Folate[1]

Folate is a generic descriptor for a family of structurally related compounds that share a common pteroylglutamic acid core and function in the acceptance, redox processing, and transfer of one-carbon units (1). Pteroylglutamic acid, or "folic acid," is seldom found in nature but due to its stability and low cost, is commercially synthesized and used in dietary supplements and as a fortificant in foods. Folic acid consists of a pteridine ring linked by a methylene bridge to para-amino-benzoic acid (PABA)[2] and a single glutamic acid molecule. It is completely oxidized and doesn't contain a carbon substitution. In contrast, folates found in nature are typically reduced to either di-hydro- or tetrahydrofolate and may carry a carbon moiety (i.e., methyl, formyl, methylene, or methenyl). A significant proportion of folates are polyglutamylated, meaning they have several glutamates linked together creating what is commonly referred to as a polyglutamate tail. Folates are best known for their role in nucleotide biosynthesis (purines and thymidine); hence, the synthesis and repair of DNA and re-methylation of homocysteine to produce methionine. Methionine can be used for protein synthesis or subsequently converted to S-adenosylmethionine, the primary methyl donor in the body. Given these roles, the requirement for folate and risk of deficiency is highest during anabolic stages of the life-cycle, which include pregnancy, lactation, and fetal development.

Deficiencies: Folate status is assessed routinely by measuring blood concentrations of the nutrient and review of the size and morphology of blood cells (2). The first sign of suboptimal folate intake is a reduction in serum folate, which is followed by a decrease in RBC folate and a rise in serum homocysteine concentration. Whereas serum folate reflects recent dietary intake, RBC concentrations are thought to reflect tissue stores and approximates folate status over the previous 120 days. Serum homocysteine can be a useful functional indicator of folate status; however, concentrations may also be elevated for other reasons, including vitamin B-12 and vitamin B-6 deficiency. Concomitant with blood folate depletion, hypersegmented neutrophils may appear in peripheral blood, followed sometime later by the manifestation of anemia characterized by the appearance of macroovalocytic RBCs. Since folic acid fortification of the food supply, folate deficiency leading to megaloblastic anemia is rare among healthy North Americans. Common cut-off values used to define risk of suboptimal folate status are < 7 nmol/L (3 μg/L) for serum folate and 317 nmol/L (140 μg/L) for RBC folate concentration (3).

A higher RBC folate cut-off (906 nmol/L [400 μg/L]) for women capable of becoming pregnant has been proposed to reduce their risk of a pregnancy being affected by a neural tube defect (NTD, e.g., spina bifida, anencephaly) and perhaps other congenital anomalies (e.g., congenital heart defects, oral cleft lip and palette) (4). Elucidation of the absolute RBC cut-off, however, requires further research. NTDs specifically occur when the neural tube doesn't close properly in the third and fourth week after conception, often before a woman is aware she is pregnant. Although the mechanisms are not completely understood, it is clear from evidence from randomized control trials (RCT) and national population data pre- and post-folic acid fortification of the food supply that consumption of folic acid during periconception reduces NTD; 25–75% reductions have been observed. Folate deficiency after the first trimester has also been linked to maternal megaloblastic anemia, low infant birth weight, still birth, and premature delivery.

Suboptimal intakes of folate are also negatively associated with the development of various types of cancer including colorectal, prostate, and breast cancer. Mechanisms proposed include folate's role in mediating the transfer of one-carbon moieties necessary for DNA synthesis, stability and integrity, and repair (5). In vitro and in vivo evidence suggest folate deficiency may induce DNA strand breaks, chromosomal and genomic instability, uracil misincorporation, impaired DNA repair, and increased mutations, and that folic acid supplementation can correct some of these defects. Other conditions associated with suboptimal folate status, particularly elevated serum homocysteine concentration, include development of cognitive impairment and stroke (2).

Diet recommendations: The RDA for adults for folate is 400 μg/day of dietary folate equivalents (DFEs) (1). For children, the RDA (μg DFE) is 150 for 1–3 y, 200 for 4–8 y, 300 for 9–13 y and 400 for 14–18 y. The RDA for pregnant and lactating women is 600 and 500 μg/day DFEs, respectively. An adequate intake level of 65 and 80 μg DFE was established for infants 0–6 mo and 7–12 mo of age, respectively. The concept of DFEs was introduced in the 1998 Dietary Reference Intakes (DRIs) for folate as a mechanism to account for the differences in the bioavailability of synthetic folic acid and naturally occurring folate. According to the DRI, synthetic folic acid is 100% bioavailable ingested on an empty stomach. Folic acid, added to foods as a fortificant, is thought to be $\sim$85% bioavailable and naturally occurring food folates about 50% bioavailable. Taken together, the following equation was developed to calculate DFEs: 1 μg of DFEs = 1 μg of naturally occurring food folate = 0.5 μg folic acid taken in the form of supplements on an empty stomach = 0.6 μg folic acid ingested with foods. For women capable of becoming pregnant, the DRI recommends 400 μg of folic acid/day from fortified foods and supplements in addition to a varied diet to decrease the risk of NTD. Health Canada recommends women of childbearing age eat folate-rich

foods and take a multivitamin supplement containing folic acid (400 μg/day) to further reduce the risk of folate-dependent NTD (4).

