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REVIEW ARTICLE

# Folate, folic acid and 5-methyltetrahydrofolate are not the same thing

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**Abstract**

1. Folate, an essential micronutrient, is a critical cofactor in one-carbon metabolism. Mammals cannot synthesize folate and depend on supplementation to maintain normal levels. Low folate status may be caused by low dietary intake, poor absorption of ingested folate and alteration of folate metabolism due to genetic defects or drug interactions.
2. Folate deficiency has been linked with an increased risk of neural tube defects, cardiovascular disease, cancer and cognitive dysfunction. Most countries have established recommended intakes of folate through folic acid supplements or fortified foods. External supplementation of folate may occur as folic acid, folinic acid or 5-methyltetrahydrofolate (5-MTHF).
3. Naturally occurring 5-MTHF has important advantages over synthetic folic acid – it is well absorbed even when gastrointestinal pH is altered and its bioavailability is not affected by metabolic defects. Using 5-MTHF instead of folic acid reduces the potential for masking haematological symptoms of vitamin $B_{12}$ deficiency, reduces interactions with drugs that inhibit dihydrofolate reductase and overcomes metabolic defects caused by methylenetetrahydrofolate reductase polymorphism. Use of 5-MTHF also prevents the potential negative effects of unconverted folic acid in the peripheral circulation.
4. We review the evidence for the use of 5-MTHF in preventing folate deficiency.

**Keywords**

Cardiovascular disease, cognitive dysfunction, folate deficiency, methylenetetrahydrofolate reductase gene, neural tube defects

**History**

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## Introduction

Folate, also known as vitamin $B_9$, is the generic term given to a family of chemically similar compounds that have been recognized as beneficial for the prevention of a range of conditions. Folate is an essential micronutrient that is vital for normal cellular function: adequate folate intake is a critical factor in preventing some neural tube defects (NTD), has been implicated in some forms of anaemia and numerous other adverse health conditions such as cardiovascular disease and cancer (Blom & Smulders, 2011; Czeizel & Dudas, 1992; Klerk et al., 2002; Lee et al., 2011; Medical Research Council Vitamin Study Research Group, 1991; van der Put & Blom, 2000; Webb et al., 2011). Plasma levels of folate are inversely related to plasma homocysteine levels at concentrations <40 mM, suggesting a link between folate intake and reduced risk of vascular disease (Forman et al., 2005; Jardine et al., 2012; Smulders & Stehouwer, 2005; Zhou et al., 2011). Furthermore, there is growing evidence that folate may play a role in the prevention of colorectal cancer, which represents the second leading cause of death due to malignancies (Sanjoaquin et al., 2005; Stolzenberg-Solomon et al., 2006).

Conversely, other evidence supports a positive association between increased risk of breast cancer and high folate intake, generally attributable to supplemental folic acid rather than a diet high in folate-rich foods (Stolzenberg-Solomon et al., 2006). This matter is still under debate. Because of the complexity of folate function, hypothetically, it is possible that both deficiency and abundance or over-supplementation of folate, in addition to other conditions, may contribute to breast carcinogenesis at different stages of tumour development or in different neoplastic or tumour phenotypes (Stolzenberg-Solomon et al., 2006).

Studies of folate supplementation indicate a role in the prevention of other diseases, including neurological, cognitive and psychiatric diseases, such as cognitive dysfunction in the elderly, and in protection against degeneration of ulcerative colitis (Carrier et al., 2003; Hooshmand et al., 2012; Hwang et al., 2012; Kelly, 1998; Morris, 2012; Perez et al., 2012).

Mammals, as well as all other animals, do not have the ability to synthesize folate and therefore must absorb it from the diet, however, daily dietary intake of folates is generally lower than the dosage recommended by national health authorities (Mitchell et al., 2004). In fact, although natural food folates are abundant in the normal range of foods available in developed countries, many people do not eat folate-rich diets due to the cost of fresh fruit and vegetables, plus naturally occurring folates are unstable, with as much as 30% lost as a result of food processing, depending on the type of cooking used (Bergström, 1994; Bjorkegren & Svardsudd, 2003). Rich sources of folate are green, leafy vegetables, sprouts, fruits, brewer's yeast and liver. However, a large

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proportion of population in lower socioeconomic groups have limited access to folate-rich foods. For this reason, most countries have established increased recommended intakes of folates, introducing mandatory food fortification with synthetic folic acid (Crider et al., 2011). Although there is no a general consensus, in most countries, the recommended dietary allowance (RDA) for folate is 300 μg/day for adults and 400 μg/day for women of childbearing age. The US Food and Nutrition Board suggested a level of 400 μg/day for folic acid, expressed in terms of dietary folate equivalents (DFE) (Dietary Guidelines Advisory Committee, 2010). The introduction of folic acid-fortified primary foods has effectively decreased the prevalence of NTD (Daly et al., 1995) and stroke mortality (Yang et al., 2012).

