

## Folate Metabolism and Requirements<sup>1,2</sup>

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**ABSTRACT** Folate functions in multiple coenzyme forms in acceptance, redox processing and transfer of one-carbon units, including nucleotides and certain amino acids. Folate-requiring metabolic processes are influenced by folate intake, intake of other essential nutrients, including vitamins B-12 and B-6, and at least one common genetic polymorphism. Estimates of folate requirements have been based on intakes associated with maintenance of normal plasma and erythrocyte folate concentrations and functional tests that reflect abnormalities in folate-dependent reactions. Dietary Reference Intakes for folate that have been developed recently are based primarily on metabolic studies in which erythrocyte folate concentration was considered the major indicator of adequacy. For adults  $\geq 19$  y, the Recommended Dietary Allowance (RDA) is 400  $\mu\text{g}/\text{d}$  of dietary folate equivalents (DFE); for lactating and pregnant women, the RDAs include an additional 100 and 200  $\mu\text{g}$  of DFE/d, respectively. *J. Nutr.* 129: 779–782, 1999.

**KEY WORDS:** • folate • one-carbon metabolism • requirements • dietary reference intakes

Adequate folate intake is vital for cell division and homeostasis due to the essential role of folate coenzymes in nucleic acid synthesis, methionine regeneration, and in the shuttling, oxidation and reduction of one-carbon units required for normal metabolism and regulation (Wagner 1995). The supply of folate coenzymes in vivo depends primarily on the quantity and bioavailability of ingested folate and the rate of loss by urinary and fecal routes and through catabolism. During periods of inadequate folate intake or malabsorption, biochemical changes associated with inadequate folate status allow the onset of abnormalities in one-carbon metabolism. These abnormalities (e.g., hyperhomocysteinemia or DNA hypomethylation) may result in deleterious consequences, including increased risk for certain types of chronic diseases (Boushey et al. 1995, Mason 1995) and developmental disorders (e.g., neural tube defects) (Scott et al. 1995). The long-term goal in defining folate requirements involves identifying intakes that minimize deleterious processes associated with inadequate intake and optimize folate-dependent reactions in metabolism and cellular development.

Recently, new Dietary Reference Intakes (DRI)<sup>4</sup> for folate

have been reported [Food and Nutrition Board (FNB) 1998]. The DRIs include recommendations based primarily on data from controlled metabolic studies in which blood folate concentrations were measured, along with data from population-based studies. Several newer functional status assessment methods have been proposed to further define folate requirements. In addition, data from whole-body folate kinetic studies will enhance understanding of how changes in folate intake influence many phases of folate metabolism and nutritional status (Gregory et al. 1998).

**Metabolism.** Folate-requiring reactions, collectively referred to as one-carbon metabolism, include those involved in phases of amino acid metabolism, purine and pyrimidine synthesis, and the formation of the primary methylating agent, S-adenosylmethionine (SAM) (Fig. 1). The central folate acceptor molecule in the one-carbon cycle is a polyglutamyl form of tetrahydrofolate (THF) (Wagner 1995). The principal function of folate coenzymes is to accept or donate one-carbon units in key metabolic pathways (Fig. 1). The conversion of THF to 5,10-methylene-THF is a crucial first step in the cycle that employs the 3-carbon of serine as a major carbon source. This one-carbon unit is transferred from serine to THF via pyridoxal phosphate (PLP)-dependent serine hydroxymethyltransferase (SHMT) to form 5,10-methylene-THF and glycine. A portion of the 5,10-methylenetetrahydrofolate thus produced undergoes irreversible enzymatic reduction to the methyl oxidation state (as 5-methyl-THF) by methylene tetrahydrofolate reductase (MTHFR). The N-5-methyl group of 5-methyl-THF can only be used metabolically for transfer to homocysteine, which results in the (re)generation of methionine. MTHFR serves a key role in one-carbon metabolism by converting methylene-THF to 5-methyl-THF, thus irreversibly directing this one-carbon moiety to methylation of homocysteine synthesis. Between 50 and 80% of the homocysteine generated is remethylated, depending on the dietary content of methionine and choline. In the methionine synthase reaction, a methyl group is removed from 5-methyl-THF, which functions as a substrate, and is sequentially transferred to the vitamin B-12 coenzyme before homocysteine, thus forming methionine. In addition to protein synthesis, methionine serves as a methyl group donor through conversion to SAM, a key biological methylating agent involved in  $>100$  methyltransferase reactions with a wide variety of acceptor molecules.

