Chapter

# Folates: An Introduction

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## Abstract

Folate is a naturally occurring essential nutrient which is vital for DNA replication and a necessary substrate in various enzymatic reactions which are involved in synthesis of amino acids and vitamin metabolism. The synthetic and oxidized form of folate is folic acid, it is mainly incorporated into fortified foods and dietary supplements for preventive measures against folate deficiency. Folate deficiency has been linked with several abnormalities in both mother (anemia, peripheral neuropathy) and fetus (congenital abnormalities). Folic acid supplementation taken around the time of conception has been known to alleviate the risk of neural tube defects in the off springs. Optimal intake and absorption of folates is required for the maintenance of the human body's normal functioning and keeping the genomic integrity intact.

**Keywords:** folate, folic acid, neural tube defects, folate deficiency, congenital abnormalities

## 1. Introduction

Around 90 years ago anemic pregnant women in India were treated with a yeast extract used by a physician named Lucy Wills [1]. Later, the active compound in the yeast was identified as folate and at that time it was named as anti-anemia factor. The term folates refer to the group of various forms of water-soluble vitamin B9 namely folic acid, dihydrofolate (DHF), tetrahydrofolate (THF), 5,10-methyl tetrahydrofolate and 5-methyl tetrahydrofolate (5-MTHF) [2]. Folate cannot be synthesized by human body due to which we have to obtain it from exogenous diet sources such as beans, citrus fruits, leafy green vegetables, brewer's yeast, and cow's liver. It is available as folic acid in fortified foods, supplements, and multivitamins. It is available as folic acid in its synthetic oxidized form having only one glutamate residue in fortified in fortified foods, supplements, and multivitamins. Folate is essential for the normal functionality of human body as it provides one-carbon groups for biosynthesis of nucleotides, amino acids metabolism, and methylation of DNA [2]. A number of scientific studies have showed that folic acid is effective in the prevention of neural tube defects (NTD's) [3, 4]. NTD's are congenital malformations of structures of central nervous system which occur due to the failure of neural tube closure after conception between 21 and 28 days [5]. Hence optimal intake of folate during early pregnancy is necessary for the developing fetus; there is a higher risk of NTD'S occurrence in offspring during early pregnancy due to folate deficiency. Recently research have shown that normal folate metabolism reduces the concentration of blood homocysteine which in high concentrations may increase the risk of stroke and coronary heart diseases [6]. Studies suggests that during early pregnancy low maternal blood folate might be related to behavioral disorders in childhood and folate may also be a factor in cognitive functions such as

Alzheimer's disease [7, 8]. In order to prevent these disorders and complications it is recommended to keep optimal blood folate levels and homocysteine levels below the accepted cutoff values, the recommended daily allowance of folate is 300 μg/day while there is a recommendation of 400 μg/day for women of reproductive age for prevention of the risk of NTD'S occurrence [9].

### 1.1 Structure of folate

The term folate represents the group of B vitamins (Vitamin B9) which have similar biological activity to folic acid. There are three major components in the parent structure of folic acid: a pteridine ring that can be either oxidized or reduced, coupled with para-aminobenzoic acid (PABA) through a methylene bridge, which is bound to glutamic acid or polyglutamate by a γ-peptide link [10]. Structure of an oxidized form of folate is illustrated in **Figure 1**, which can be converted to DHF after being reduced at the double bond present at the N-8 position. Further reduction at N-5 double bond leads to the formation of THF and in this state the N-5 of the pteridine moiety and N-10 of the PABA group may act as acceptors of single carbon units [11].

