Detailed Study Notes: Episode 444

Folate: Intake, Genetics & Health Outcomes

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Introduction to this Episode

Folate (also known as vitamin B9) actually relates to a collection of folates; both natural dietary folates and synthetic forms, primarily folic acid. This folate/folic acid that is consumed via the diet or supplementation is a precursor for the formation of tetrahydrofolate (THF), which is a carbon donor and acts a cofactor for a number of enzymes that play important roles in several processes.

In this episode, Alan and Danny discussed:

- the role of folate in the methylation cycle
- the impact of folate insufficiency/deficiency
- genetics variants of the MTHFR gene (and other genes) that impact folate metabolism
- the impact of folate on health outcomes; including:
    - heart disease
    - birth defects
    - cancer
    - brain health & cognition.

Connection to Previous Episodes

- Folate and folic acid have been discussed in previous episodes related to pregnancy, specifically episode 441 with Dr. Julie Abayomi.
- One-carbon metabolism, methylation and homocysteine were discussed in episode 443 with Dr. Kevin Klatt.
- The impact of diet on brain health, including the role of folate, was discussed in episode 438.
- The difference between a systems biomarker and a causal risk factor (that Alan referenced in this episode), was discussed in episode 381 with Prof. Chris Packard.

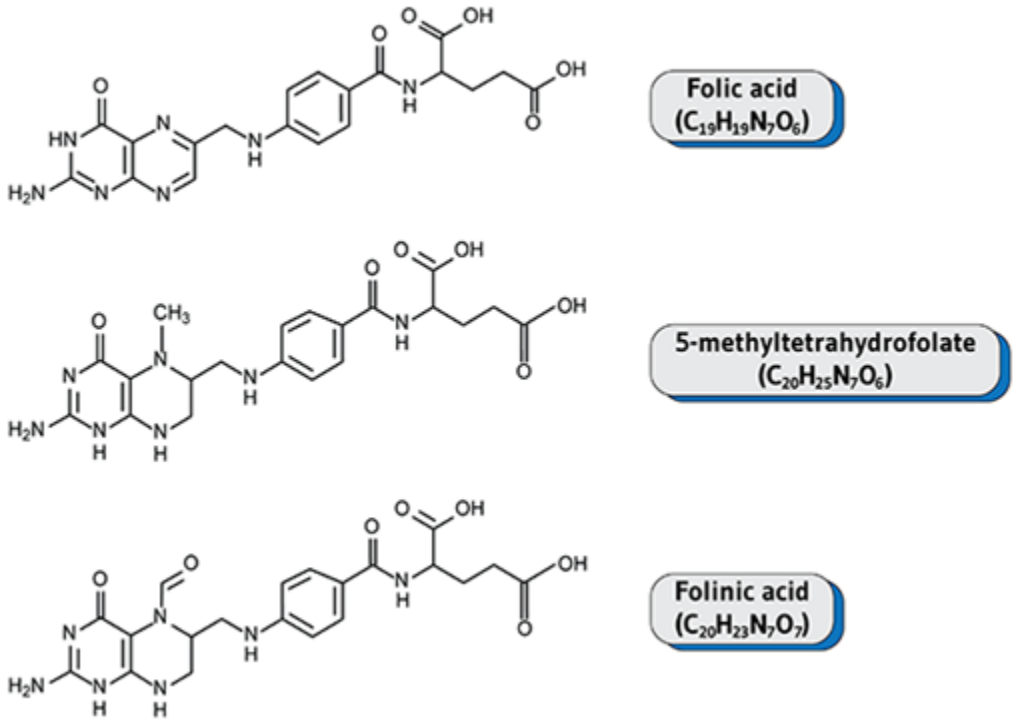
Glossary/Terminology

- **One-carbon (1C) unit** = a biochemical term for functional groups containing only one carbon in addition to other atoms. A number of one-carbon units (e.g. methyl) are transferred by folate coenzymes.
- **Methylation** = a biochemical reaction resulting in the addition of a methyl group (-CH3) to another molecule.
- **Homocysteine** = an amino acid, which is an intermediate in the metabolism of another amino acid, methionine. Elevated homocysteine levels in the blood have been associated with increased risk of cardiovascular disease.
- **MTHFR** = Methylenetetrahydrofolate reductase (MTHFR) is an enzyme that breaks down the amino acid homocysteine. The MTHFR gene that codes for this enzyme has the potential to mutate, which can either interfere with the enzyme's ability to function normally or completely inactivate it.
- **Anemia** = Anemia is the general term for having either fewer red blood cells than normal or having an abnormally low amount of hemoglobin in each red blood cell. There are several different types of anemia and each one has a different cause.
- **Megaloblastic anemia** = Megaloblastic anemia is a type of anemia characterized by very large red blood cells (technically, megaloblasts), with an overall low red blood cell count. It has several different causes but deficiencies of either vitamin B12 or folate are the two most common.
- **Pernicious anemia** = A disorder characterized by the inability of the body to properly utilize vitamin B12, which is essential for the development of red blood cells. Most cases result from the lack of the gastric protein known as intrinsic factor, without which vitamin B12 cannot be absorbed.
- **Neural Tube Defect (NTD**) = a birth defect caused by abnormal development of the neural tube, the structure which gives rise to the central nervous system. Neural tube defects include anencephaly and spina bifida.
- **Spina bifida** = a birth defect, also known as a neural tube defect, resulting from failure of the lower end of the neural tube to close during embryonic development. Spina bifida, the most common cause of infantile paralysis, is characterized by a lack of protection of the spinal cord by its membranes and vertebral bones.

What is Folate?

- Folates are essential vitamins derived from dietary sources and the microbiome.
- Folate relates to both natural folates and synthetic folic acid. Other synthetic forms include folinic acid
- Folate = also known as vitamin B9
- Dietary sources = green leafy vegetables, fortified foods
- Fortification and supplementation often in the form of folic acid
- Folic acid has no biological activity unless converted into folates
- Folate/folic acid is a precursor for the formation of **tetrahydrofolate (THF)**
- THF is a carbon donor and acts a cofactor for a number of enzymes that play important roles in several processes discuss later in these notes.
- Severe deficiency in either folate or vitamin B12 can lead to **megaloblastic anemia**, which causes fatigue, weakness, and shortness of breath.