The methionine synthase reaction also regenerates THF required for the formation of 5,10-methylene-THF and 10-formyl-THF used directly in thymidylate and purine synthesis, respectively. In the thymidylate synthase reaction, 5,10-methylene-THF donates its  $\text{CH}_2$  unit (becoming the thymidine methyl group). In the de novo purine synthesis pathway, two separate steps utilize 10-formyl-THF.

**Metabolic Control Mechanisms.** The synthesis of methyl groups and other one-carbon units is tightly controlled (Shane 1995, Wagner 1995). Folate coenzymes and relevant enzymes are compartmentalized between the cytosol and the mitochondria. Metabolic products are readily transported between compartments, but the folate coenzymes are not. The cytosolic form of

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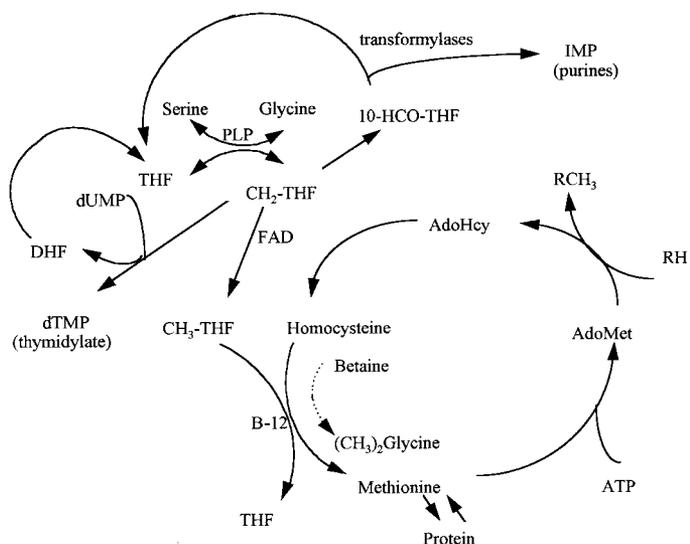
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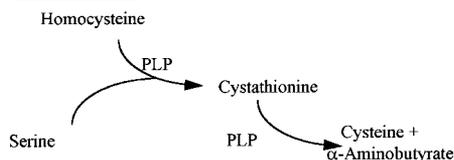
<sup>4</sup> Abbreviations used: AI, adequate intake; ApABG, N-acetylated para-aminobenzoylethylglutamate; DFE, dietary folate equivalents; DRI, Dietary Reference Intakes; EAR, estimated average requirement; FNB, Food and Nutrition Board; MTHFR, methylene tetrahydrofolate reductase; pABG, para-aminobenzoylethylgluta-

mate; PLP, pyridoxal phosphate; RDA, Recommended Dietary Allowance; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate.

### Main Reactions of One-Carbon Metabolism



### Transsulfuration Pathway



**FIGURE 1** Overview of one-carbon metabolism and transsulfuration pathways. Abbreviations: Ado, adenosyl; CH<sub>3</sub>-THF, 5-methyl-THF; DHF, dihydrofolic acid; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; FAD, flavin adenine dinucleotide; 10-HCO-THF, 10-formyl-THF; IMP, inosine monophosphate; PLP, pyridoxal phosphate; RCH<sub>3</sub>, methylated product; RH, methyl acceptor molecule (e.g., DNA, protein, phospholipids); THF, tetrahydrofolate.

SHMT, although reversible, is thought to function mainly in the transfer of carbons from serine to THF, yielding 5,10-methylene-THF under most circumstances. A mitochondrial form of SHMT also exists (Garcia-Martinez and Appling, 1993, Kastanos et al. 1997). Mitochondrial SHMT has been hypothesized to convert glycine to serine and to serve as a source of mitochondrial THF. In contrast to one-carbon metabolism in cytosol, mitochondrial one-carbon metabolism appears to derive much of the one-carbon transfer from folate-mediated serine oxidation (to formate) rather than via SHMT (Graham et al. 1997). In cytosol, the carbon transfer from serine to THF by SHMT is inhibited by 5-methyl-THF and by 5-formyl-THF (Stover and Schirch 1991); 5-formyl-THF formation is a secondary reaction catalyzed by SHMT (Stover and Schirch 1990). Regulation of this pathway also occurs by inhibition of MTHFR by SAM, which suppresses the production of 5-methyl-THF. Thus, the intracellular presence of excess methyl groups (i.e., increased intracellular 5-methyl-THF and SAM as in periods of high intake of methionine, choline and adequate supply of other relevant coenzymes) curtails the further de novo biosynthesis of methyl groups.