## The folate family of compounds

Folate is the generic term for a family of compounds including folic acid and its derivatives which include 5-methyltetrahydrofolate (5-MTHF), 5-formyltetrahydrofolate (5-FTHF or folinic acid), 10-formyl-THF, 5,10-methylene-THF and unsubstituted THF. Deficiency of folate can be a direct result of low dietary intake, poor absorption of ingested folate by the intestine and increased use (i.e. physical activity, pregnancy); it can also be caused by pathological liver conditions (Halsted, 1989; Wright et al., 2005) and folate dysmetabolism, due to genetic defects or drug interactions.

Folic acid is the synthetic, parent compound of this family. It is an oxidized synthetic water-soluble member of the vitamin-B complex family which does not exist in nature, although oxidation of folates to folic acid is seen in stored or cooked foods (Forssen et al., 2000). It is composed of two main units: a pteroyl group linked to a glutamic acid residue (Figure 1A). Folic acid itself is not active as a coenzyme and has to undergo several metabolic steps within the cell in order to be converted into the metabolically active THF form. In most cases, folic acid shows greater stability than the reduced folates (Forssen et al., 2000).

Folinic acid is a 5-formyl derivative of THF (Figure 1B). Unlike the synthetic folate, folinic acid is naturally found in food. It is readily converted to THF without requiring the action of the enzyme dihydrofolate reductase (DHFR). Therefore its function as a vitamin is unaffected by drugs inhibiting this enzyme, such as methotrexate (Rajagopalan et al., 2002). 5-MTHF (Figure 1C) is a biologically active form of folate and is the most abundant form found in plasma, representing >90% of folate and is the predominant active metabolite of ingested folic acid.

## Folate metabolism

Folate plays an essential role in one-carbon metabolism, facilitating the transfer of one-carbon units in reactions required for the synthesis of purine and pyrimidine precursors of nucleic acids, for the metabolism of methionine, serine, glycine and histidine and for the formation of methylating agents required for normal metabolism and gene regulation (Bottiglieri et al., 1994; Lucock, 2004; Mischoulon & Fava, 2002; Reynolds, 2002; Wagner, 1995). Folates ingested with the diet mainly exist as polyglutamates, which must be

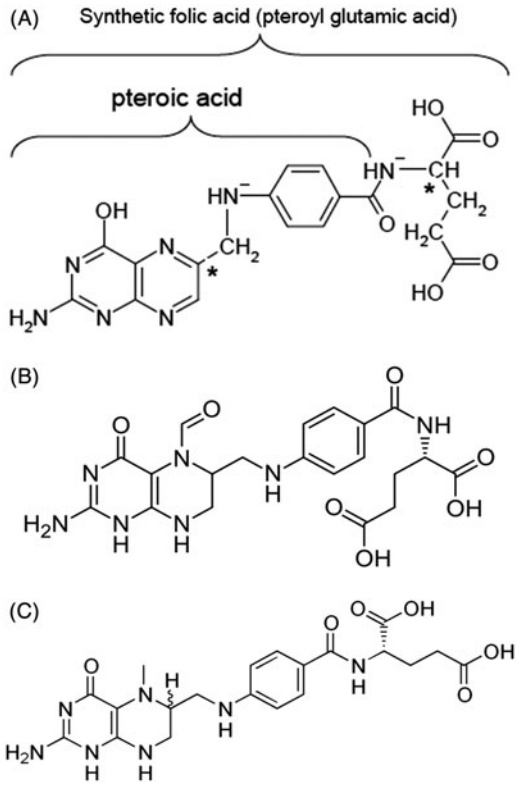


Figure 1. Structure of folic acid (A) and its derivatives, folinic acid (B) and L-5-methyltetrahydrofolate (C).

hydrolysed to monoglutamates in order to be transported. This first step of folate metabolism occurs in the intestinal mucosa.