Figure 1. Chemical Structures



From: Oregon State University

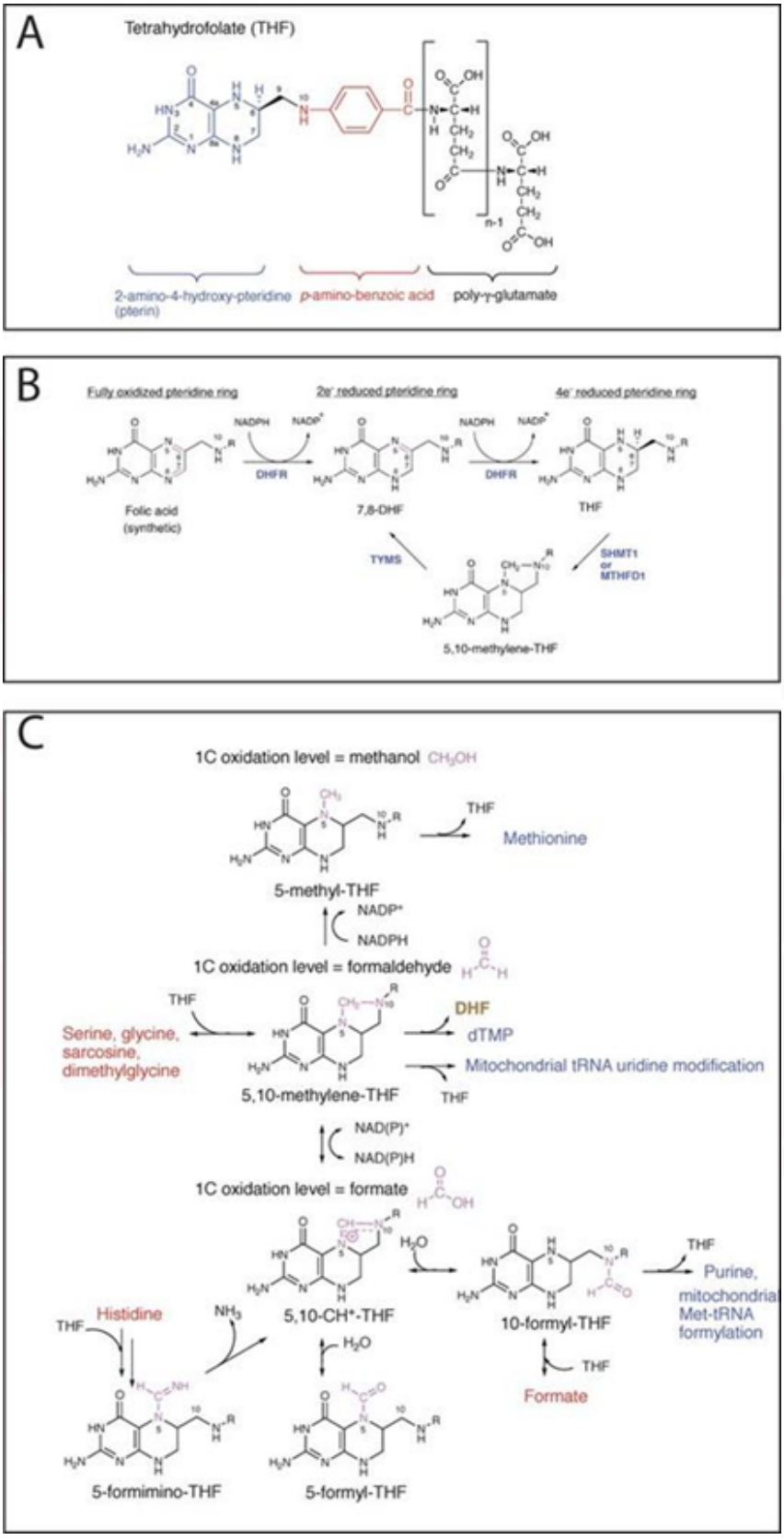


Image from: [Zheng & Cantley, J Exp Med (2019) 216 (2): 253–266.]

Dietary Folate Equivalents (DFEs)

- Folate recommendations are expressed as Dietary Folate Equivalents (DFEs).

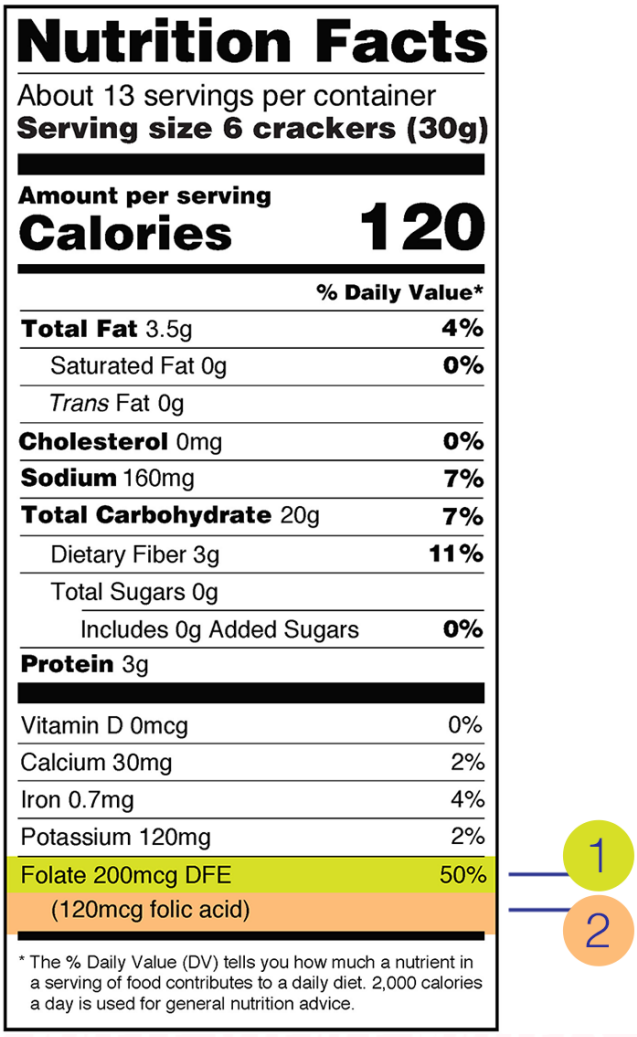
Table 1: Recommended Dietary Allowances (RDAs) for Folate [2]

