

CARDIOVASCULAR

5-METHYLTETRAHYDROFOLATE - THE ACTIVE FORM OF FOLIC ACID

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Introduction

The generic term folates refers to both folic acid (pteroylmonoglutamic acid) and the naturally occurring reduced folates (polyglutamates), e.g. 5-methyltetrahydrofolate(5-MTHF), 10-formyl-tetrahydrofolate(THF), THF and 5,10-methylene-THF. Folic acid is the most oxidised and hence stable form of folate. Folic acid does not occur naturally, but it is widely used in vitamin supplements, and for the fortification of food. The natural folates are present as polyglutamates, with the glutamate moieties linked via γ -carboxyl peptide bonds. Dietary sources rich in folates are green leafy vegetables such as spinach and broccoli, and oranges and liver. The most abundant natural folates are 5-MTHF and 10-formyl-THF (Fig. 1).

The folate polyglutamates need to be converted to monoglutamates before absorption. This conversion is catalysed by the enzyme folate conjugase in the brush border of the small intestine. The folate monoglutamates are subsequently actively transported across the proximal small intestine by a saturable process. Alternatively, folates can also be absorbed by a passive diffusion mechanism. During absorption, all natural folates are converted to 5-MTHF, which is the only form of folate to enter the human circulation under normal conditions. Folic acid from supplements or fortified food is already present as a monoglutamate and is therefore easily absorbed. During transit through the gut wall, folic acid is reduced to THF and then converted to 5-

MTHF. This conversion capacity, however, is limited. It has been shown that, even after intake of only 200 μg of folic acid, unmetabolised folic acid appears in the human circulation (1). The monoglutamate form of 5-MTHF is also readily absorbed and appears in plasma without biotransformation. 5-MTHF is then bound to plasma proteins or folate-binding protein, transported to the liver and then distributed to the various tissues (Fig. 2). 5-MTHF is taken up by target cells in a receptor-mediated regulated fashion, then converted to THF and subsequently to the polyglutamate form of THF.

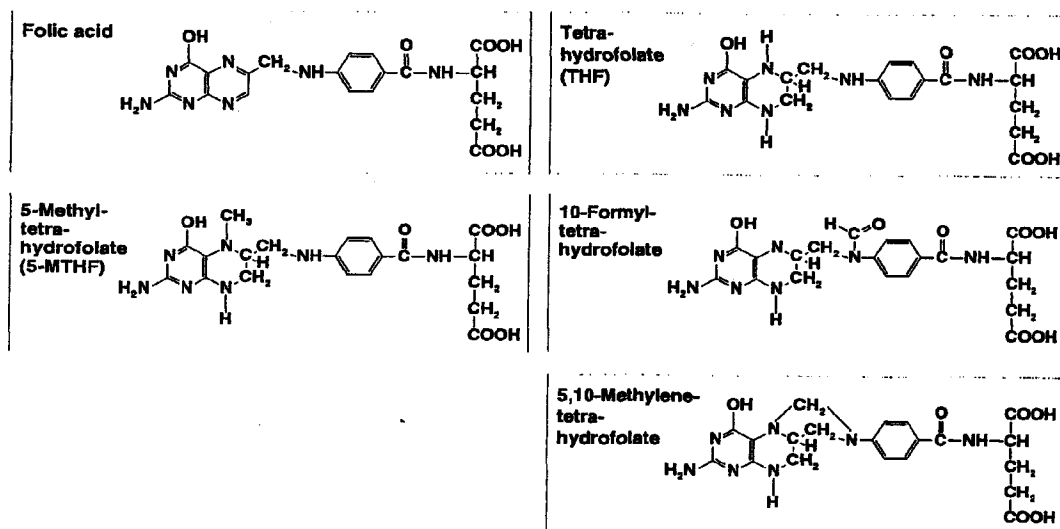


Fig. 1. Structure of folates (monoglutamates)

The main function of folates is to participate in DNA synthesis and to donate methyl groups. THF functions as a one-carbon unit donor (formyl group) for purine biosynthesis. The C₈ and C₂ positions in the purine ring are derived from the folate one-carbon pool. Additionally, a methylene group attached to THF is utilised for the conversion of uracil to thymine. This reaction is catalysed by thymidylate synthase. Furthermore, folates in the form of 5-MTHF provide methyl groups for methyltransferases; for example, 5-MTHF participates in the remethylation of homocysteine to methionine (2). This reaction also depends on the presence of vitamin B₁₂, and is catalysed by methionine synthase (Fig. 3, adapted from (2)). Folate deficiency slows down purine and pyrimidine biosynthesis, and hence DNA synthesis and cell division. The first site where reduced DNA synthesis becomes apparent is rapidly dividing tissues such as bone marrow. Folate deficiency therefore results in anaemia (2).