Knowledge of nutritional regulation of one-carbon metabolism is slowly evolving. The influence of intracellular folate concentration (as governed largely by dietary intake) on the entry and processing of substrate in one-carbon metabolism is uncertain. Because folates serve as carbon acceptors, carriers and donors, inadequate folate status would impair one-carbon metabolism during severe deficiency. However, because of the role of 5-methyl and 5-formyl-THF as SHMT inhibitors, marginal folate

deficiency may (at least transiently) have little adverse effect on carbon flux because the effects of these physiologic inhibitors may be diminished. Flux through SHMT would be predicted to be a function of PLP concentrations (Jones and Priest 1978).

Regulation by SAM is disrupted in response to a folate deficiency (Miller et al. 1994). In poor folate status, S-adenosylhomocysteine (SAH) concentration would tend to increase due to impairment of methyl group synthesis and homocysteine remethylation. Resulting product inhibition by SAH would suppress many of the SAM-dependent methyltransferase reactions (Selhub and Miller 1992), thus illustrating the far-reaching effects of impaired one-carbon metabolism during such a nutritional deficiency.

Another aspect of nutritional influences on one-carbon metabolism is the effect of cobalamin status on the activity and in vivo action of methionine synthase. Methionine synthase catalyzes the cobalamin-dependent transfer of a methyl group from 5-methyl-THF to regenerate methionine from homocysteine. The co-dependence of methionine synthase on folate and vitamin B-12 provides a biochemical explanation of why a single deficiency of either vitamin leads to the same hematological abnormalities. THF must be regenerated in the methionine synthase reaction before conversion to 5,10-methylene-THF required for thymidylate and, thus, DNA synthesis. Another aspect of the interrelationship between folate and vitamin B-12 is the elevation of plasma homocysteine concentrations by deficiencies of folate and/or vitamin B-12. Thus hyperhomocystemia is not specific for folate deficiency. Homocysteine has two primary metabolic fates as follows: 1) conversion to methionine, and 2) catabolism via the transsulfuration pathway that involves PLP-dependent enzymes cystathionine  $\beta$ -synthase and  $\gamma$ -cystathionase. Vitamin B-6 deficiency inhibits homocysteine catabolism, which tends to increase plasma homocysteine and intracellular SAH concentrations. In summary, folate and vitamin B-12 function in the methylation of homocysteine, whereas vitamin B-6 and folate act in the acquisition (and reduction to methyl level) of one-carbon units from serine, and vitamin B-6 is involved in homocysteine catabolism. Although other sources of one-carbon units exist (e.g., choline, formate, glycine or betaine), serine appears to be the primary carbon donor for the diverse processes of one-carbon metabolism (Pasternack et al. 1996, Shane 1995, Wagner 1995).

### Metabolic and Clinical Manifestations of Folate Deficiency

In the case of folate deficiency, all of the reactions in one-carbon metabolism will be compromised to varying degrees depending on the relative affinities of the enzymes for the respective folate molecules involved. When reactions of one-carbon metabolism are affected by folate deficiency, various substrates and metabolic intermediates will accumulate and may have negative consequences. For example, elevated plasma homocysteine during a folate deficiency has been associated with a significantly increased risk for numerous types of vascular diseases (Boushey et al. 1995, Rimm et al. 1998, Selhub et al. 1995). The pathologic mechanisms involved are still actively debated.

Clinically, severe folate deficiency yields a specific type of anemia, a megaloblastic anemia (Lindenbaum and Allen 1995). Megaloblasts are large, abnormal, nucleated cells that are precursors of erythrocytes; in a folate deficiency, they accumulate and are found in the bone marrow. These cells arise as a result of a failure of the red cell precursors to divide normally. The resulting anemia is not the only manifestation of diminished cell division. There are also decreased numbers of white cells and platelets. There is general impairment of cell division related to folate's role in nucleic acid synthesis, which is more apparent in tissues that turn over rapidly, such as the hematopoietic system and the cells lining the digestive tract (Lindenbaum and Allen 1995).