### 1.2 Folate absorption and one-carbon metabolism

The difference between synthetic folic acid and naturally occurring is that the former is the oxidized monoglutamate form while the latter being the reduced polyglutamate form. Dietary forms of folates are predominantly reduced polyglutamates in order get transported across the intestinal wall these polyglutamates need to be hydrolyzed via glutamate carboxypeptidase II (GCPII) inside the gut into monoglutamate forms which can cross the cell membranes [12]. There are three different types of proteins that helps in transport of monoglutamate folate form across cell membrane, such as proton-coupled folate transporter (PCFT), reduced folate carrier (RFC) and folate receptor proteins (FRα & FRβ). Intestinal uptake of folate appears to be PCFT dependent which is a pH dependent transporter because even if RFC is expressed in the intestine, mutations in the genes encoding for the RFC are not found to be linked with deficiency in folate absorption from intestine; the optimal pH required for folate transport is pH 5.5 [13]. RFC employs reduced folate carrier (RFC) as anion exchanger, cellular uptake of folate is dependent on RFC (supports low- affinity high-capacity uptake system) and folate receptor proteins (supports high- affinity low-capacity uptake system) while folate transport across blood brain barrier appears to require both PCFT and FRα [14]. Both the PCFT and RFC are

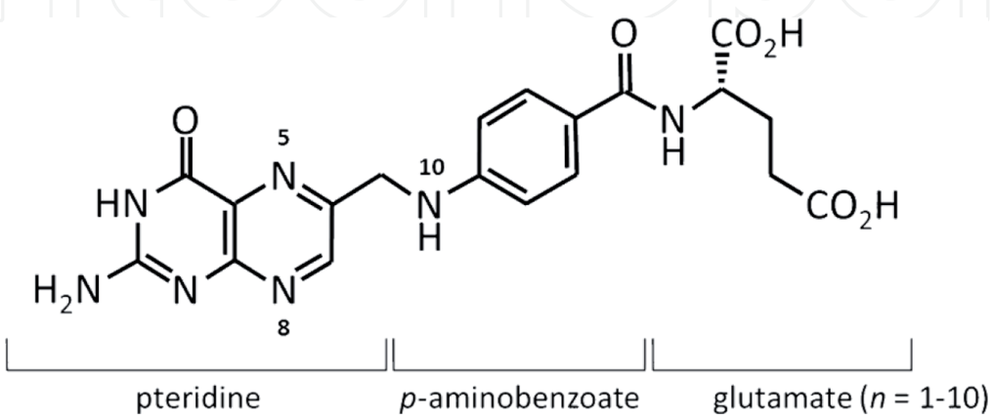


**Figure 1.**
*The structure of folic acid, N-5, N-8, and N-10 are one carbon unit and/or hydrogen acceptors, glutamate may be n = 1 (monoglutamate form) or n = 2–10 (polyglutamate form [11].*

different in their specificities towards reduced and oxidized folate species and their expression inside gut, mutations in the genes encoding transporter proteins, leads to complications in the dietary folate uptake from intestine such mutations at the gene ALC46A1 (encodes PCFT) on chromosome 17q11.2, its treatment involves parenteral folate administration as orally administration of folate has been successful in some cases and it is really important to maintain the CSF (cerebrospinal fluid) folate above the levels associated with deficiency which are 15 ng/mL [15, 16].

The set of biochemical reactions mediated by folate cofactors is known as one carbon metabolism because there is transfer of one carbon units inside the enterocytes, firstly conversion from monoglutamate folic acid to dihydrofolate (DHF) occurs and then DHF is converted into tetrahydrofolate (THF) via dihydrofolate reductase (DHFR) which is NADPH dependent enzyme. Once reduction into THF occurs, the N-5 and/or N-10 serve as acceptors for one carbon units and these carbons are transferred at varying oxidation states which depends on their sources and the enzymes which catalyzes the reactions. In the next step THF is converted into 5,10-methylene tetrahydrofolate, where one carbon unit is accepted by THF from serine hydroxymethyl transferase (SHMT), vitamin B6 has a role of cofactor for SHMT, and glycine is released as the final product. Part of 5,10-methylene tetrahydrofolate can be consumed for the synthesis of thymidine by donating its methylene unit or can be used in the de novo purine synthesis where it undergoes oxidation to 10-formyl-THF. Another part of 5,10-methylene tetrahydrofolate leads to the production of 5-methyl-THF in a reduction catalyzed by methylenetetrahydrofolate reductase (MTHFR).