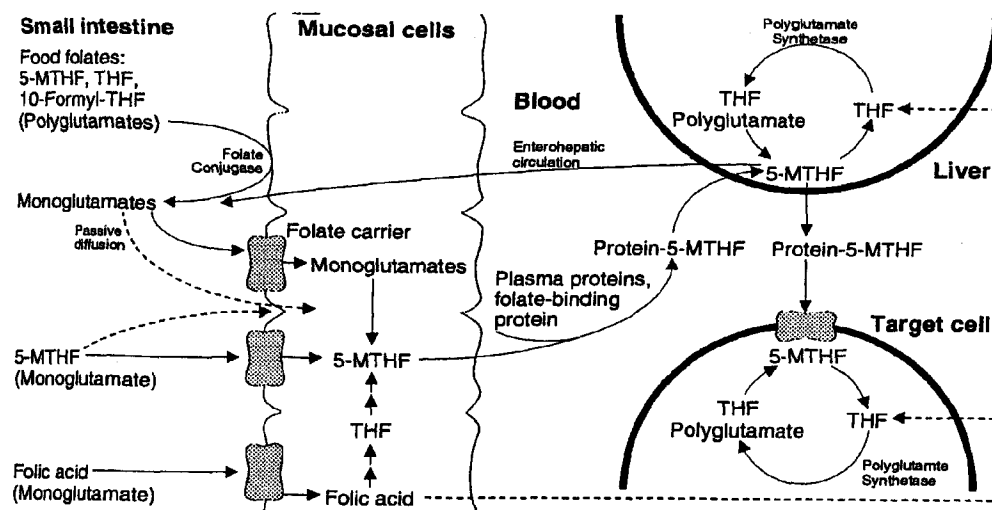


Fig. 2. Absorption and metabolism of folates

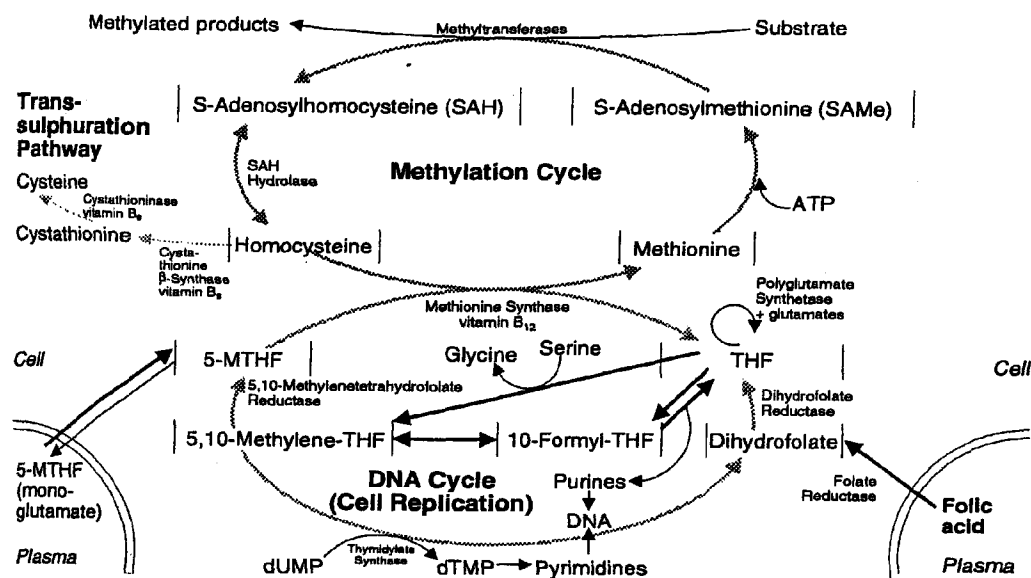


Fig. 3. Absorption and metabolism of folates (adapted from (2) with kind permission by Lippincott Williams & Wilkins, Philadelphia, USA)

