

Let Them Eat Cake

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Abstract: Folate, a B vitamin found almost ubiquitously in foods, is required for *de novo* nucleotide synthesis and methylation reactions in our body. A shortage of folate can lead to macrocytic anemia, however, extra folic acid, the synthetic form of folate, is considered to be a modern day panacea for a variety of diseases, specifically vascular disease. This review summarizes the evidence from randomized controlled trials and illustrates the weak foundation on which health claims regarding the possible beneficial effect of extra folic acid for vascular disease are based. It is prudent for public health policy makers to await trials--the conventional golden standard by which to test causality--before deciding on folic acid fortification for the general population.

PANACEA

Folic acid, the synthetic form of folate, a B vitamin, has been called the leading contender for the panacea of the 21st century [1]. The speed at which folate has reached new heights as a cure-all for many modern day ills is astonishing, even faster than the introduction of sugar to the common man's diet [2]. Lucy Wills, in 1931, reported on a factor in yeast which could correct macrocytic anemia in pregnant women, later revealed to be folate. Macrocytic anemia in patients with celiac disease, idiopathic steatorrhoea, sprue and undernutrition responded better to folic acid, newly synthesized by Bob Stockstad in 1943, than to liver or yeast extracts [3]. The historical context may explain why definitions of folate deficiency are based on abnormal changes in blood such as macrocytic anemia. Since the discovery of folate, low concentrations of folate have been linked to a multitude of diseases and chronic conditions, like vascular disease, dementia and age-related hearing loss, either as a cause or a consequence [4, 5].

FOLATE FROM FOOD

Folate is present almost ubiquitously in natural foods, but still many adults do not consume enough folate to meet the dietary reference intake. The estimated folate intake in Dutch adults aged 20 to 65 years is approximately ~200-250 µg/d, lower than the dietary reference intake of 300 µg of folate per day [6]. One can achieve adequate folate status eating a diet high in folate-rich foods such as fruits and vegetables [7-10]. Poor dietary habits e.g. diets high in refined grains, high-fat dairy products and red meat rather than fruits and vegetables, whole grains and poultry, may help explain low dietary folate intake and low folate concentrations [11-18].

Dietary patterns are known to change with age, and aging has been associated with lower folate intake and low folate concentrations [19-21]. In addition to poor dietary habits, aging may influence one's folate metabolism. With increasing age, the activity of enzymes involved in folate

metabolism may decrease and the incidence of malabsorptive intestinal disease, like atrophic gastritis increases. Furthermore, folate metabolism is impaired as a consequence of secondary nutrient deficiencies in iron and vitamins B₆ and B₁₂ [22].

To ensure optimal folate status, many countries have adopted folic acid fortification programs. The impetus for this political action spawned from the results of a series of trials conducted in the 1980s and 1990s, which demonstrated that maternal folic acid supplementation reduced the risk of giving birth to a baby with defects such as spina bifida [23]. Since its inception in 1998, folic acid fortification in the United States of America has resulted in an increase in folate concentrations and appears to decrease risk of neural tube defects [24] and cardiovascular disease [25] without increasing the number of elderly with low vitamin B₁₂ status without anemia [26]. The latter is of concern, as folic acid supplementation may mask signs of vitamin B₁₂ deficiency such as megaloblastic anemia. For this reason, the Dutch government has refrained from fortification of food products with folic acid meant for the general population [27]. Monitoring for adverse effects of folic acid is also necessary: maternal folic acid supplementation may increase risk of spontaneous abortion [28, 29] and may increase the number of babies born with the 5,10-methylenetetrahydrofolate reductase (MTHFR) 677TT genotype [30]. Adverse effects of folic acid on risk of cancer are unknown [31].