Age	Male	Female	Pregnancy	Lactation
Birth to 6 months*	65 mcg DFE*	65 mcg DFE*		
7–12 months*	80 mcg DFE*	80 mcg DFE*		
1–3 years	150 mcg DFE	150 mcg DFE		
4–8 years	200 mcg DFE	200 mcg DFE		
9–13 years	300 mcg DFE	300 mcg DFE		
14–18 years	400 mcg DFE	400 mcg DFE	600 mcg DFE	500 mcg DFE
19+ years	400 mcg DFE	400 mcg DFE	600 mcg DFE	500 mcg DFE

Table: NIH

- This unit was created in order to account for the greater bioavailability of folic acid, compared to naturally-occurring dietary folate.
- The DFE amount differs based on the form of folate used (*µg = microgram*):
    - 1 µg of dietary folate = 1 µg of DFEs
    - 1 µg of folic acid (taken with meals or as fortified food) = 1.7 µg of DFEs
    - 1 µg of folic acid (supplement taken on an empty stomach) = 2 µg of DFEs
- Or put the opposite way:
    - 1 µg DFE = 1 µg dietary folate
    - 1 µg DFE = 0.6 µg of folic acid (taken with meals or as fortified food)
    - 1 µg DFE = 0.5 µg of folic acid (supplement taken on an empty stomach)

On US food labels, the total amount of folate (in DFE) and its % of daily value (DV) is shown for the product (see image on next page). And if any of the total folate comes from folic acid, that amount of folic acid is listed in parentheses.



Nutrition Facts

About 13 servings per container
Serving size 6 crackers (30g)

Amount per serving
Calories **120**

	% Daily Value*
Total Fat 3.5g	**4%**
Saturated Fat 0g	**0%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 160mg	**7%**
Total Carbohydrate 20g	**7%**
Dietary Fiber 3g	**11%**
Total Sugars 0g	
Includes 0g Added Sugars	**0%**
Protein 3g	
Vitamin D 0mcg	0%
Calcium 30mg	2%
Iron 0.7mg	4%
Potassium 120mg	2%
Folate 200mcg DFE	50%
(120mcg folic acid)	

* The % Daily Value (DV) tells you how much a nutrient in a serving of food contributes to a daily diet. 2,000 calories a day is used for general nutrition advice.

Source: fda.gov

One-Carbon Metabolism & the Folate Cycle

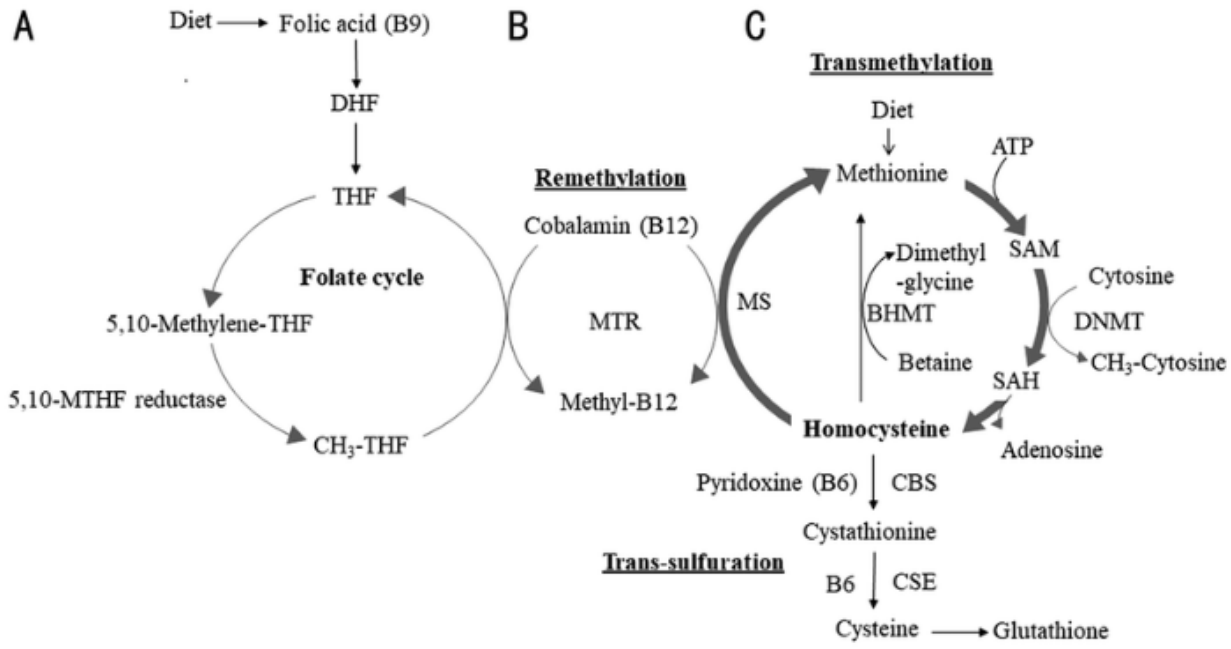
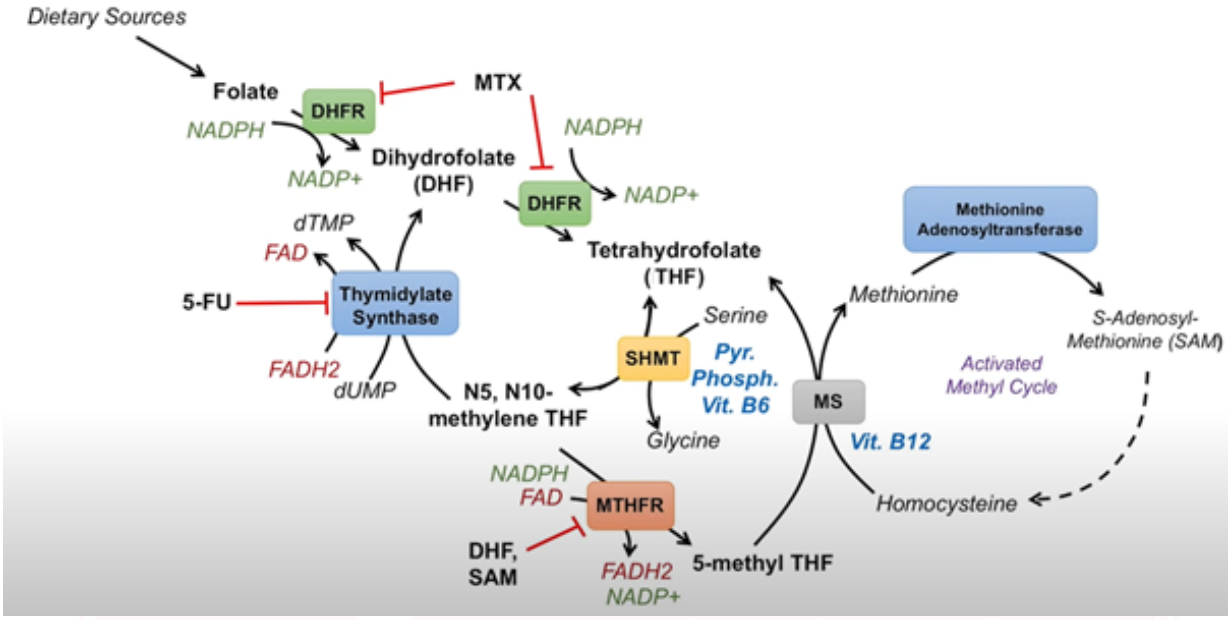