Beneficial Effects of Folates

The benefit of folic acid supplementation for the prevention of neural tube defects (NTDs) has been confirmed by several intervention trials (3). NTDs are caused by a failure of the neural tube to close and present as spina bifida and anencephaly. The prevalence varies between countries and ranges from 0.1% in the US to 1.1% in northern China. About 50-70% of NTDs can be prevented by daily intake of 400 µg of folic acid during the periconceptual period and early pregnancy.

Folates also play an important role in the prevention of cardiovascular disease (CVD). The reduction in cardiovascular risk by folates is attributed to a decrease in plasma homocysteine, a major independent risk factor for CVD (4). Homocysteine is a sulfur-containing amino acid and intermediate in the methionine metabolism. Approximately 80% of homocysteine is bound to plasma proteins, whereas the remaining 20% is present as dimer homocysteine, mixed disulfides or free homocysteine. Normal plasma homocysteine levels range from 5-15 µmol/litre. An increase in homocysteine by 5 µmol/litre increases cardiovascular risk by 60-80% (5). Folic acid (0.4-5.0 mg/day) lowers homocysteine by 25%. Further lowering (7%) can be achieved by adding vitamin B₁₂, but not vitamin B₆ (6). Although the studies demonstrating the above-mentioned benefits used folic acid for supplementation, it is highly likely that 5-MTHF rather than folic acid accounts for the beneficial effects, as folic acid is converted to 5-MTHF during mucosal transit. Furthermore, only 5-MTHF, not folic acid, can participate in the remethylation of homocysteine. Hence, it is not surprising that oral administration of 5-MTHF also significantly reduced plasma homocysteine in patients on haemodialysis (Fig. 4) (7) and in healthy subjects (Fowler, B. - personal communication).

There are as yet no intervention trials that show the benefits of folates and folic acid in the prevention of CVD. However, nine such intervention trials are ongoing at present, and there is sufficient evidence for a causal relationship between high plasma homocysteine and CVD:

- ❖ Three rare genetic mutations (cystathionine β-synthase, methionine synthase and methylenetetrahydrofolate reductase (MTHFR)) are associated with severely elevated homocysteine and result in CVD and stroke.
- ❖ Cases with CVD or stroke have higher plasma homocysteine than matched controls.

- ❖ Prospective studies demonstrate that cases of cardiovascular death had higher homocysteine levels in blood samples taken 20 years ago compared to controls (8).
- ❖ Progression to death from CVD is predicted by plasma homocysteine at diagnosis (9).
- ❖ Stenosis of carotid artery increases in parallel with plasma homocysteine.
- ❖ Lowering plasma homocysteine by folic acid is associated with reduced arterial plaque (10).
- ❖ In most studies, the relationship between plasma homocysteine and CVD was independent from other known risk factors for CVD (smoking, cholesterol, BMI, age, blood pressure, and diabetes).

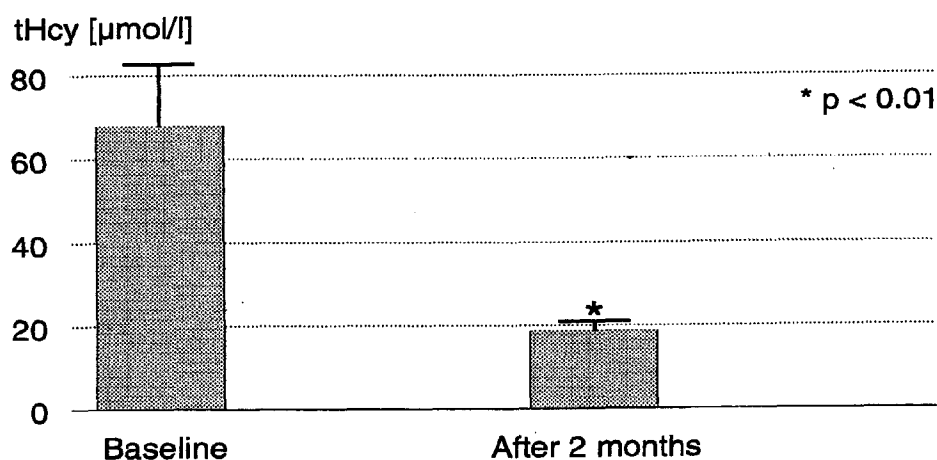


Fig. 4. Plasma total homocysteine in 14 patients on haemodialysis treated with 15 mg 5-MTHF (7)