5-MTHF serves as a substrate for the formation of methionine by donating its methyl group to homocysteine (Hcy) and converting it to methionine in a reaction catalyzed by methionine synthase which is a B-12 dependent enzyme. In mammalian cells methionine can be regenerated from homocysteine in a manner which is independent of folate and instead betaine (product of degradation of choline) is used via an enzyme called betaine homocysteine methyltransferase (BHMT), the expression of BHMT is limited only to liver and kidney [17]. Methionine has a vital role in protein synthesis as it can be converted to S-adenosylmethionine (SAM) which is a universal methyl donor and methylates major biomolecules such as adrenaline, carnitine or phosphatidylcholine, SAM is converted into S-adenosylhomocysteine (SAH) which undergoes hydrolysis to produce homocysteine for beginning a new cycle as shown in **Figure 2**. Along with methionine synthase pathway, homocysteine also goes into the transsulfration pathway where in a cystathionine-β-synthase (CBS) catalyzed reaction, it is converted into cystathionine (Cys), cystathionine can be hydrolyzed into cysteine by cystathionine-γ-lyase enzyme as shown in **Figure 2**.

One-carbon metabolism is compartmentalized, and it takes place in compartments inside cell, such as cytosol and mitochondria with nucleus having very low levels of folates [19]. These two subcellular compartments (cytosolic and mitochondrial) have different redox states due to which inside the mitochondria generation of formate from serine and glycine is favored while in cytoplasmic folate metabolism there is incorporation of one-carbon units derived from mitochondrial produced formate into 10-formyl-THF and then to 5,10-methylene-THF, and 5-MTHF as well as synthesis of purines, thymidine along with homocysteine remethylation to form methionine [14]. Some of the metabolic steps such as the interconversion of serine and glycine occurs in both cytosolic and mitochondrial compartments [20]. In nucleus dUMP (deoxyuridine monophosphate) is converted to deoxythymidine monophosphate (dTMP) by thymidylate synthase (TYMS).

Folate one-carbon metabolism depends majorly on factors such as dietary folate intake and genetic polymorphism in the associated genes due to which any