FUNCTIONS OF FOLATE

Folate is essential for many physiological functions. Recent evidence suggests an antioxidant-like function of folate, but the traditional function of folate is that it accepts and donates one-carbon units (Fig. 1). Various folate derivatives fulfill roles for one-carbon transfer for *de novo* nucleotide and formate synthesis and amino acid interconversions. The bulk of research on folate is attributed to its role with homocysteine, an amino acid formed in cells when the essential amino acid, methionine, is catabolized [4]. An elevated concentration of plasma total homocysteine is an independent risk factor for vascular disease [32]. Folic acid supplementation and extra dietary folate intake from the

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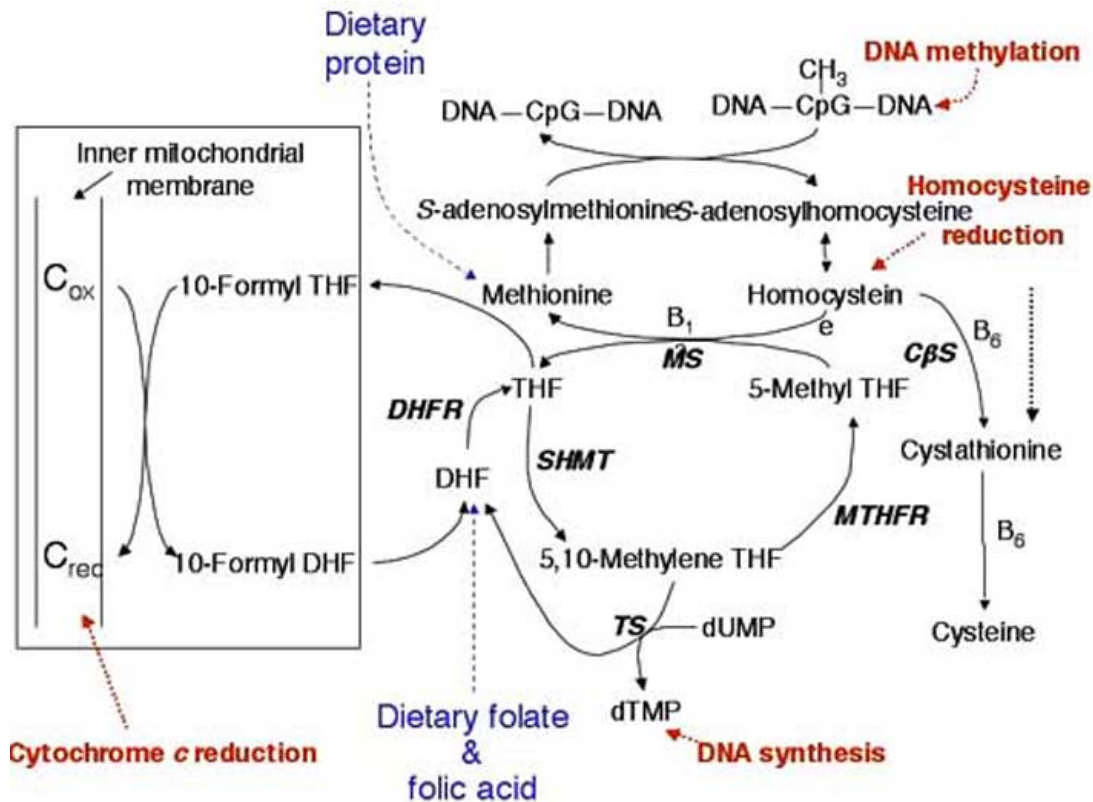


Fig. (1). Metabolism and function of folate.

The MTHFR 677C→T polymorphism, involving a C to T substitution at the 677 base of the gene encoding for MTHFR, is associated with reduced enzyme activity *in vitro*. Subjects with the MTHFR 677TT genotype have in theory a reduced capacity of methyl transfer and greater capacity of formyl folate for i.e. mitochondrial cytochrome *c* reduction (mitochondria are the greatest producers of reactive oxygen species in the cell) and methenyl folate for thymidylate synthase, preventing dUMP accumulation, which has been associated with increased uracil misincorporation in DNA. Alongside the MTHFR 677TT genotype, low intracellular concentrations of folate can influence competition for 5,10-methylene-tetrahydrofolate, which favors commitment of one-carbon moieties for formylfolate and methenylfolate reactions[38]. Subjects with the MTHFR 677TT genotype have increased risk of cardiovascular disease and decreased risk of cancer. The association of the MTHFR 677TT genotype with dementia and neural tube defects is unclear. Abbreviations. DNA-CpG-DNA DNA CpG island; THF tetrahydrofolate; DHF dihydrofolate; MS methionine synthase; C S cystathionine -synthase; DHFR dihydrofolate reductase; SHMT serine hydroxymethyltransferase; MTHFR methylenetetrahydrofolate reductase; TS thymidylate synthase; dUMP deoxyuridylate; dTMP thymidylate; C_{ox} oxidized cytochrome *c*; C_{red} reduced cytochrome *c*.

food may decrease concentrations of plasma homocysteine. Effect of low folate concentrations or folic acid supplementation on coenzyme Q₁₀ has received less research.