Folic acid itself has no coenzyme activity until it is reduced to THF by a two-step enzymatic reaction involving a DHF intermediate and the DHFR (Blakley, 1984). THF is then metabolized by the enzyme serine hydroxymethyltransferase (SHMT) to generate glycine and 5,10-methylene-THF (Blakley, 1984; Gregory et al., 2000). 5,10-methylene-THF is in turn converted into L-5-methyl-THF [the predominant folate form found in plasma (Blom & Smulders, 2011)] by the action of the riboflavin-dependent enzyme methylenetetrahydrofolate reductase (MTHFR).

Intracellular folate metabolism is at the branch of two major inter-related metabolic cycles: synthesis of thymidylate and purines and synthesis of methionine from homocysteine (Figure 2). 5-Methyl-THF acts as a methyl donor for homocysteine remethylation which is catalysed by the vitamin $B_{12}$-dependent enzyme methionine synthase. The resulting THF can be converted into 10-formyl-THF and then into 5,10-methylene-THF by the action of the trifunctional enzyme—tetrahydrofolate dehydrogenase (MTHFD1). The 10-formyl-THF serves as donor of one-carbon groups required for purine biosynthesis. THF can also be directly converted into 5,10-methylene-THF by the action of the enzyme SHMT. 5,10-Methylene-THF serves as a cofactor for the conversion of dUMP into dTMP which is catalysed by the enzyme thymidylate synthase (Blakley, 1984; Blom & Smulders, 2011). DHF, which is formed as a co-product of this reaction, is then converted to THF via DHFR. The cycle is completed with THF accepting another one-carbon unit and regenerating 5,10-methylene-THF by the action of SHMT. Within this metabolic cycle, the reaction catalysed by the enzyme

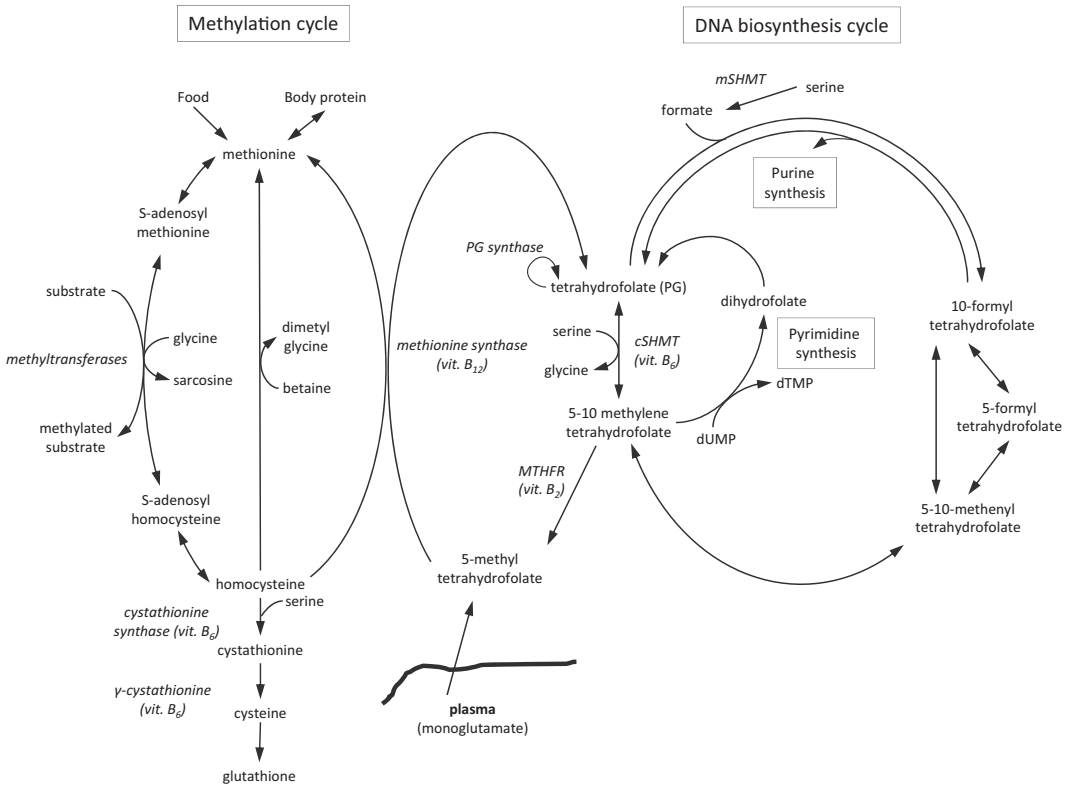


Figure 2. Intracellular folate metabolic pathways. mSHMT: mitochondrial SHMT; PG: polyglutamyl. Reproduced with permission from Smulders & Stehouwer (2005).