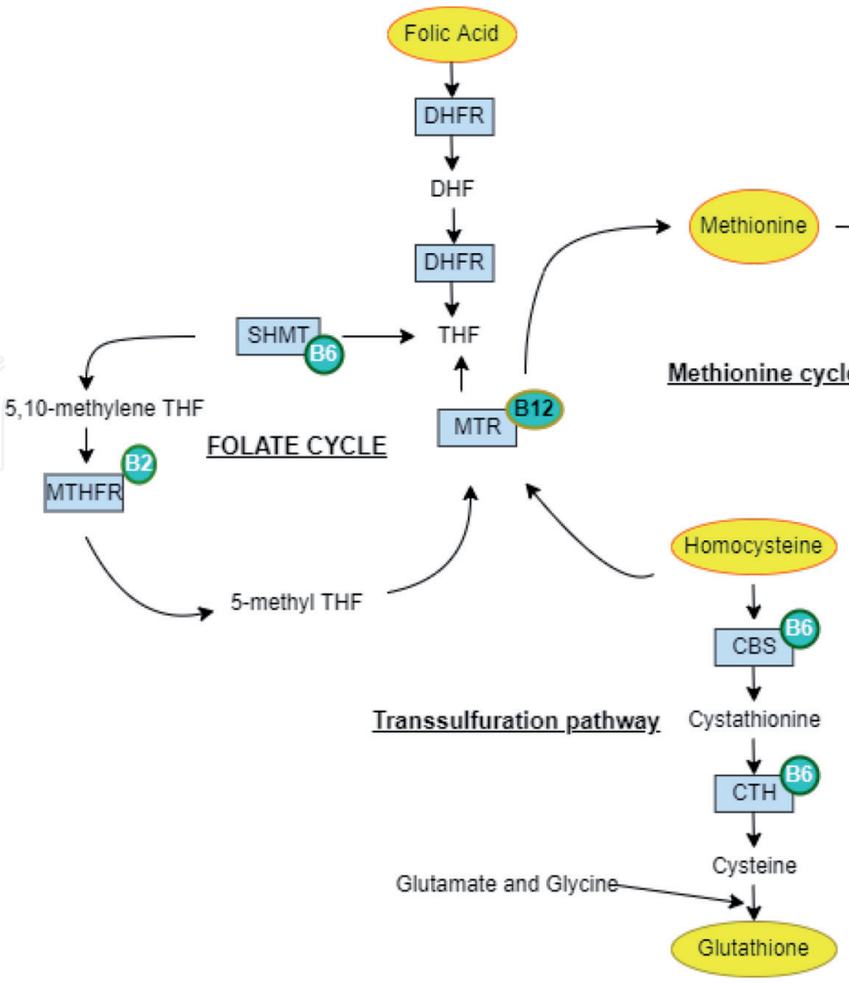


**Figure 2.**
*Folate one-carbon metabolism [18].*

disturbances in folate metabolism can lead to disruptions in regulation of DNA synthesis, abnormal homocysteine levels followed by subsequent pathological consequences. Deleterious mutations in associated genes such as a 19 bp deletion in gene encoding DHFR and single base substitutions in gene encoding MTHFR such as C677T and G1958A results in the formation of a thermolabile MTHFR enzyme and ultimately increasing levels of unmetabolized folic acid (UMFA) in blood, hyperhomocysteinemia and underlying health conditions such as cardiac complications and CNS malformations [21, 22].

## 2. Different species of folate

As we discussed in the introduction that folate is an umbrella term used for a diverse forms of water-soluble Vitamin B9, we will focus on two major folate species the first being the synthetic form called folic acid and the second one called 5-methyl tetrahydrofolate (5-MTHF) which is the active form.

### 2.1 Folic acid

Folic acid is synthetic form and oxidized state of folate, it is mainly incorporated in fortified foods and dietary supplements for preventive measures against folate deficiency especially during pregnancy. Folic acid is different from naturally occurring folate as shown in **Figure 3** as it has a monoglutamate residue unlike the natural

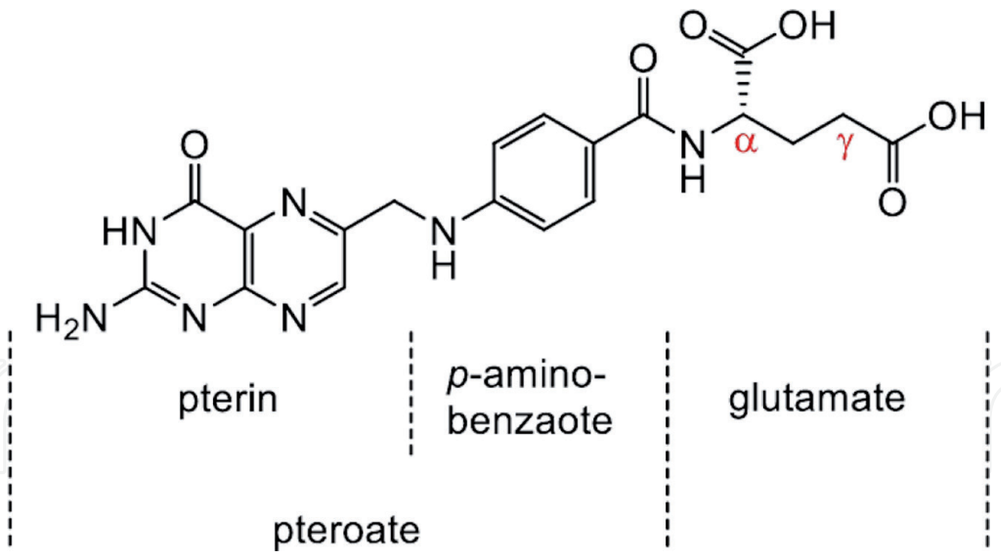


**Figure 3.**
*Structure of folic acid [23].*

folates which have polyglutamate residues. In order to be active metabolically folic acid needs to be converted into its reduced form THF, the oxidized form of folate has the advantage of being more heat stable as compared to the natural food form which is more heat labile and light sensitive [24].