SCIENTIFIC OPTIMISM

But will folic acid prevent disease? There is much optimism for folate as a panacea. Governments hesitant with folic acid fortification have even been accused of public health malpractice [33]. Reviews have summarized the wealth of literature from *in vitro*, *in vivo*, animal and human studies, which have shown adverse changes in vascular and neurological tissue associated with low concentrations of folate and elevated concentrations of homocysteine. Likewise, observational studies have shown an independent association of folate and homocysteine with cardiovascular disease. A recent meta-analysis has found that a 2.6 μmol/L higher homocysteine was associated with a 13% (95% CI 8 to 19%) increase in risk of cardiovascular disease [32]. Cohort and case-cohort studies suggest that low folate intake [34-37] and low levels of serum or erythrocyte folate[36, 37,

39-46], independent of homocysteine concentrations [37, 39, 40], are associated with vascular disease morbidity and mortality.

Some believe the evidence on the benefit for homocysteine-lowering by B vitamins is so strong that ethical issues have arisen in B vitamin trials. For example, patients enrolled in a B vitamin trial in the control group were not offered placebo, rather low-doses of B vitamins [47]. Alternatively, subjects with high homocysteine concentrations were excluded from folate trials as it was deemed unethical to allocate them to the placebo treatment (FACIT¹ trial) [48]. A combination pill of 0.8 mg folic acid plus a statin, three antihypertensive agents and aspirin has been brought forth as a therapeutic strategy capable of reducing risk of stroke by 80% and risk of ischemic heart disease by 88% in individuals above 55 years of age [49]. In

¹ FACIT is an acronym for Folic Acid and Carotid Intima-media Thickness. The FACIT trial investigates the effects of 0.8 mg folic acid supplementation for three years on carotid intima-media thickness, cognitive function and hearing.

the case of folic acid, this risk reduction was based solely on observational evidence [50]. Some governments have endorsed health claims for folate. The USA, for example, has authorized the 'qualified health claim' for folic acid, vitamin B₁₂ and vitamin B₆. "As part of a well-balanced diet, rich in fresh fruits and vegetables, daily intake of at least 400 µg folic acid, 3 mg vitamin B₆ and 5 µg vitamin B₁₂ may reduce the risk of vascular disease" [51]. Food manufacturers have put forth health claims suggesting benefit of extra folic acid (in their product) for the 'heart and mind.'

There is surprisingly little evidence from randomized controlled trials to support the B vitamin health claims for the reduction of vascular disease risk, let alone for improving cognitive function (Table 1). One study found a beneficial effect of B vitamin supplementation on restenosis after percutaneous transluminal coronary angioplasty after two years; this effect was confined to those subjects responsive to therapy as measured by homocysteine reduction ($n=553$) [52]. In contrast, in a larger study, percutaneous transluminal coronary angioplasty patients administered B vitamin therapy, initially intravenously and thereafter orally, had smaller lumen diameters and increased rates of restenosis compared with patients taking the placebo ($n=636$) [53]. Three studies found a positive effect of B vitamin supplementation on cardiovascular disease risk as measured by carotid intima-media thickness or abnormal exercise electrocardiography test [54-56]. The former is a validated surrogate marker of vascular disease [57] whereas the latter may be inappropriate as marker for cardiovascular disease risk in the general population [58].