MTHFR is crucial for the regulation of available 5-methyl-THF, which is required for methionine synthesis. Methionine, in turn can be metabolized to S′-adenosyl methionine (SAM), which acts as the principal methyl donor in many reactions, including methylation of DNA, histones and other proteins. These methylation reactions play important roles in development, gene expression and genomic stability (Wolffe et al., 1999). The methionine cycle is highly sensitive to inadequate folate status (Basten et al., 2006). When folate status is poor, the ability of the cell to remethylate cellular homocysteine is impaired and this results in increased plasma homocysteine levels. Therefore, plasma homocysteine levels are an indirect indicator of folate level (Blom & Smulders, 2011).

## Pharmacokinetics

### Absorption

Dietary folate exists in the polyglutamate form, which must be converted into the monoglutamate form to be absorbed from the intestinal lumen. This reaction is catalysed by the folate conjugase such as the intestinal brush border pteroylpolyglutamate hydrolase (BB-PPH) and the intracellular hydrolase (IC-PPH) (Halsted, 1989). Intestinal absorption occurs by both passive and carrier-mediated mechanisms, with the second process predominating in the proximal small intestine. Passive absorption occurs mainly at higher doses of folate. Carrier-mediated transport occurs via three systems, namely, the reduced folate carrier (RFC), the folate receptors (FRs) and the proton-coupled folate transporter (PCFT), which transports oxidized and reduced folates with similar efficiency (Selhub et al., 1984; Sirotnak & Tolner, 1999; Subramanian et al., 2008; Zhao et al., 2009). Intestinal folate transport is a saturable process with a pH optimum between 5.5 and 6.0, explaining why antacids appear to reduce folate absorption (Russell et al., 1988).

### Bioavailability

Several studies aiming at estimating the bioavailability of food folates relative to folic acid have reported values ranging between 10 and 98% (Gregory, 1995; Hannon-Fletcher et al., 2004; Tamura & Stokstad, 1973) depending on the assessment method used. Such discrepancies may be due to differences in study design, variation in the test food used, inter-subject variability, genetic and metabolic differences, lack of standardized reference methods for sample preparation and folate quantification and use of non-certified reference material (Finglas et al., 1999; Gregory, 1995; Melse-Boonstra et al., 2004; Pfeiffer et al., 2010; Summers et al., 2010; Vahteristo et al., 1996; Wright et al., 2003). In order to study folate bioavailability, both long- and short-term trials have been conducted. Long-term trials have generally focused on the analysis of folate status parameters, such as plasma folate levels, concentration of folate in red blood cells (RBC) and plasma total homocysteine. Short-term studies have evaluated the availability of folate and its active metabolites using the area-under-the-serum-response-curve (AUC) method (Konings et al., 2002).

Several trials report a higher relative bioavailability of supplemental folic acid compared with food folates, concluding that consumption of extra folate as natural food folate is relatively ineffective at increasing folate status (Cuskelly et al., 1996; Hannon-Fletcher et al., 2004). When external supplementation is taken into consideration, folate may be given as