Biochemical evidence suggests that the association between elevated homocysteine and CVD is attributable to endothelial damage and increased clotting. These effects may be mediated by increased formation of reactive oxygen species in hyperhomocysteinaemia (Fig. 5). For example, it has been shown that 5-MTHF infusion restored endothelial function in familial hypercholesterolaemia *in vivo*, possibly as a result of reduced catabolism of NO (11).

Hence, optimal folate intake may reduce cardiovascular risk substantially. Owing to the clear relationship between folate intake and health, many

governmental agencies have established a recommended dietary allowance (RDA) for folate. The FDA established a reference daily intake of 400 μg for adults. The RDA for pregnant women is 600 $\mu\text{g}/\text{day}$ (12).

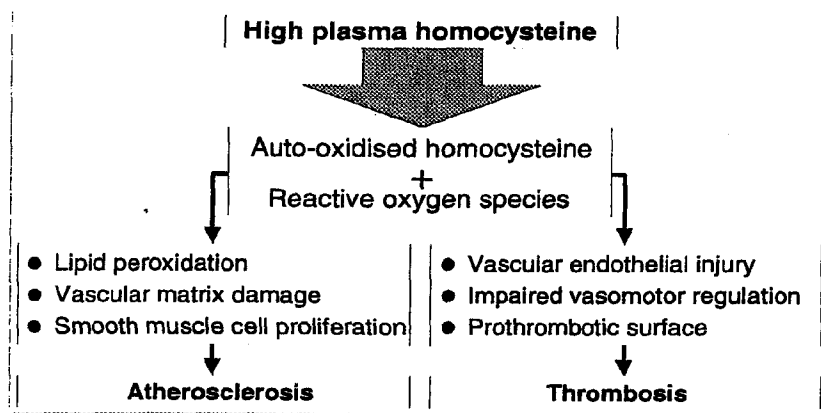


Fig. 5. Possible role of homocysteine in atherosclerosis and thrombosis

Advantages of 5-MTHF over Folic Acid

Vitamin B₁₂ deficiency

Because of the proven benefit of folic acid for the prevention of NTDs, the FDA implemented the fortification of flour with folic acid by law at a dosage of 140 $\mu\text{g}/100\text{ g}$. This fortification programme commenced in 1996 and had to be completed by 1 January 1998. The level of fortification is estimated to add an additional 80-100 μg of folic acid daily to the US diet, hence raising the average daily intake of folates from approximately 200 to about 300 μg . This is still short of the 400 $\mu\text{g}/\text{day}$ required to prevent NTDs. Likewise, at least 400 $\mu\text{g}/\text{day}$ of folates are necessary to reduce cardiovascular risk. Hence, higher fortification of flour with folic acid is considered desirable, but would expose a substantial proportion of the population to excessive intake of folic acid. Thus, the FDA is still looking for ways to improve folate intake in targeted subgroups, particularly females of childbearing age, while not putting other population groups at risk of excessive intake (13). This risk relates to the limited capacity of mucosal cells to convert folic acid to 5-MTHF. Thus, excessive intake of folic acid results in unaltered folic acid appearing in the blood. Even after intake of only 200 μg of folic acid at one time, unaltered

folic acid appeared in the human circulation (1). This unmodified folic acid may mask vitamin B₁₂ deficiency.