**TABLE 1**  
*Folate dietary reference intakes<sup>1</sup>*

Group	Adequate intake <sup>2</sup>	Recommended dietary allowance
$\mu\text{g}$ of DFE/d		
Infants <sup>3</sup>		
0-5	65	
6-11	80	
Children and adolescents <sup>3</sup>		
1-3		150
4-8		200
9-13		300
14-18		400
Adults <sup>3</sup>		
$\geq 19$		400
Pregnant women		
All ages		600
Lactating women		
All ages		500

<sup>1</sup> FNB (1998).

<sup>2</sup> Dietary folate equivalents.

<sup>3</sup> Males and females.

Inadequate folate intake has been implicated in the development or enhancement of certain types of cancer. Proposed hypotheses regarding folate's role in carcinogenesis relate to DNA structure, stability and transcriptional regulation; they include increased susceptibility of DNA to strand breakage, uracil misincorporation in DNA and hypomethylation of DNA. Misincorporation of uracil into DNA with chronic folate deficiency is expected to stress the mechanism of DNA repair and thus result in subsequent increases in DNA strand breaks and chromosomal instability (Blount et al. 1997). The sensitivity of human thymidylate synthesis to a folate deficiency was reported by Blount et al. (1997) who observed increased misincorporation of uracil into lymphocyte DNA. Folate-deficient humans had elevated incorporation of uracil into DNA, accompanied by an increased frequency of cellular micronuclei, a measure of DNA and chromosome damage (Blount et al. 1997). Uracil misincorporation was markedly reduced by folate supplementation of folate-deficient subjects, which restored thymidylate synthesis.

Methylation reactions are required for the biosynthesis of many important products, but the methylation of DNA has been shown to regulate the expression of genes in eukaryotic cells. Methyl groups are transferred to the N-5 position of cytosine in DNA by a specific DNA methylase. The extent of methylation of specific genes varies from tissue to tissue and changes during development. It has been shown that, in most cases, undermethylation favors gene expression, whereas increased methylation is associated with gene silencing (Wagner 1995).

The reduction in risk of neural tube defects by folic acid supplementation has been definitively shown by controlled intervention trials, as reviewed previously (Scott et al. 1995). The mechanism by which adequate folate intake reduces risk during the crucial developmental phase of the embryonic neural tube is unknown. Increasing folate intake, which increases the concentrations of folate coenzymes in tissues, may overcome an unidentified metabolic defect in the production of proteins and/or DNA or regulation of gene expression at the time of neural tube development and closure (FNB 1998).

Folate metabolism must adapt during pregnancy to multiple fetal and maternal physiologic influences that change throughout gestation. Adequate folate intake is essential throughout gestation to ensure normal growth and development. Evidence

associating impaired folate status with reduced infant birth weight has been reported in the U.S. (O'Scholl et al. 1996).

**Dietary Intake Recommendations.** Folate requirements historically have been defined as the quantity of intake necessary to prevent a severe deficiency with clinical symptoms (FNB 1989). More recently, the focus has shifted to identifying intakes associated with maintenance of normal one-carbon transfer reactions as described above. Inadequacies identified by abnormalities in the metabolic pathways such as hyperhomocysteinemia, hypomethylation of DNA (Jacob et al. 1998) and uracil misincorporation (Blount et al. 1997) are characterized as functional indicators of folate status (Green and Jacobsen 1995, Selhub et al. 1993). In vivo kinetics, which are readily studied using stable isotopically labeled tracers, also will aid in defining the folate requirement more precisely (Gregory et al. 1998). In addition, a great deal of current research is focused on characterizing optimum folate intake associated with risk reduction for chronic disease (Boushey et al. 1995) and developmental defects (e.g., neural tube) (Daly et al. 1997).

The FNB recently reported new DRI (Bailey 1998, FNB 1998) that include a folate requirement estimate for population groups referred to as the Estimated Average Requirement (EAR). The EAR for folate is defined as the amount of folate that is needed to meet the requirement of 50% of the population. This requirement estimate was based primarily on the ability of specified intakes of folate to maintain normal red cell folate concentration. Red blood cell folate concentration was defined as the primary indicator of adequacy because of its correlation with liver folate and thus tissue stores (Wu et al. 1975). Maintenance of normal homocysteine concentration was also evaluated in relation to folate intake and was considered an ancillary functional indicator of adequacy. The Recommended Dietary Allowances (RDA) were estimated from the EAR by correcting for population variance and were defined as the average level of daily dietary intake sufficient to meet the nutrient requirement of ~98% of the population.