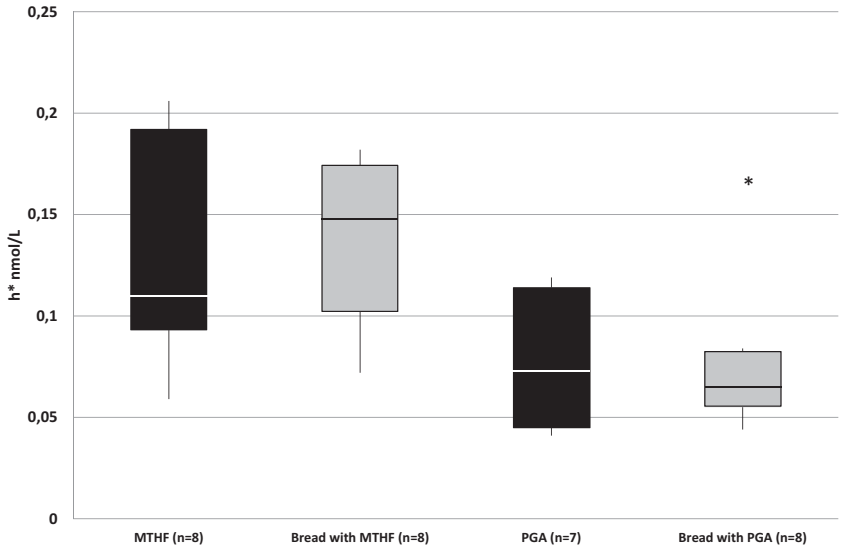


Figure 3. Dose-normalized AUC of plasma [$^{13}C_5$]5-MTHF (h* nmol/L) after single oral equimolar folate doses (~450 nmol = 200 μg) in the form of pharmaceutical preparation with (6S)-[$^{13}C_5$]5-MTHF (black) or [$^{13}C_5$]folic acid (pteryol glutamic acid (PGA), black) or as bread fortified with (6S)-[$^{13}C_5$]5-MTHF (bread with MTHF, grey) or [$^{13}C_5$]folic (bread with PGA, grey). Asterisk indicates an outlier (adapted from Ohrvik & Witthoft, 2011).



folic acid or as the naturally occurring form [6S]-5-MTHF. Several studies have focused on comparing the efficacy of these two compounds in modulating folate-related parameters. Lamers et al. (2006) conducted a 24-week double-blind, randomized, placebo-controlled intervention study aimed at assessing the efficacy of daily supplementation with the naturally occurring [6S]-5-MTHF compared with folic acid in increasing RBC folate in healthy women of child-bearing age. RBC folate concentrations are useful in determining long-term folate status as they respond very slowly to changes in folate intake. This is because erythrocytes, which have a 120-day lifespan, accumulate folate only during erythropoiesis. Low serum folate is considered an indicator of folate deficiency; however, a single measurement cannot be used to differentiate between a transitory decrease in dietary folate intake and chronic deficiency states. After treatment, increases in RBC and plasma folate concentrations were significantly higher in the group receiving [6S]-5-MTHF compared with the folic acid group (Lamers et al., 2006). The results this study support the use of [6S]-5-MTHF as an effective and safe alternative to synthetic folic acid. Fohr et al. (2002) performed an 8-week trial in which equimolar amounts of folic acid and [6R,S]-5-MTHF were administered to 160 healthy women of child-bearing age. After treatment, folate levels were measured in plasma and in RBC at time zero, and at 4 and 8 weeks. Folate plasma concentrations were significantly higher in the 5-MTHF group compared with the folic acid group, whereas the increase in RBC folate was similar in both treatment groups (Fohr et al., 2002). Similarly, Houghton et al. (2006) conducted a 16-week trial to evaluate the effectiveness of folic acid versus [6S]-5-MTHF on RBC folate concentration during lactation. At the end of the study, the RBC folate concentration in the [6S]-5-MTHF group was higher than that in the folic acid group (Houghton et al., 2006). In short-term trials, folate availability is determined using the AUC method. A number of authors have validated this method for assessing food folate bioavailability compared with supplemental folic acid in short-term trials (Konings et al., 2002; Prinz-Langenohl et al., 1999; Wright et al., 2005).

A combined approach of various short-term techniques has recently been reported to determine acute absorption of equimolar doses of either stable isotope-labelled [6S]-([$^{13}C_5$)5-MTHF or [$^{13}C_5$] folic acid from bread. Following the ingestion of bread fortified with [6S]-([$^{13}C_5$)5-MTHF the plasma AUC of this labelled folate was significantly higher than that for food labelled with folic acid. Similar results were obtained with supplemental (6S)-[$^{13}C_5$]5-MTHF when compared with [$^{13}C_5$] folic acid (Buttner et al., 2011; Ohrvik et al., 2010; Ohrvik & Witthoft, 2011) (Figure 3). These data differ from previous works reporting no difference in short-term availability between folic acid and 5-MTHF (Pentieva et al., 2004; Prinz-Langenohl et al., 2003) and further support the influence of the methodological approach in determining folate bioavailability.

## Clinical pharmacokinetic and metabolic considerations

As reported above, polyglutamate folates ingested with the diet must be converted to the monoglutamate form by the conjugase enzymes in order to be absorbed. Since these enzymes have an optimum activity at pH 6–7, alteration of the intestinal pH may determine an incomplete deconjugation of folate thus leading to reduced absorption (Halsted, 1989; Wei & Gregory, 1998). There are several conditions in which the luminal pH changes, such as atrophic gastritis and situations with altered biliary–pancreatic secretions (Russell et al., 1986). In addition, treatment with drugs such as proton pump inhibitors (PPI) and $H_2$-antagonists and ingestion of foods rich in citrate, malate and ascorbate may lead to alteration of luminal pH (Halsted, 1989; Russell et al., 1986). In all these conditions, supplementation with folates like folic acid is effective and generally recommended (Inskip et al., 2009; Knudsen et al., 2004). Moreover, there are conditions in which drug treatment causes defects in folate metabolism thus impairing its conversion to the active form. This is the case for treatment with drugs such as methotrexate, aminopterine, pyrimethamine and trimethoprim which inhibit DHFR. In these conditions, folic acid supplementation is ineffective and folinic acid or 5-MTHF can be a good alternative to folic acid (Figure 4).