Vitamin B₁₂ deficiency affects 10-15% of people above the age of 65 (pernicious anaemia, achlorhydria), and results in megaloblastic anaemia (reversible) and neuropathy (irreversible). The megaloblastic anaemia in vitamin B₁₂ deficiency is caused by the reduced activity of methionine synthase, which catalyses the vitamin B₁₂-dependent conversion of 5-MTHF to THF (Fig. 3, adapted from (2)). As THF is required to maintain DNA synthesis, particularly in rapidly dividing cells, such as bone marrow cells, anaemia occurs at a relatively early stage of vitamin B₁₂ deficiency and allows timely diagnosis. The reduced activity of methionine synthase in vitamin B₁₂ deficiency also results in blocking of the methylation cycle and trapping of 5-MTHF in the so-called "folate trap". As a consequence, reduced methylation (e.g. of myelin basic protein) results in neuropathy. If the vitamin B₁₂ deficiency remains untreated, the neuropathy will persist and eventually become irreversible (14).

In vitamin B₁₂ deficiency, unaltered folic acid in the circulation can be incorporated into bone marrow cells, where it is reduced in a vitamin B₁₂-independent reaction by folate reductase to dihydrofolate (DHF), and further reduced to THF (Fig. 3, adapted from (2)). THF can then restart DNA synthesis and red blood cell formation, thereby correcting the haematological signs of the vitamin B₁₂ deficiency. In contrast, the neurological symptoms are unaffected and may eventually become irreversible. Thus, folic acid may impede the diagnosis of the vitamin B₁₂ deficiency, by masking the anaemia (14). This masking effect of folic acid has been reported in numerous cases (1948-60) of pernicious anaemia, where oral folic acid (0.3-50 mg/day) was administered incorrectly and improved haematology, but resulted in manifestation of neuropathy (12). Further evidence comes from studies *in vitro*. These studies with vitamin B₁₂-deficient bone marrow cells showed that folic acid triggered DNA synthesis, whereas 5-MTHF was ineffective (15-17). The safety concerns associated with high intakes of folic acid resulted in the decision by the FDA to implement food fortification at levels that, on the one hand would not convey any risk, but on the other hand failed to increase folate intake to 400 µg/day, the amount required for the prevention of NTDs and CVD.

The disadvantages of folic acid can be overcome by the use of 5-MTHF instead. 5-MTHF does not mask vitamin B₁₂ deficiency, because it is converted to THF via the vitamin B₁₂-dependent methionine synthase reaction before it can be utilised for DNA synthesis. Thus, in vitamin B₁₂

deficiency, 5-MTHF becomes trapped ("folate trap") (Fig. 6). Hence, unlike folic acid, 5-MTHF cannot correct the haematological signs of vitamin B₁₂ deficiency and does not interfere with the timely diagnosis of vitamin B₁₂ deficiency.

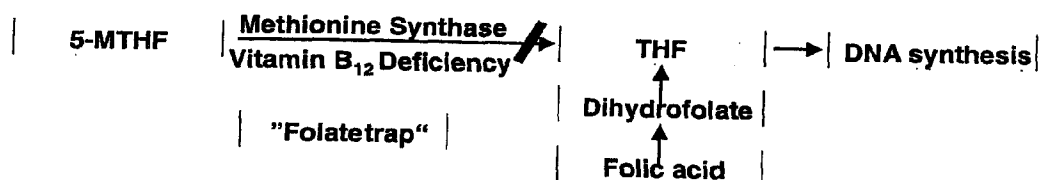


Fig. 6. 5-MTHF vs folic acid in vitamin B₁₂ deficiency

Subjects with thermolabile variant MTHFR

MTHFR catalyses the conversion of 5,10-methylene-THF to 5-MTHF (Fig. 3, adapted from (2)). Between 5 and 15% of the population are homozygous for a genetic variant of MTHFR resulting from a point mutation (C → T substitution at nucleotide 677) in the coding region of the gene. This mutation is associated with a thermolabile variant and about half of the normal enzyme activity. Furthermore, the thermolabile variant has been positively associated with CVD in some studies and increased risk of NTDs (18).

Subjects homozygous for the T/T thermolabile variant have moderately increased plasma homocysteine, particularly individuals with low folate status (4). These subjects may require higher intake of folates to achieve a reduction of homocysteine. 5-MTHF is potentially more effective in decreasing plasma homocysteine in individuals with thermolabile MTHFR variant than folic acid, because it can directly remethylate homocysteine to methionine. Folic acid, however, upon entering the cell has to be reduced to THF and converted to 5-MTHF (via 5,10-methylene-THF) before it can participate in the remethylation of homocysteine. The conversion from 5,10-methylene-THF to 5-MTHF, though, depends on the activity of MTHFR (Fig. 7). Hence, 5-15% of the population with reduced MTHFR activity may benefit significantly from the use of 5-MTHF instead of folic acid.