Moreover, folic acid is 70% more bioavailable as compared with the other polyglutamates which needs to be hydrolyzed to monoglutamate form in order to get absorbed, it is absorbed more readily because of its monoglutamate nature, there is very minute probability of any adverse effects or hypersensitivity thus making it the best choice for food fortification. Folic acid is vital for necessary metabolic functions and its optimal intake is recommended to maintain normal folate status. In Europe, the recommended daily allowance (RDA) for folic acid is 170–300 μg/day for women and for men it is 200–300 μg/day [25]. Folic acid is the major component in prenatal nutrition as folic acid requirement is increased during pregnancy and lactation in order to prevent NTD's and other congenital complications.

## 2.2 5-MTHF: the active form of folic acid

5-Methyltetrahydrofolate (5-MTHF), also known as 'Levomefolic acid' accounts for 98% of the entire circulating folate, catalyzed by Methylene tetrahydrofolate reductase (MTHFR) and has several important roles which includes methylation,

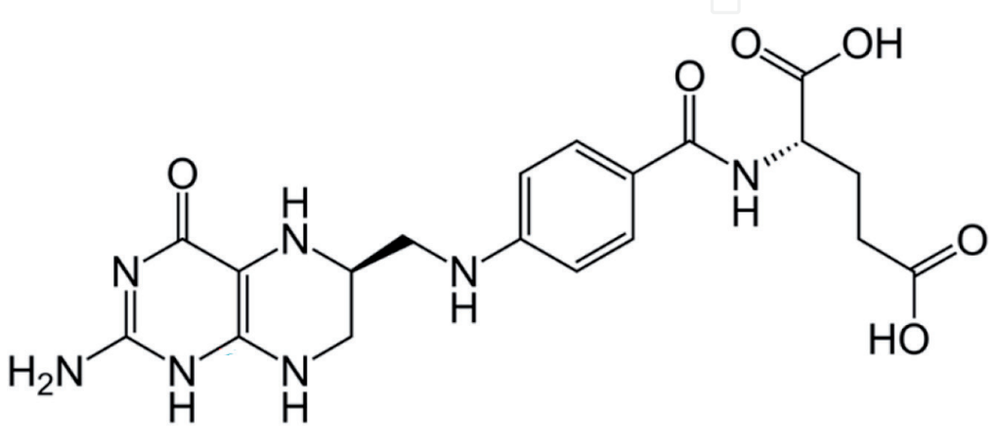

**Figure 4.**
*Structure of 5-MTHF [26].*

conversion of homocysteine to methionine, production of serotonin and melatonin. It is also responsible for the synthesis of DNA, the chemical structure of 5-MTHF is illustrated in **Figure 4**.

The L-5-methyl-tetrahydrofolate (L-5-MTHF) is present as the primary form of dietary folate and is also the only form (under the folate category) which is found normally in the systemic circulation. Thus, 5-MTHF is transported into peripheral tissues and is used for the maintenance of cellular metabolism. The crystalline form of L-5-MTHF is available as calcium salt (Metafolin (R)) and is popularly used in the commercial market as a supplement [27]. The data generated by Verhaar and colleagues, [28] indicated that administration of 5-MTHF can restore the endothelial function in hypercholesterolemia patients and this could probably because 5-MTHF has the potency to alter the cellular oxidative metabolism. These findings also tell us about the beneficial exploitation of oral folic acid therapy, which can be helpful in the reduction of cardiovascular disease risk.

It has been proven that 5-MTHF which occurs naturally is more advantageous to the body when compared to synthetic form of folic acid. This is due to the higher absorption rate of the former even when there is a change in the pH of the gastrointestinal tract. Another reason for this property of 5-MTHF is that its overall bioavailability is unaltered in the presence of metabolic defects. The usage of 5-MTHF contrary to folic acid, potentially decreases the masking of certain hematological symptoms of vitamin B12 deficiency. The 5-MTHF is also capable of minimizing the interaction with drugs that cease the action of dihydrofolate reductase and thereby is successful in overcoming metabolic defects caused by methylene tetrahydrofolate reductase polymorphism [29].