Image from: George et al., 2019

A quick explainer of 1C metabolism and the role of the folate and methionine cycles:

- Dietary sources are where we get our folate/folic acid
- This folate is then acted on by the enzyme dihydrofolate reductase to covert it into dihydrofolate (DHF)
- The same enzyme (i.e. DHFR) now acts on DHF to convert it to tetrahydrofolate (THF).
- THF can then be converted to 5, 10- methylene THF (via the action of an enzyme SHMT)
- Then the enzyme MTHFR can act on 5,10-methylene THF to convert it to 5-methyl THF.
- 5-methyl THF can be recycled to THF (due to the action of methionine synthase, MS)
- In another cycle (see cycle on the right in diagram above), MS converts homocysteine into methionine.
- Methionine can then in turn be converted to SAM, which can ultimately end up being recycled to homocysteine.

Another way of representing those cycles:



> "The folate cycle provides one-carbon units for an extensive metabolic network that fuels the methionine cycle, transsulfuration pathway, de novo purine synthesis, thymidine production, serine, glycine, glutathione, and NADPH pools, and thereby regulates cellular redox state, growth, and proliferation" – *Annibal et al., Nature Communications volume 12, Article number: 3486 (2021)*

Folate Deficiency

Folate deficiency can be caused by several factors:

- Dietary insufficiency
    - Severe deficiency in either folate or vitamin B12 can lead to megaloblastic anemia
- Chronic, heavy alcohol consumption
- Smoking
- Pregnancy increases folate needs
- Cancer/inflammation can increase demands
- Malabsorptive conditions – IBD and Coeliac
- Medications

Genetic Variants

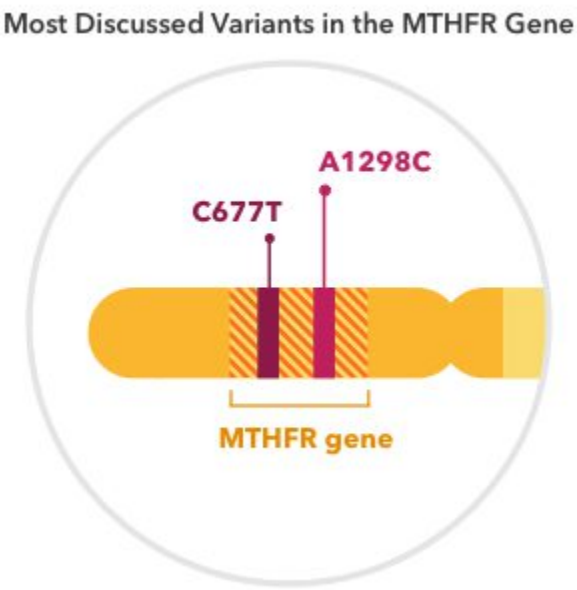


Image credit: 23andme.com

- Folate status is influenced by the presence of genetic variations in folate metabolism. Of particular importance are variations found in the gene encoding for **5,10-methylenetetrahydrofolate reductase (MTHFR)**.
- Most commonly the focused on a variation in what DNA base is found at nucleotide 677. (Another is A1298C).
- Usually, at this position a cytosine (C) should be found.
- However, a variation can occur whereby a thymine (T) base is found at this position instead of a C. This polymorphism is sometimes written as MTHFR c.677C>T or MTHFR c.677C→T
- Each of us has two copies of this gene, therefore meaning someone may be:
    - MTHFR 677 CC
    - MTHFR 677 CT (heterozygous for the variant)
    - MTHFR 677 TT (homozygous for the variant)