LIMITATIONS IN EVIDENCE

Evidence for the health claims has not come from the trials, with ~8,000 subjects showing no effect of folic acid on cardiovascular disease risk, but has been based on evidence from observational epidemiology [51]. Observational epidemiology suffers from confounding and reverse causality. Confounding, because people with high folate levels differ in many aspects from those with lower levels. These aspects may be difficult to measure and may highly correlate with folate, hindering statistical adjustment [70]. Diets high in folate tend to be high in other nutrients and macromolecules such as antioxidants and other vitamins, minerals, mono- and polyunsaturated fatty acids, n-3 fatty acids, fiber, phytochemicals and plant protein, which could explain its inverse association with risk of cardiovascular disease. Subjects with low folate and high homocysteine concentrations tend to have poor dietary patterns, which have been associated with risk of cardiovascular disease, and more unhealthy lifestyle risk factors, e.g. smoking and low physical activity [11, 12, 16, 17, 71-73]. Secondly, associations from observational epidemiology may be explained by reverse causality. Stronger associations of homocysteine with risk of cardiovascular disease are found from case-control (prone to bias by existing disease influencing homocysteine) rather than from cohort studies [74] and the association from cohort studies is stronger in patient populations than the in the general population [75]. This suggests that homocysteine may be a marker of vascular damage.

MENDELIAN RANDOMIZATION

Genetics, specifically common polymorphisms of folate-dependent enzymes, may give insight into the temporal relationship and may help understand etiology of disease. Mendelian randomization, the random assortment of e.g. MTHFR 677T alleles to offspring, reduces bias and confounding, as exposure (genotype) is not likely to influence other risk factors. An association found between MTHFR 677T allele and disease may support the causal relation of folate or homocysteine with disease [76]. Moreover, Mendelian randomization reduces imprecision; genotype is more representative of 'usual' homocysteine concentrations than a one-off homocysteine measurement with its inherent measurement error [77]. An ideal polymorphism for Mendelian randomization is one that results in appreciable inter-genotype variation in serum markers, like that of a 25% increase in homocysteine concentrations in subjects with the 5,10-methylenetetrahydrofolate reductase (MTHFR) 677TT genotype compared with the CC genotype (Fig. 1) [78]. If indeed folate or homocysteine is causally associated with risk, then support for such a role comes from the 16% (95% CI 5 to 28%) greater risk of coronary heart disease in ~30,000 patients with the MTHFR TT genotype compared with CC genotype [78, 79]. This estimate is largely unconfounded and establishes a temporal relationship between homocysteine and cardiovascular disease. Genetic association studies, as illustrated by Klerk *et al.*, [78] require large sample sizes to avoid spurious results and assumes that MTHFR is not a pleiotropic gene [80].

HEALTH CLAIMS

Neither observational nor genetic studies can tell us whether folic acid supplementation will lead to a reduction in vascular disease. Health claims for folate need to be based on convincing evidence from randomized controlled trials to generate and maintain consumer confidence. To date the majority of trials have been conducted in patients with vascular disease; however, evidence must also come from trials conducted in the general population, if only because the vast majority of consumers are not patients. Such a trial would require a large study population and a lengthy duration to collect enough cases with incident cardiovascular disease. Validated surrogate markers of disease for the general population are needed to make such trials feasible.

Trials will make or break the enthusiasm around folate, just like antioxidant vitamin trials did in the 1990s. Supplementation with β -carotene increased incidence of cardiovascular disease, especially pronounced in smokers [81], a surprise for the research community considering that in observational epidemiology and *in vitro* studies, β -carotene was inversely associated with risk of cardiovascular disease. The lessons from the β -carotene trials have taught us that complex diet rather than single nutrient may be important [82]. The beneficial actions of nutrients from our diet may be the result of food synergy, which reflects the interplay between a prudent dietary pattern, food groups like whole grains, fruits and vegetables and vegetable constituents [82]. Randomized controlled trials show that

Table 1. Summary of Studies Investigating the Effect of Folic Acid on Vascular Disease Endpoints.^a