Food sources: Excellent sources of naturally-occurring folates are green leafy vegetables, dark green vegetables such as broccoli and brussel sprouts, and orange juice, beans, and other legumes (4). As part of a public health strategy to reduce NTD, in 1998 mandatory folic acid fortification of white wheat flour (140–150 μg/100 g) and grain products labeled enriched was fully implemented in North America (2). A number of other countries including Chile, Costa Rica, Australia, and South Africa have also implemented mandatory fortification policies and still others, including New Zealand and the United Kingdom, have voluntary fortification programs in place. Because folic acid fortification of the food supply in North America, the largest reported component of dietary folate now comes from fortified "grains" instead of fruits and vegetables.

Clinical uses: Women who have had a pregnancy affected by a NTD and are planning a pregnancy again may be prescribed higher levels of supplemental folic acid (4000–5000 μg/d) for at least 3 months prior to conception through the first trimester of pregnancy (6). Medications known to elevate folate requirements include chemotherapeutic agents (e.g., methotrexate), anti-epileptic medications (phenytoin, carbamazepine, valproate), sulfa drugs (e.g., sulfasalazine), and treatment for high cholesterol (cholestyramine). Other conditions that may require a folic acid supplement include alcohol abuse, malabsorption, anemia, and gastric by-pass surgery.

Toxicity: The 1998 DRI reported no adverse effects associated with consumption of folate from foods; hence the Tolerable Upper Intake Level (UL) for folate was based on supplemental folic acid intakes alone and its relationship with vitamin B-12 (1). Folate and vitamin B-12 are both co-enzymes involved in the conversion of homocysteine to methionine. Supplementation with $\geq$ 5000 μg/day folic acid can correct the characteristic megaloblastic anemia associated with vitamin B-12 deficiency thereby potentially obscuring and delaying diagnosis of vitamin B-12 deficiency. Untreated vitamin B-12 deficiency can lead to permanent nerve damage and cognitive decline, and high folic acid intakes may precipitate the neurological complications of vitamin B-12 deficiency while correcting the anemia. The Institute of Medicine established a UL of 1000 μg/day for folic acid. The UL was calculated using the lowest observed adverse effect level (5000 μg/day) divided by an uncertainty factor of 5.

Recent research: Although the impact of mandatory folic acid fortification on the reduction of NTD is undisputed for the target population, there has been significant recent research activity on the possible unintended negative consequences of higher circulating folates. Compared to prefolic acid fortification of the food supply, serum folate concentrations have doubled and RBC folate concentrations have increased by at least 60% (2). Additionally, postfortification, unmetabolized folic acid is frequently detected in the circulation of healthy North Americans, even among those not consuming a supplement (7).

Recent research suggests it is unlikely that intakes from dietary sources alone (natural folate and folic acid as a fortificant), exceed the UL and hence there is little risk that consumption of folic acid-fortified foods on their own present a risk of masking a vitamin B-12 deficiency (2,8). Use of folic acid supplements, on the other hand, can result in intakes above the UL, particularly among children (9). Most multivitamins will contain both folic acid and vitamin B-12. As well, several large-scaled, population-based studies suggest that high folate status is a risk factor for developing cognitive impairment in individuals with low vitamin B-12 (10).

One of the most hotly debated undesirable effects associated with high folate intake is its affect on the incidence of cancer. As it has been described in the Deficiencies section above, adequate folate intake and modest intakes of folic acid appear protective against the initiation of carcinogenesis in healthy tissue (5). However, high doses of folic acid consumed after the establishment of neoplastic lesions has been shown in rodents models, at least, to promote cancer development and progression. Available data in humans are mixed. In a recent meta-analyses of 10 RCTs, Wien et al. (11) reported that there was no benefit of folic acid supplementation ($\geq$ 400 μg/day) but a borderline significant increase 1.07 (95% CI 1.00 to 1.14) in incidence of overall cancer in the folic acid group compared to controls. Further, meta-analyses of six RCTs reporting prostate cancer incidence showed an RR of prostate cancer of 1.24 (95% CI 1.03 to 1.49) for the men receiving folic acid compared to controls. In contrast, Stevens et al. (12) recently reported among 43,512 American men and 56,011 American women in the Cancer Prevention Study II Nutrition Cohort that total folate intake was significantly associated with lower risk of colorectal cancer even after folic acid fortification of the food supply (RRQ5vsQ1 = 0.81; 95% CI: 0.66–0.99; P = 0.047). Additional research is needed to fully understand the relationship between folate intake and cancer and the role of supplementation timing, particularly at high intake levels. Given that the prevalence of folate insufficiency in children and adult males is extremely low in North America, consideration should be given to removing folic acid from multivitamin supplements designed for these population subgroups.