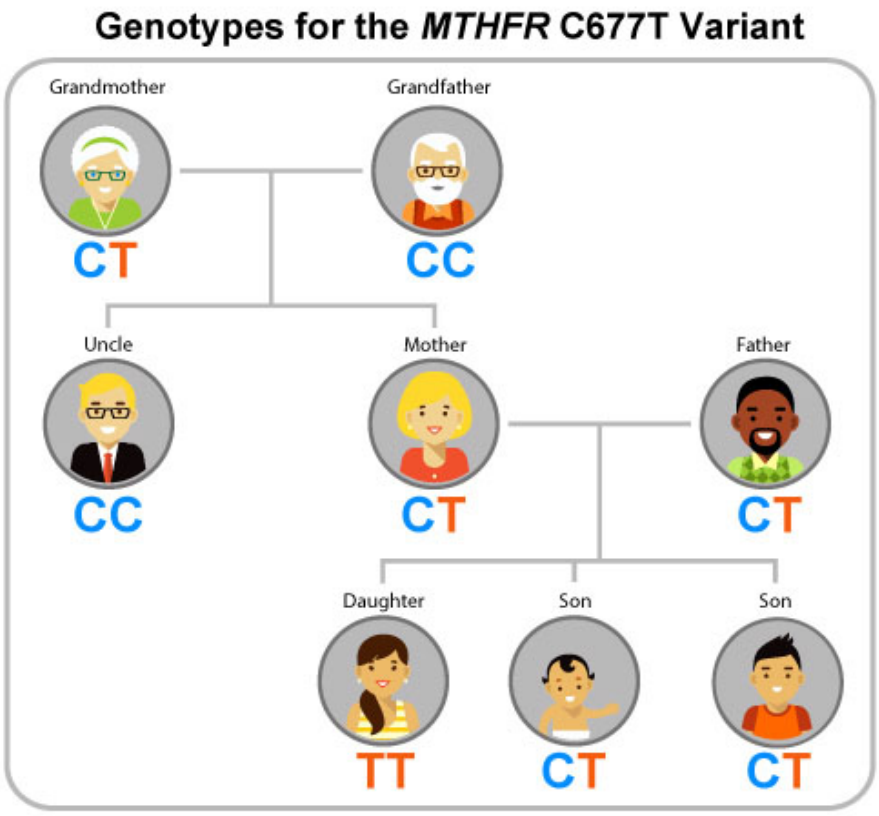


Image from: cdc.gov

- Have a variant in the gene is common. Depending on the population (Wilcken et al., 2003):
    - 20 - 53% of individuals may be MTHFR 677 CT
    - 3 - 32% of individuals may be MTHFR 677 TT
- MTHFR activity is impaired by:
    - 30% in heterozygous 677C/T
    - 65% in homozygous 677T/T
- How much extra folate (if at all) individuals with a polymorphism should consume is still debated.

Neural Tube Defects

Neural tube defects (NTDs) are the result of the embryonic neural tube failing to close (The neural tube in the fetus develops into the brain and spinal cord). This is usually between day 21 and 27 after conception. As this is a time when many women may not even realize they are pregnant, folic acid supplementation is recommended for all women of child-bearing age.

The most common NTDs are:

1. Anencephaly (which results in stillbirth or death soon after delivery)
2. Spina bifida (which may lead to a range of physical disabilities including partial or total paralysis)

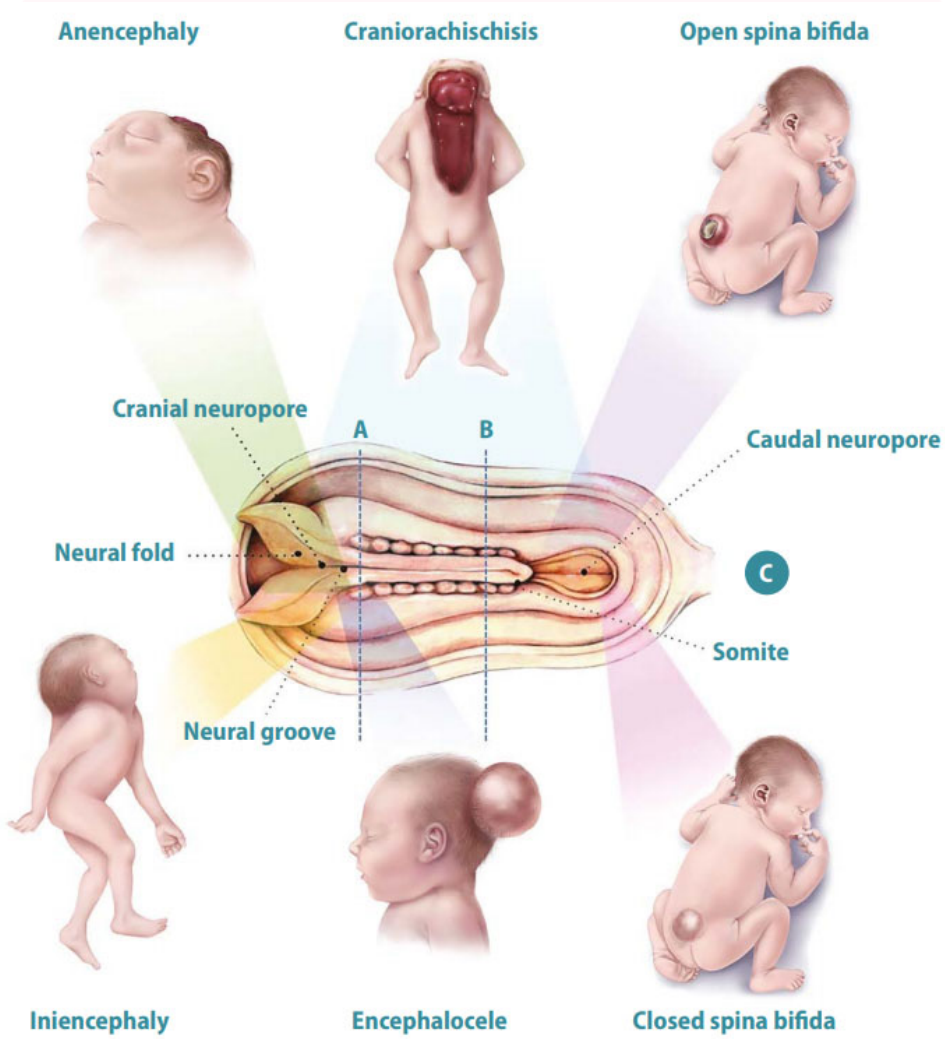


Image credit: cdc.gov
Adapted from: Botto et al. Neural tube defects. N Engl J Med. 1999;341(20):1509–19