Design	Duration	Sample size (n) and Treatment	Relative risk (95% confidence interval)
Vascular disease patients			
RCT [59]	2 y	942 940 5 mg FA placebo	1.0 (0.7 to 1.3) Revascularization, recurrent myocardial infarction, cardiac-cause death
RCT [47]	2 y	1827 1853 2.5 mg FA, 25 mg B ₆ , 0.4 mg B ₁₂ 0.02 mg FA, 0.2 mg B ₆ , 6 µg B ₁₂	1.0 (0.8 to 1.1) Recurrent stroke
T [60]	20 m ^b	79 194 5 mg FA, 250 mg B ₆ ^c standard care ^d	0.96 (0.6 to 1.6) Recurrent peripheral disease, coronary or cerebrovascular disease
T [61]	57 m ^b	52 151 5 mg FA, 250 mg B ₆ ^c standard care ^d	1.03 (0.6 to 1.9) Recurrent cerebrovascular disease, coronary or peripheral disease
RCT ^e	6 m	353 348 5 mg FA, 50 mg B ₆ , 0.4 mg B ₁₂ placebo	0.8 (0.6 to 1.3) Recurrent venous thrombosis
T [62, 63]	5 y ^{b,f}	101 2.5 mg FA, 25 mg B ₆ , 0.25 mg B ₁₂	-0.2 cm ² /y change in rate of carotid plaque progression
RCT [53]	6 m	316 320 1.2 mg FA, 48 mg B ₆ , 0.06 mg B ₁₂ ^g placebo	1.5 (1.0 to 2.3) Revascularization target vessel, myocardial infarction, cardiac-cause death
RT [64]	1 y	140 143 5 mg FA, statin statin	0.98 (0.7 to 1.4) Recurrent myocardial infarction, stroke, invasive vascular intervention, all-cause death
RT [65]	2 y ^b	300 293 0.5 mg FA standard care	1.1 (0.6 to 1.8) Recurrent myocardial infarction, stroke, invasive vascular intervention, all-cause death
RCT [52]	6 m	272 281 1 mg FA, 10 mg B ₆ , 0.4 mg B ₁₂ placebo	0.5 (0.3 to 0.9) Restenosis
RCT [66]	6 m	58 55 1 mg FA, 10 mg B ₆ , 0.4 mg B ₁₂ placebo	0.3 (0.2 to 0.7) Restenosis
RCT [67]	1 y	272 281 1 mg FA, 10 mg B ₆ , 0.4 mg B ₁₂ placebo (6 m treatment only)	0.7 (0.5 to 0.96) Revascularization, myocardial infarction, all-cause death
RCT [54]	1 y	50 2.5 mg FA, 25 mg B ₆ , 0.5 mg B ₁₂ or placebo	-0.15 mm in carotid intima-media thickness
Renal disease patients			
RCT [55]	6 m	25 28 5 mg FA, 50 mg B ₆ , 0.4 mg B ₁₂ placebo	55% regression in carotid intima-media thickness
RT [68]	2 y	174 177 177 15 mg FA 5 mg FA 1 mg FA	1.6 (0.6 to 1.9) Cardiovascular events, death (15 mg FA vs. 1 mg FA) 0.9 (0.2 to 4.2) Cardiovascular events, death (5 mg FA vs. 1 mg. FA)
Non-patient population			
RCT [56]	2 y	78 80 5 mg FA, 250 mg B ₆ placebo	0.9 (0.6 to 1.3) Ankle brachial index 1.0 (0.3 to 4.1) Peripheral stenosis 0.9 (0.5 to 1.6) Carotid stenosis 0.4 (0.2 to 0.9) Abnormal exercise ECG
RCT [69]	13 m ^b	68 73 5 mg FA, 250 mg B ₆ placebo	0.5 (0.2 to 1.4) Abnormal magnetic resonance angiography

^a Abbreviations. R randomized; C controlled; T trial; FA folic acid; ECG electrocardiography.^b Follow up time.^c Hyperhomocysteinemic.^d Non-hyperhomocysteinemic.^e M den Heijer. Personal communication.^f Follow up time before treatment 2.7 - 4.5 y, follow up time after treatment 1.6 - 1.8 y.^g First day treatment 1 mg FA, 5 mg B₆, 1 mg B₁₂ intravenously.

food-base interventions can decrease risk of cardiovascular disease [83]. Whether strategies that increase consumption of folate-rich foods rather than relying on folic acid supplementation are more successful in effectively preventing cardiovascular disease remains to be seen. Like Marie Antoinette's solution to the hungry women who stormed the Palace of Versailles in Paris of 1789, "Let them eat cake," foods fortified with folic acid may prove to be just as frivolous.

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