Among the available pharmaceutical preparations, 5-MTHF shows several important advantages over folic acid. As reported above, 5-MTHF displays better performance

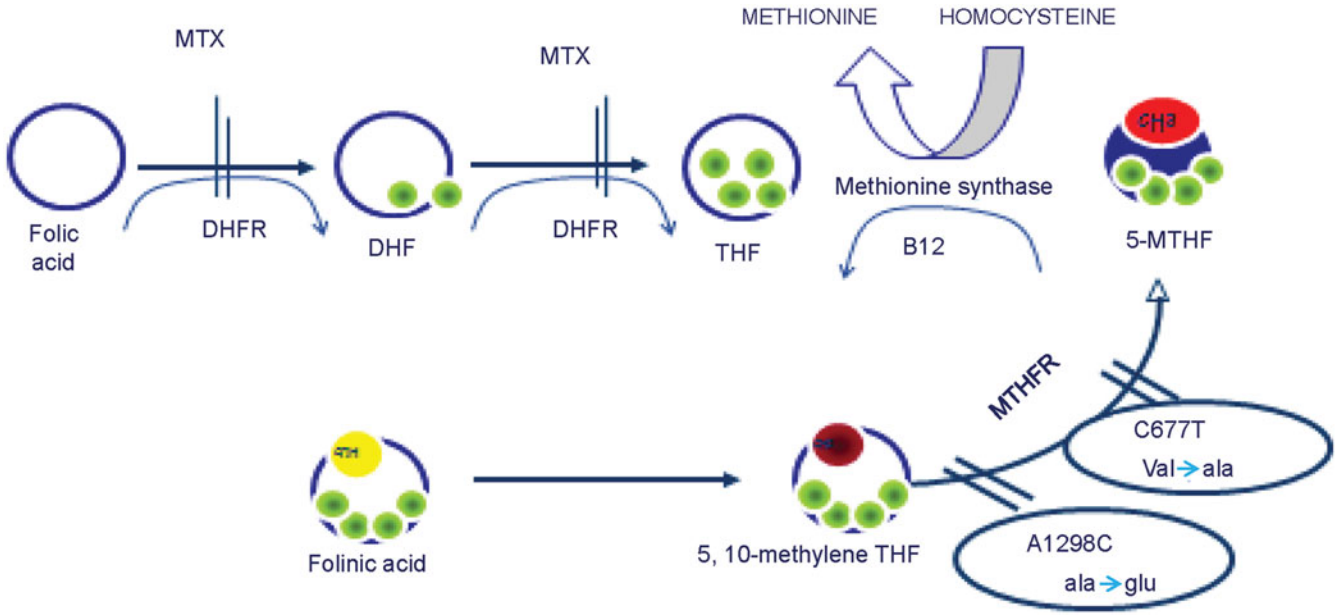


Figure 4. Genetic polymorphisms of L-5-methyltetrahydrofolate reductase (MTHFR) and implication in methotrexate depletion of systemic folate.

compared to folic acid in terms of plasma concentrations of folate (Fohr et al., 2002; Houghton et al., 2006; Lamers et al., 2006). In addition, the reaction catalysed by DHFR, which is required to reduce folic acid to THF, is slow and easily reaches saturation. Bailey & Ayling (2009) have shown that the reduction of folic acid by DHFR per gram of human liver is on average, <2% of that in rat liver at physiological pH. Moreover, in contrast to rats, there was almost a five-fold variation of DHFR activity among the human samples. This extremely low rate of conversion of folic acid suggests that the benefit of its use in high doses will be limited by saturation of DHFR, especially in individuals possessing lower than average activity. Thus with the ever-increasing exposure to folic acid from fortification of foods and the use of supplements a total folic acid intake >1 mg is now not uncommon in USA and the low activity of DHFR in human liver is the fundamental cause of exposure to relatively high transients of plasma unmetabolized folic acid at doses greater than the RDA. Finally, a major risk of folic acid supplementation is that it may mask vitamin $B_{12}$ deficiency (Savage & Lindenbaum, 1994). In this respect, 5-MTHF would reduce this risk because, unlike folic acid, it is not able to induce a haematological response in cells from patients with vitamin $B_{12}$ deficiency (Ganeshaguru & Hoffbrand, 1978; Zittoun et al., 1978).