Results of randomized trials have demonstrated anywhere between 60% to 100% of NTD cases can be avoided, if women are supplemented with folic acid in the periconceptual period. This is about one month before and at least one month after conception.

A Cochrane systematic review found a 70% reduction in risk for neural tube defects from folic acid supplementation in the 2-3 months prior to, and after, conception.

Trials in the early 90s shed significant light on this impact, notable examples being:

1. Medical Research Council Vitamin Study, 1991 – Lancet
2. NEJM Trial from Hungary – Czeizel & Dudás, 1992

Medical Research Council Vitamin Study, 1991 – Lancet

1. 33 centers in seven countries
2. 1817 women at high risk of having a pregnancy with a neural tube defect, because of a previous affected pregnancy
3. 4 groups:
    - Folic acid
    - 7 other vitamins (A, D, B1, B2, B6, C, and nicotinamide)
        i. [nicotinamide = a water-soluble form of vitamin B3 or niacin]
    - Both
    - Neither
4. 27 of these had a known neural tube defect:
    - 6 in the folic acid groups
    - 21 in the two other groups
5. 72% protective effect (relative risk 0.28, 95% CI =l 0.12-0.71)

TABLE V—PREVALENCE OF NEURAL TUBE DEFECTS ACCORDING TO RANDOMISATION GROUP AMONG WOMEN WITH INFORMATIVE PREGNANCIES: SUBORDINATE ANALYSIS EXCLUDING WOMEN WHO STOPPED TAKING CAPSULES (ON-TREATMENT ANALYSIS)

Randomisation group			All women			Women not already pregnant at randomisation*		
	Folic acid	Other vitamins	NTD/all		Relative risk: folic acid *vs* non-folic acid (95% CI)	NTD/all		Relative risk: folic acid *vs* non-folic acid (95% CI)
A	+	−	1/280	4/558 (0·7%)	0·21 (0·07–0·62)	1/242	3/483 (0·6%)	0·17 (0·05–0·59)
B	+	+	3/278			2/241		
C	−	−	11/281	19/558 (3·4%)		10/243	17/477 (3·6%)	
D	−	+	8/277			7/234		

*First day of last menstrual period was 14 days or more after admission.

Table from: MRC Vitamin Study Research Group, Lancet. 1991 Jul 20;338(8760):131-7.
Copyright © 1991 Published by Elsevier Ltd.

NEJM Trial – Czeizel & Dudás, 1992

- RCT in women planning a pregnancy (in most cases their first)
- Two groups:
    - vitamin supplement (containing 12 vitamins, including 0.8 mg of folic acid; 4 minerals; and 3 trace elements)
    - trace-element supplement (containing copper, manganese, zinc, and a very low dose of vitamin C)
- Daily supplementation for at least one month before conception and until the date of the second missed menstrual period or later.
- Pregnancy was confirmed in 4753 women.
- Congenital malformations:
    - trace-element supplement = 22.9 per 1000
    - vitamin-supplement group = 13.3 per 1000
- Neural-tube defects:
    - trace-element supplement = 6 total cases
    - vitamin-supplement group = zero cases

Table from: Czeizel & Dudás, N Engl J Med 1992; 327:1832-1835

Table 3. Congenital Malformations, According to Study Group.

Malformation	Vitamin Group	Trace-Element Group
	number	
Neural-tube defect	0	6
Congenital hydrocephalus	0	2
Cardiovascular malformation	6	9
Cleft palate	0	2
Cleft lip (with or without cleft palate)	4	3
Hypospadias	1	1
Obstructive defects of urinary system	1	2
Congenital postural deformity	2	0
Limb-reduction defect	1	5
Foramina parietale permagna	0	2
Exomphalos and gastroschisis	1	1
Large hemangioma on face	3	1
Down's syndrome	2	3
Unidentified multiple malformations	3	3
Other*	4	7
Total	28	47