In the human body the folate molecule acts as a one-carbon unit carrier, thus it serves as an essential component for the biosynthesis of nucleotides and also in the process of DNA replication. 5-MTHF has a vital role in methionine cycle as it donates a –CH3 group for homocysteine methylation to produce methionine and any imbalance in this process will act as a contributing factor to hyperhomocysteinemia [30].

### 2.3 Folate deficiency

Folate is an essential nutrient vital for DNA replication and it is a necessary substrate in various enzymatic reactions which are involved in synthesis of amino and vitamin metabolism. The demand for folate increases during pregnancy because it is required for nourishment of the fetus. Folate deficiency has been linked with several abnormalities in both mother (anemia, peripheral neuropathy) and fetus (congenital abnormalities). Folic acid supplementation taken around the time of conception has been known to alleviate the risk of NTD's in the offspring [2].

The term 'folate deficiency anemia' is given to the medical condition in which there is a decrease in the number of red blood cells (anemia) in the blood. It is characterized by the presence of large-sized, abnormal RBC's (megaloblasts), which are formed due to disrupted DNA synthesis. Folate deficiency occurs when the body's demand for folate is not fulfilled, or when there is insufficient dietary intake or inadequate absorption of folate, and when the body loses more folate than usual.

Folate deficiency is also known to have many adversities, such as 'megaloblastic anemia', resulting from deranged cell maturation during erythropoiesis. NTDs, which is caused by failure of neural tube fusion during the early month of pregnancy. Another complication due to folate deficiency is hyperhomocysteinemia, which is a risk factor for cardiovascular and metabolic diseases especially in patients with end stage renal diseases. Folate deficiency usually occurs due to insufficient dietary intake, but other possible reasons are intestinal malabsorption, deranged

folate metabolism and elevated demand of folate during pregnancy, or even due to chronic alcoholism. Dietary folate intake can be assessed by the plasma folate concentration, and it is the most widely used method. For long-term status and tissue folate stores, the erythrocyte folate proved to be the best indicator [31].

Both in animals and humans, the methylation status of certain genes at birth can be modified due to folate deficiency, with probable pathogenic and tumorigenic effects in the offspring. The metabolic network of folates can be modified when there is a presence of pre-existing genetic polymorphisms and will also increase the risk of cancer, which include childhood leukemias. The protective effects of folic acid might be dosage dependent, as excessive (hyper) folic acid may have the adverse effect of nourishing certain types of tumors. Thus, it was concluded that the right amount of folate was required for the maintaining normal functioning of the human body and keeping the genomic integrity intact [32].

## Abbreviations

| | |
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| DHF | dihydrofolate |
| THF | tetrahydrofolate |
| 5-MTHF | 5-methyl tetrahydrofolate |
| 5,10-MTHF | 5,10-methyl tetrahydrofolate |
| MTHFR | methylene tetrahydrofolate |
| NTD'S | neural tube defects |
| RDA | recommended daily allowance |
| UMFA | unmetabolized folic acid |
| PABA | para amino benzoic acid |
| GCPII | glutamate carboxypeptidase II |
| PCFT | proton-coupled folate transporter |
| RFC | reduced folate carrier |
| CSF | cerebrospinal fluid |
| DHFR | dihydrofolate reductase |
| NADPH | reduced nicotinamide dinucleotide phosphate |
| SHMT | serine hydroxymethyl transferase |
| BHMT | betaine homocysteine methyl transferase |
| SAM | S-adenosyl methionine |
| SAH | S-adenosyl homocysteine |
| CBS | cystathionine-β-synthase |
| CYS | cystathionine |
| TYMS | thymidylate synthase |
| DTMP | deoxythymidine monophosphate |
| DUMP | deoxyuridine monophosphate |

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