## Genetic polymorphisms of the *MTHFR* gene

Genetic alterations of genes codifying for key enzymes of folate metabolism may affect their activity and reduce folate availability. This would increase folate requirement and contribute to the risk of several disease conditions linked to folate status, such as NTD and cardiovascular diseases (Christensen et al., 1999; Morin et al., 2003; Rozen, 2004). In 1995, Frosst et al. observed that a thermolabile variant of MTHFR is due to a polymorphism of the *MTHFR* gene (677C → T polymorphism). This mutation results in an amino acid change from alanine to valine (A222V) at a site that is critical for flavin adenine dinucleotide (FAD) binding activity and enzyme stability (Frosst et al., 1995; Wilcken et al., 2003). Such a mutation in the *MTHFR* gene of a developing embryo is the most established genetic risk factor for NTD and causes elevated plasma level of homocysteine (Brattstrom et al., 1998; Gudnason et al., 1998; Klerk et al., 2002). The distribution of the polymorphism varies considerably worldwide. In the European population, up to 12% are homozygous (TT), 43% heterozygous (CT) and 45% wild-type (CC) for that polymorphism (Brattstrom et al., 1998; Gudnason et al., 1998; Klerk et al., 2002; Meleady et al., 2003). TT homozygous frequency is lower among the African American population (~1%) and higher in the Hispanic population, reaching up to 30%. In the TT genotype, the *in vitro* enzyme activity is reduced by ~75% compared with that of the wild-type enzyme (Frosst et al., 1995; Kang et al., 1988).

The 677C → T variant of the *MTHFR* gene has been associated with increased risk of NTD and increased cardiovascular risk (Christensen et al., 1999; Klerk et al., 2002; Shields et al., 1999; van der Put & Blom, 2000). In a study published by Christensen et al. (1999) the authors observed that 18–20% of the analysed sample population with NTD were homozygous for the 677C → T MTHFR polymorphism, compared to 11% for controls, implying an increased risk of NTD associated with the 677C → T polymorphism. Another study from Shields et al. (1999) conducted on Irish population led to similar conclusions. The authors detected the homozygous TT polymorphism in 19% of NTD cases versus 8% of controls, once again supporting that the homozygous MTHFR polymorphism is an important genetic determinant in MTHFR-derived NTD risk (Shields et al., 1999). Besides the increased risk of developing NTD, individuals with TT homozygosis present a significantly higher cardiovascular risk due to the higher concentrations of homocysteine, especially in populations with a low dietary folate intake