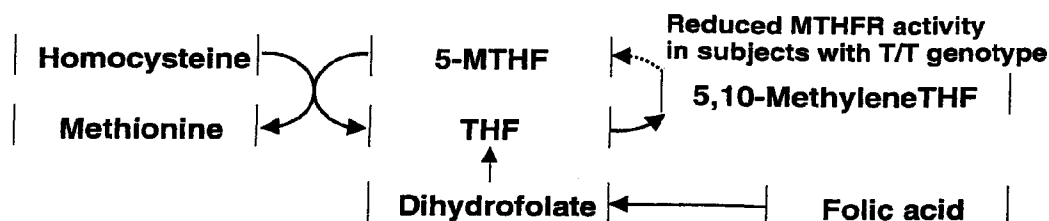


Fig. 7. 5-MTHF vs folic acid in individuals with thermolabile variant MTHFR

The advantages of 5-MTHF vs folic acid can be summarised as follows:

- ❖ Unlike folic acid, 5-MTHF occurs in nature.
- ❖ 5-MTHF is the active form of folic acid and is absorbed and utilised without biotransformation. It is present in blood and tissues.
- ❖ 5-MTHF is the only form of folate able to cross the blood-brain barrier and to concentrate in cerebrospinal fluid. 5-MTHF has been demonstrated to exert anti-depressant properties (19, 20).
- ❖ Bioavailability of 5-MTHF is higher than that of folic acid.
- ❖ 5-MTHF is likely to be better utilised in subjects with thermolabile MTHFR variant.
- ❖ Unlike folic acid, 5-MTHF does not mask vitamin B₁₂ deficiency and does not exacerbate polyneuropathy caused by vitamin B₁₂ deficiency.

Practical Aspects of 5-MTHF

- ❖ Patented for the treatment of depression (USA) and the prevention of vascular damage in hyperhomocysteinaemia (Italy).
- ❖ Manufactured under GMP from folic acid and available as Ca salt (improved stability).
- ❖ Recommended dosage: 200-1,000 µg/day.
- ❖ Stability at room temperature:
 - 24 months in bulk
 - 36 months in single-entity tablets.
- ❖ Approval in Europe as novel food in progress.
- ❖ Work on improved formulations in progress, primarily for the fortification of foods, e.g. beverages and flour.

- ❖ Higher stability in beverages than folic acid (e.g. sports drinks; Fig. 8).

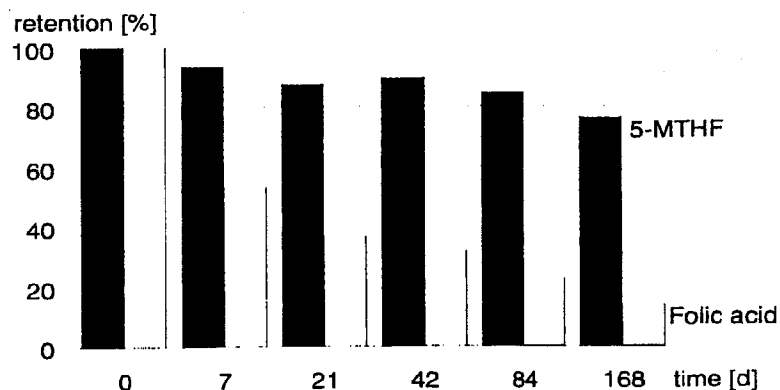


Fig. 8. Stability of 5-MTHF vs folic acid in a sports drink

Conclusions

Folic acid reduces the risk of NTD and CVD. Hence, the intake of 400 µg folic acid per day is highly recommended, and can be achieved by fortification of staple foods and dietary supplements. 5-MTHF is the active form of folic acid and can overcome the disadvantages associated with folic acid, such as the masking effect of vitamin B₁₂ deficiency or the reduced effectiveness in individuals with thermolabile MTHFR variant.

5-MTHF = Activated Folic Acid → Superior

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