma homocysteine concentrations than did those with the *MTHFR CC* genotype. Although significant increases in plasma and RBC folate concentrations were observed in all *MTHFR*genotype groups, those with the *MTHFR TT* genotype never attained the concentrations achieved by the *MTHFR CC* genotype group. The *TT* genotype was significantly associated with response to supplementation even at the highest daily doses. These findings have had implications for the “rescue” of folic acid–sensitive NTDs through food-fortification programs. Thus, the effects of the reduced enzymatic activity in the *TT* genotype group affected the response to folic acid supplementation across folic acid doses.

The design of this large, population-based study of reproductive-age women included 6 mo of folic acid only supplementation and follow-up at 3 mo after supplementation discontinuation in a population without exposure to fortified foods or supplements. A major strength of the study was the design, which allowed for a precise determination of the effect of *MTHFR* genotype on long-term exposure to folic acid alone. However, a more extended period of supplementation and a longer duration of observation after discontinuation of supplementation may have been needed to detect the stabilization of all the biomarkers in each of the folic acid dose groups. Shorter and more frequent sampling intervals, especially early in the interventions, would have provided more data on the timing and trajectories of genotype-dependent responses to supplementation regimens. In addition, because this study only included women of reproductive age, without anemia or vitamin B-12 deficiency, it is not known whether these results can be generalized to other groups (eg, men).

In conclusion, elevated plasma homocysteine concentrations in those with the *TT*genotype were unresponsive to the 100-µg/d and 4000-µg/wk doses of folic acid. The plasma and RBC folate and homocysteine concentrations during 6 mo of folic acid supplementation and 3 mo after discontinuation of supplementation were strongly associated with *MTHFR* genotype, irrespective of folic acid dose.

Online Misinformation Fuels a Fight Over Folic Acid
-Some nutritionists and complementary health practitioners recommend avoiding folic acid. Christa Biegler, a registered dietician and podcaster, tells people with MTHFR variations to supplement with a synthetic version of L-methylfolate because it bypasses the MTHFR enzyme in the process of converting folate, so people with less functional MTHFR enzymes can put the supplement straight to use.
-But Shane counters that this isn't how folates are processed; rather than an assembly line, it is a cycle, so even if MTHFR is skipped once, it will be involved as the folates get cycled through the pathway again and again. Because this cycling occurs hundreds or thousands of times a day, Shane explained that the advantage of giving L-methylfolate, compared to folic acid, is "absolutely minimal."

What do medical organizations say about MTHFR gene variants?

- ACOG and NSGC
    - Due to the lack of evidence associating MTHFR variants independently with thrombosis, recurrent pregnancy loss (RPL), or other adverse pregnancy outcomes (APO), MTHFR genotyping or fasting homocysteine levels is not recommended for the work up of VTE or thrombophilia
        - Only antiphospholipid syndrome has shown consistent associations with RPL
    - There is lack of association between heterozygosity or homozygosity for C677T and any APOs

    - The ACOG and NSGC do not recommend evaluation of MTHFR polymorphism status or plasma homocysteine testing for the evaluation of recurrent pregnancy loss
- ASRM and NSGC
    - Testing for MTHFR variants for RPL is not recommended
    - A study reported in ASRM's "Fertility and Sterility" journal analyzed the proportion of embryo aneuploidy in patients detected as carriers of MTHFR gene mutations
        - This study demonstrated the presence of the most common MTHFR genotype variants are not associated with the rate of embryo aneuploidy
        - Even after controlling for age and other potential cofounders, patients who have a MTHFR polymorphism did not experience increased odds of embryo aneuploidy
- ACMG
    - MTHFR polymorphism genotyping should not be ordered as part of the clinical evaluation for thrombophilia or RPL or for at-risk family members
    - Do not adjust preventative FA supplementation dose for neural tube defects based on MTHFR results
- The chance for NTDs in a fetus may be slightly higher for a pregnant person with 2 copies (homozygous) of the C677T variant
    - These studies are generally conducted in countries without FA fortification
        - This means that persons in these studies are not getting FA in their diets from fortified foods
    - This chance can be lowered by taking FA before getting pregnant and during early pregnancy
    - Routine screening for the MTHFR C677T variant is not recommended by ACOG as a risk determinant of a NTD
- Experts recommend that anyone who is able to get pregnant should take a daily supplement that has 400 to 800 mcg of FA
    - There have been no recommendations to increase FA supplementation, alter the type of FA supplementation, or to perform additional screening based on MTHFR genotype alone
    - Studies have shown that a person who consumes 400 mcg of FA each day generally has enough folate in their blood to help prevent NTDs regardless of MTHFR C677T genotype (CC, CT, or TT)
        - The FA intake is more important than MTHFR genotype for determining the amount of folate in the blood

MTHFR, FA and tongue tie

Anne Parle McDermott Nutritition and Genomics group at DCU. Experts in genetics, folate metabolism and its influence on human health.

Claim: parents with MTHFR gene variants like 677TT are more likely to have babies with tongue tie. This is from the blog, “MTHFR.net” by Ben Lynch, and Sara Hornsby, blogger and myofunctional therapist, and other MTHFR blogs.

There are no peer reviewed published studies on tongue tie and MTHFR gene variations. There is no biological plausibility for this.

Other blogs claim this happens due to X-linked inheritance and MTHFR variation. This is impossible as MTHFR gene is on chrom 1. Cannot be caused by both.

TBx22 is an X-linked gene may be linked to inherited forms of tongue tie, bit research has not confirmed.

Tongue tie is likely caused by a variety of factors, both genetic and environmental.

Maternal folic acid supplementation and the risk of ankyloglossia (tongue-tie) in infants; a systematic review
Recent public-facing communications have raised concerns about a causal relationship between folic acid supplementation, particularly after the first trimester, and ankyloglossia (tongue-tie) in infants. Non-evidence-based communications are potentially harmful because they could adversely affect adherence to folic acid supplementation, increasing NTD occurrence.
One case-control study reported that regular intake of folic acid supplements was higher in women with infants with ankyloglossia. However, this study has limitations regarding design, selection bias, and confounding, calling the findings into question.

Insufficient evidence exists for a relationship between folic acid supplementation and ankyloglossia. Currently, the benefits of folic acid supplementation far outweigh the risks. This must be clearly communicated to patients by their clinicians during preconception and antenatal care.