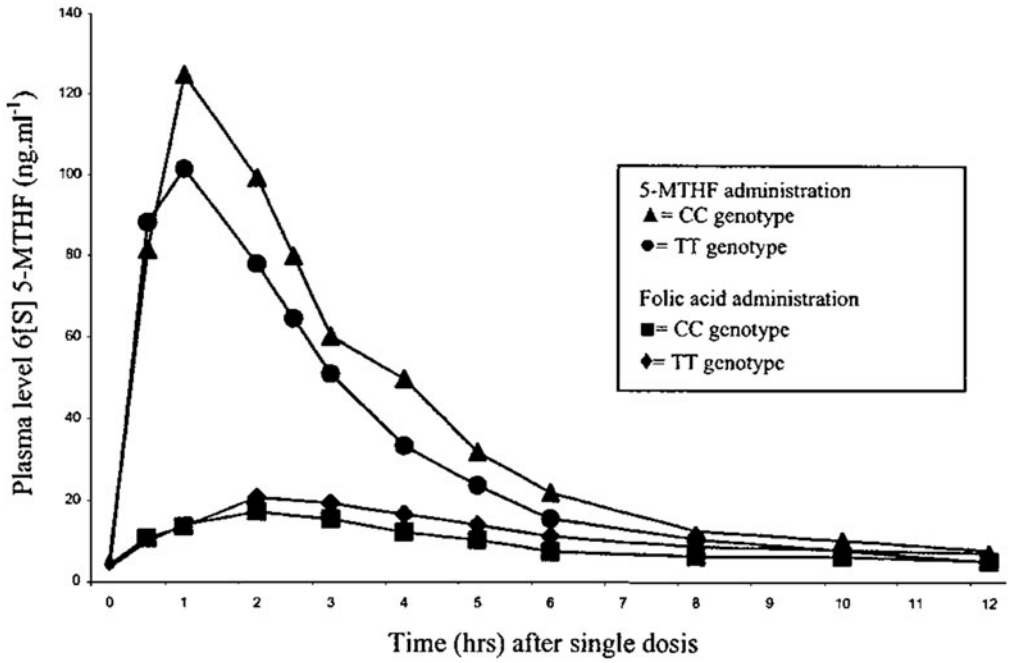


Figure 5. Genotype and treatment: 6[S] 5-MTHF plasma concentration (ng/mL) in patients with MTHFR CC genotype or TT genotype following the administration of 6[R,S]-5-MTHF. 6[S]-5-MTHF plasma concentration (ng/mL) in patients with MTHFR CC genotype or TT genotype following the administration of folic acid. Reproduced with permission from Willems et al. (2004).



(Klerk et al., 2002; Rallidis et al., 2008). The methylated form of folate, N5-methyltetrahydrofolate, is required for the remethylation of homocysteine to methionine. By inhibiting this remethylation pathway, folate deficiency induces homocysteine efflux into the circulation. Studies show a negative correlation between plasma folate, particularly N5-methyltetrahydrofolate, and circulating homocysteine levels (Durand et al., 1998).

Another polymorphism that has been studied in relation to folate metabolism and NTD is the dihydrofolate reductase (DHFR) 19-bp deletion polymorphism [a 19-bp deletion of intron 1a (DHFR19bpdel); rs70991108] (Parle-McDermott et al., 2007). The association between this polymorphism and the NTD risk was inconsistent between studies (Johnson et al., 2004; van der Linden et al., 2007).

## Supplementation

External supplementation of folate may occur as folic acid, folinic acid or 5-MTHF. Supplementation of folic acid has been proven to reduce the risk of NTD and helps in re-establishing the correct levels of homocysteine in individuals with TT homozygosis (Brouwer et al., 1999; Fohr et al., 2002; Lamers et al., 2004; Venn et al., 2003). It is now thought that naturally occurring 5-MTHF, as well as being more or at least as effective as folic acid in improving folate status, may present important advantages over synthetic folic acid and, therefore, supplementation with 5-MTHF may be a valid alternative to folic acid (Czeizel et al., 2011; Mischoulon & Fava, 2002; Obeid et al., 2013; Pietrzik et al., 2010; Reynolds, 2002; Scott, 2001). In fact, independent studies have demonstrated that [6S]-5-MTHF displays higher bioavailability compared to folic acid, irrespective of the patient's genotype (Bottiglieri et al., 1994; Li et al., 2008; Prinz-Langenohl et al., 2009; Willems et al., 2004) (Figure 5). Therefore, this natural form of folate should be considered a valid alternative to folic acid supplementation or in the fortification of food products. However, specific clinical trials investigating the prevention of NTD using 5-MTHF are required. Such studies could ascertain whether the risk of NTD would be sufficiently decreased simply by increasing folate status using any folate (such as 5-MTHF) rather than specific folic acid supplementation. Daly et al. (1995) compared two approached to raise folate levels: targeting high-risk individuals or targeting the population (in which only 5% were existing users of folic acid supplements). They found, that supplementation of high-risk women decreased the individual risk while the population approach of food fortification reduced population and suggested that the two strategies should be considered complementary in prevention of NTDs (Daly et al., 1995).

## Conclusions

Low folate status is considered to be one of the most common nutritional deficiencies and although inadequate dietary intake is the primary cause genetic alterations and interactions of drugs with folate metabolism may contribute to lower folate availability. In addition, folate deficiency may be due to low levels of vitamin $B_{12}$ since this vitamin serves as a cofactor in folate metabolism. Folate deficiency has been linked to an increased risk of numerous adverse health conditions such as NTD, cardiovascular disease, cancer and cognitive disorders. External supplementation of folate may occur as folic acid, folinic acid or 5-MTHF. Naturally occurring 5-MTHF is now known to present important advantages over synthetic folic acid. Therefore, the use of 5-MTHF instead of folic acid is strongly recommended for external supplementation and food fortification.

## Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article. The authors thank Chiara Cipollina and Mary Hines for providing editorial assistance on behalf of inScience Communications, Springer Healthcare. This assistance was sponsored by Zambon.

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