As primary studies on which conclusions regarding the EAR were drawn, the FNB committee considered those metabolic studies in which folate status response to defined diets was determined. Other types of supporting data were provided by epidemiologic studies in which folate intake was estimated in conjunction with plasma folate and homocysteine concentrations. The DRIs for folate are expressed as Dietary Folate Equivalents (DFE), a term adopted by the National Academy of Sciences to adjust for the generally higher bioavailability of synthetic folic acid relative to most forms of naturally occurring folate in foods (Bailey 1998, Cuskelly et al. 1996, Peiffer et al. 1997, Sauberlich et al. 1987). An example of the type of study on which the EAR for adults is based is that of O'Keefe et al. (1995); in that study, a specified quantity of dietary folate was inadequate to maintain normal indicators of folate adequacy in 50% of the experimental group. Specifically, the consumption of 320  $\mu\text{g}$  of DFE in a long-term metabolic study in young adult women was inadequate to maintain normal RBC folate concentrations ( $>140$  ng/mL; 316 nmol/L) in 50% of the group. In addition, the ancillary indicators, serum folate and plasma homocysteine concentrations, also were abnormal in 50% of the group [ $<3$  ng/mL (6.8 nmol/L) and  $>14$   $\mu\text{mol/L}$ , respectively]. A complete description of the entire database on which the new DRI are based is included in the report (FNB 1998). For male and female adults  $>19$  y of age, the folate EAR and RDA are 320 and 400  $\mu\text{g}$  DFE/d, respectively.

For pregnant women, the new EAR and RDA are 200  $\mu\text{g}$  of DFE/d higher than for nonpregnant women (i.e., 520 and 600  $\mu\text{g}$  of DFE/d, respectively) (FNB 1998). The increased requirements for folate during pregnancy are associated with the rapid rate of maternal and fetal cellular growth and development. The types of studies on

which the NAS based the EAR and RDA for pregnant women included population-based studies and one controlled metabolic study (FNB, 1998). Our research group conducted the metabolic study in which folate status was monitored for 12 wk in second trimester pregnant women and nonpregnant controls who consumed one of two quantities of folate (Bonnette et al. 1998, Caudill et al. 1997 and 1998). A folate intake of 600  $\mu\text{g}$  of DFE/d was sufficient to maintain both RBC folate concentration and serum folate in the normal range in pregnant subjects and provided blood folate concentrations that did not differ from those of nonpregnant controls. The conclusion of this study was consistent with the findings from population studies that  $\sim 600 \mu\text{g}$  of DFE/d is adequate to maintain normal folate status in pregnant women.

An additional approach to estimating the folate requirements of pregnant women is the measurement of urinary folate excretory products (McPartlin et al. 1993). This approach is based on the assumption that urinary catabolic products are representative of folate utilized daily because the major route of folate turnover is by catabolism and cleavage of the C9-N10 bond, producing pteridines and *para*-aminobenzoylglutamate (pABG), which is *N*-acetylated before excretion (ApABG). McPartlin et al. (1993) reported a twofold higher urinary ApABG excretion by pregnant women during the second trimester relative to nonpregnant controls. In contrast, data from our metabolic study in which dietary folate intake was strictly controlled indicated no change in folate catabolism due to pregnancy (Caudill et al. 1998).

Folate requirements for lactating women are increased to replace the quantity of folate secreted daily in breast milk plus the amount necessary to maintain normal folate status (Bailey 1998, FNB 1998). It is unclear whether the physiologic changes associated with lactation increase maternal folate requirements. The new EAR and RDA are 450 and 500  $\mu\text{g}$  of DFE/d, respectively (Bailey 1998, FNB 1998).

Folate DRI estimates for all age categories, including infants, children and adolescents, are not based on data from controlled metabolic studies. For infants, it was not possible to estimate an EAR or RDA due to limitations in the database. A separate DRI, designated the Adequate Intake (AI), was based on the quantity of folate consumed daily by breast-fed infants (Bailey 1998, FNB 1998). For childhood and adolescence, data were extrapolated from estimates for the EAR and RDA for adults (Bailey 1998, FNB 1998). Table 1 summarizes the AI and RDA estimates for all age and sex categories.

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