Recently, much attention has also focused on maternal folate status and its impact on disease phenotype in offspring via epigenetic mechanisms. Higher maternal circulating folate concentrations were found to be associated with a higher prevalence of insulin resistance (10) and atopic dermatitis in offspring (13). In addition, folic acid supplementation during pregnancy has been linked with compromised respiratory health in early childhood (14). It was also reported that the use of maternal folic acid supplements may be associated with

higher incidence of childhood retinoblastoma in a susceptible subset (15). A definitive conclusion about the aforementioned associations will require confirmation in future studies.

For more information: IOM. Dietary reference intakes. The essential guide to nutrient requirements. Otten JJ, Hellwig JP, Meyers LD, editors. Washington, DC: The National Academy Press; 2006. 244–53.

Bailey LB. (editor) Folate in health and disease. 2nd ed. Baca Raton: CRC Press: 2010.

Smith AD, Kim YI, Refsum H. Is folic acid good for everyone? Am J Clin Nutr 2008; 87:517–33.

Acknowledgments

All authors have read and approved the final manuscript.

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[1]Y.-M. Chan, R. Bailey, and D. L. O'Connor, no conflicts of interest.

Abbreviations used: DFE, dietary folate equivalent; DRI, Dietary Reference Intake; NTD, neural tube defect; PABA, para-amino-benzoic acid; RCT, randomized controlled trial; UL, Tolerable Upper Intake Level.

Literature Cited

1. Food and Nutrition Board, Institute of Medicine. Dietary Reference Intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin and choline. Washington, DC : National Academy Press, 1998.
2. Bailey LB. (editor)Folate in health and disease. 2nd ed. Baca Raton : CRC Press: 2010.
3. Pfeiffer CM, Hughes JP, Lacher DA, Bailey RL, Berry RJ, Zhang M, Yetley EA, Rader JI, Sempos CT, Johnson CL. Estimation of trends in serum and RBC folate in the U.S. population from pre- to postfortification using assay-adjusted data from the NHANES 1988–2010. J Nutr. 2012;142:886–93.
4. Health CanadaPrenatal nutrition guidelines for health professionals, 2009 [cited 2012 Dec 1]. Available from: http://www.hc-sc.gc.ca/fn-an/nutrition/prenatal/index-eng.php. Folate (Cat. No.: H164–109/4–2009E-PDF)
5. Kim YI. Folate acid supplementation and cancer risk: point. Cancer Epidemiol Biomarkers Prev. 2008;17:2220–5.
6. High dose folic acid supplementation - Questions and answers for health professionals, 2010 [cited 2012 Dec 1]. Available from: http://www.hc-sc.gc.ca/fn-an/nutrition/prenatal/fol-qa-qr-eng.php.
7. Bailey RL, Mills J, Gahche J, Pfeiffer C, Dodd K, Dwyer J, Yetley E, Sempos C, Picciano MF. Unmetabolized serum folic acid and its relation to folic acid intake from diet and supplements in a nationally representative sample of adults aged > 60 y in the United States. Am J Clin Nutr. 2010;92:383–9.
8. Shakur YA, Garriguet D, Corey P, O'Connor DL. Folic acid fortification above mandated levels results in a low prevalence of folate inadequacy among Canadians. Am J Clin Nutr. 2010;92: 818–25.
9. Bailey RL, Fulgoni VL, Keast DR, Dwyer J. Do dietary supplements improve micronutrient sufficiency in children and adolescents? J Pediatr. 2012.161: 837–42.
10. Smith AD, Kim YI, Refsum H. Is folic acid good for everyone? Am J Clin Nutr. 2008;87:517–33.
11. Wien TN, Pike E, Wisløff T, Staff A, Smeland S, Klemp M. Cancer risk with folic acid supplements: a systematic review and meta-analysis. BMJ Open. 2012;2:e000653.
12. Stevens VL, McCullough ML, Sun J, Jacobs EJ, Campbell PT, Gapstur SM. High levels of folate from supplements and fortification are not associated with increased risk of colorectal cancer. Gastroenterology. 2011;141:98–105.
13. Kiefte-de Jong JC, Timmermans S, Jaddoe VW, Hofman A, Tiemeier H, Steegers EA, de Jongste JC, Moll HA. High circulating folate and vitamin B-12 concentrations in women during pregnancy are associated with increased prevalence of atopic dermatitis in their offspring. J Nutr. 2012;142:731–8.
14. Håberg SE, London SJ, Stigum H, Nafstad P, Nystad W. Folic acid supplements in pregnancy and early childhood respiratory health. Arch Dis Child. 2009;94:180–4.
15. Orjuela MA, Cabrera-Muñoz L, Paul L, Ramirez-Ortiz MA, Liu X, Chen J, Mejia-Rodriguez F, Medina-Sanson A, Diaz-Carreño S, Suen IH, et al Risk of retinoblastoma is associated with a maternal polymorphism in dihydrofolate reductase (DHFR) and prenatal folic acid intake. Cancer. 2012;118:5912–9.