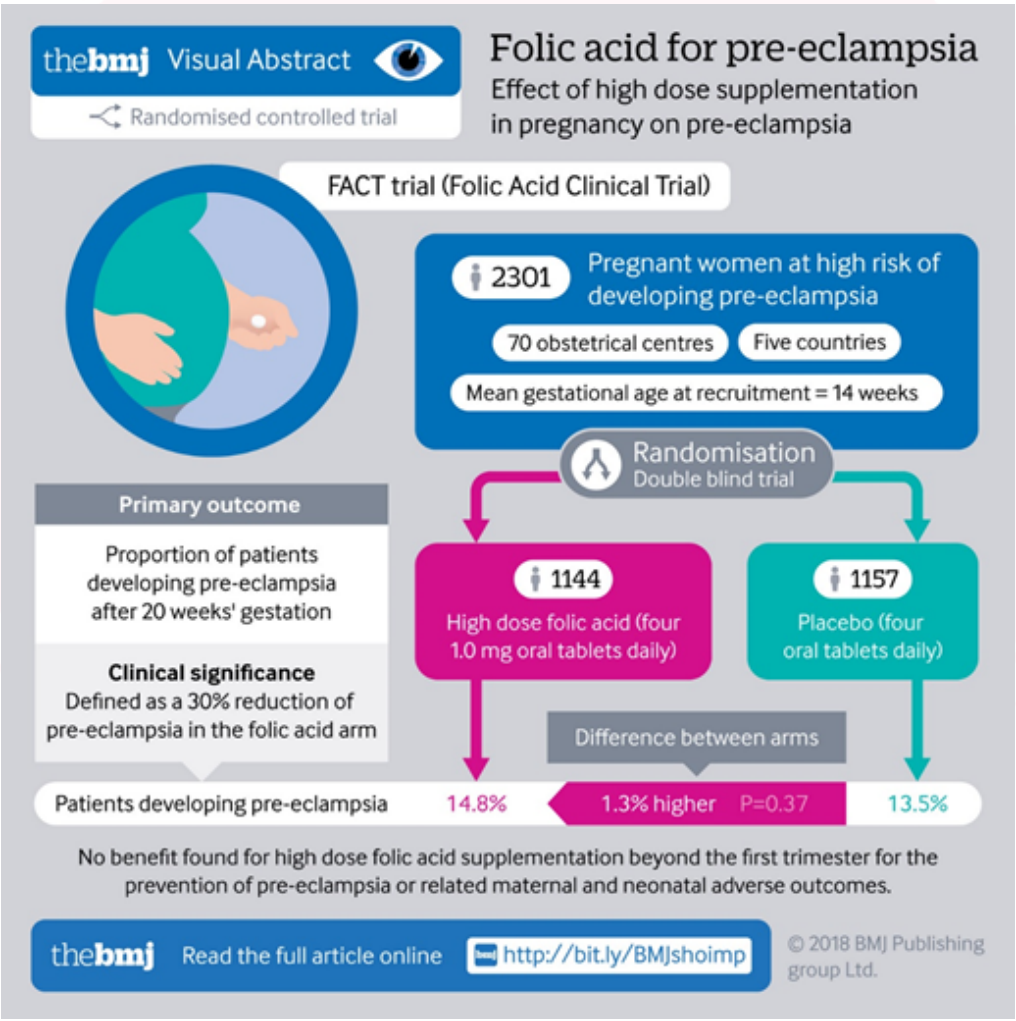
*Each congenital malformation occurred only once in either group.

The recommended folate concentration for pregnancy (>906nmol/L) is difficult to achieve through diet alone, and thus folic acid supplementation is recommended in addition to a folate-rich diet.

Folic acid fortification in the food supply is now mandated in 87 countries globally, and this policy has been associated with reduced prevalence of neural tube defects in certain countries, but is currently not mandatory in Europe.

Other Pregnancy Outcomes

- Systematic review and meta-analysis of 8 RCTs: positive association between folic acid supplementation and birth weight
- Prospective cohort study: Low folate intakes and maternal folate status during the third trimester of pregnancy were associated with higher incidence of small for gestational age births (i.e. a birth weight in the lowest 10th percentile)
- FACT clinical trial: Supplementation with 4.0 mg/day folic acid beyond the first trimester does not prevent pre-eclampsia in women at high risk for this condition (see graphic below).



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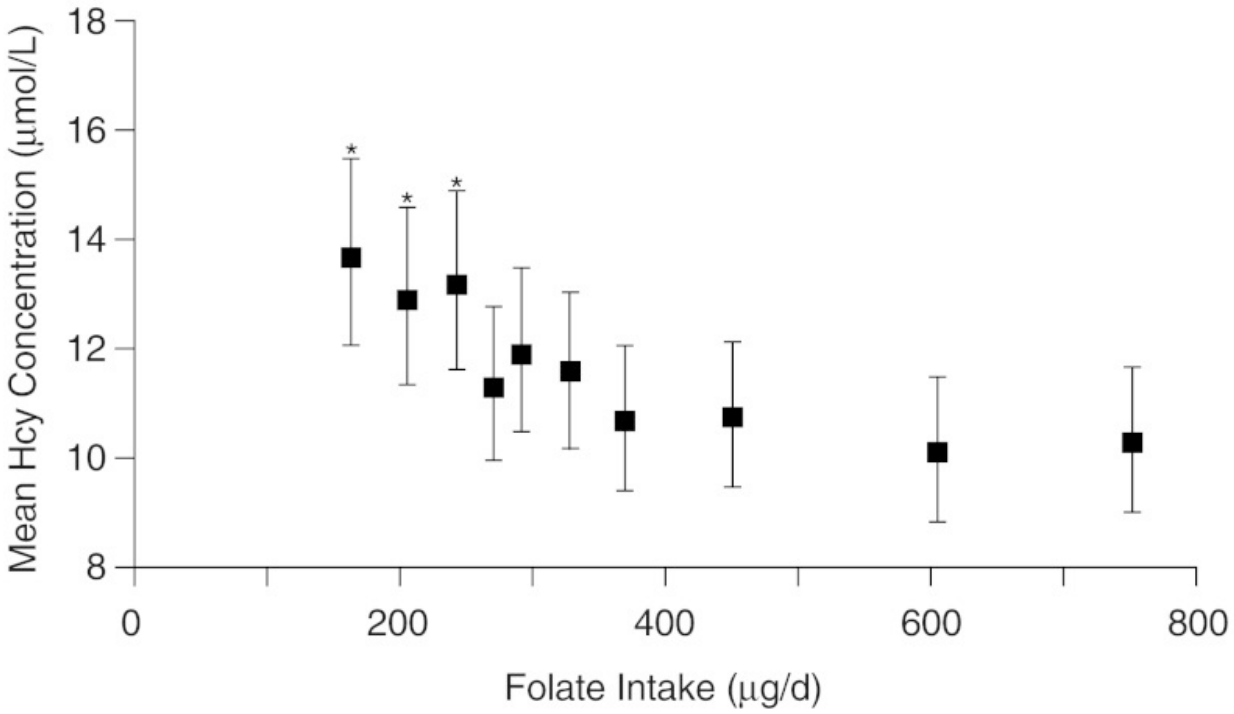
Cardiovascular Disease

Folate deficiency and elevated concentrations of homocysteine in the blood are associated with increased risk of cardiovascular disease (CVD). Although folic acid supplementation has been proven effective to control circulating homocysteine concentrations, the effect of homocysteine lowering on the incidence of CVD is still debated.



Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, Other B Vitamins, and Choline. Washington (DC): National Academies Press (US); 1998.

Homocysteine

- Elevated homocysteine is a consistent and independent risk factor for cardiovascular disease specifically stroke but cardiovascular disease generally.
- However, based on current evidence, it is not a causal risk factor.
- Folate supplementation can reduce homocysteine levels (2005 meta-analysis).
- But again, the question is; does this translate to impacts on cardiovascular disease outcomes?

Folate Supplementation & CVD

- A 2012 meta-analysis of RCTs found that homocysteine lowering through folic acid supplementation failed to reduce the incidence of CVD (even though homocysteine levels dropped considerably). Others have shown the same lack of effect.

- However, there may be an effect specifically for atherosclerosis: a meta-analysis found supplementation led to significant reductions in carotid intima-media thickness (CIMT) in those at risk for CVD.

Cancer

- Originally, some worried that widespread folic acid supplementation or fortification could actually increase cancer risk, due to folic acids role in DNA synthesis (and therefore theoretically in tumor growth promotion).
- However, evidence from food fortification programs and other human data has shown that this does not increase cancer risk.
- On the other side, some associational work has suggested a reduced risk of some cancers, specifically colorectal cancer.
- However, overall, meta-analyses (e.g. Vollset et al., 2013) of folic acid intervention trials (of doses from 500 to 5,000 µg/day for > one year) did not show any specific effect on total or site-specific cancer incidence.

Cognitive Impairment

[For more details on brain health and diet check out episode 438]

VITACOG Study:

- Combined B-vitamin supplements (high doses beyond RDI) for 2 years
- Found beneficial effects on cognitive performance in participants with mild cognitive impairment and elevated homocysteine levels.
- Participants: age ≥70 years
- The treatment group received oral tablets containing:
    - 0.8 mg folic acid
    - 0.5 mg vitamin B12
    - 20 mg vitamin B6
- Results:
    - Treatment with B vitamins significantly slowed the rate of brain atrophy.
    - After adjustment for age, the rate of brain atrophy per year was 29.6% less in the active treatment group compared to the placebo group.
- The effect of treatment was dependent on baseline homocysteine.
- Secondary analysis: Conversely, the effects of B-vitamin supplementation on protecting against brain atrophy were enhanced with the highest levels of omega-3 fatty acid levels, a relationship which was stronger overall for DHA compared to EPA.

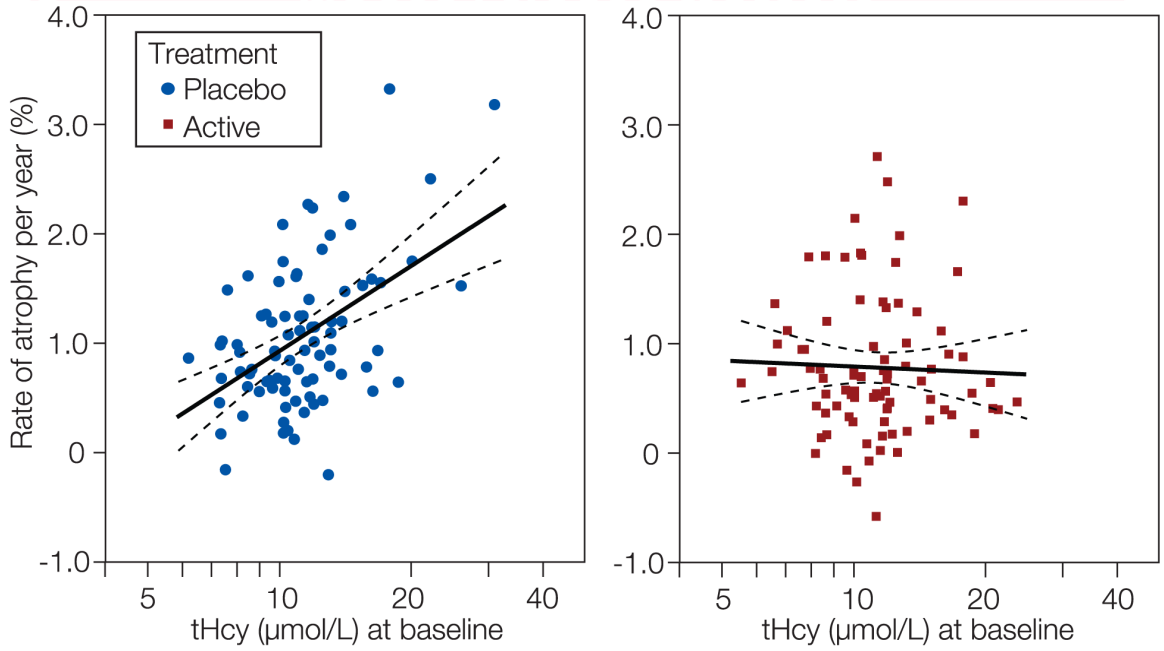


Image from: Smith et al., 2010 - VITACOG

Practical Application

- RDA:
    - Men and women ages 19 years and older should aim for 400 mcg DFE.
    - Pregnant and lactating women require 600 mcg DFE and 500 mcg DFE, respectively.
- Food sources of folate include:
    - Spinach, kale, Brussels sprouts, cabbage, broccoli
    - Beans and legumes (e.g. peas, blackeye beans)
    - Peanuts
    - Sunflower seeds
    - Fresh fruits, fruit juices
    - Wheat bran and other whole grain foods
    - Poultry, pork, shellfish and liver
    - Fortified foods
- For those that may become pregnant, the amount of folate in the diet may not be high enough to maximally reduce risk of NTDs. Therefore supplementation is recommended.