Folate bioavailability: implications for establishing dietary recommendations and optimizing status[1–4]

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ABSTRACT

The addition of folic acid to the US food supply, along with the critical role of folate in certain health outcomes, has intensified worldwide interest in the bioavailability of folate. Bioavailability is a function of absorptive and postabsorptive processes, which in turn are influenced by diet, individuality, and complex diet-host interactions. As such, it is unlikely that a single bioavailability figure will accurately reflect food folate bioavailability from every diet for every person. Although there is broad agreement that naturally occurring food folate is not as bioavailable as folic acid, questions remain as to the extent of these differences, particularly within the context of a whole diet. This article *1*) summarizes and integrates bioavailability estimates derived from studies that use whole-diet approaches; *2*) highlights the influences of genetics, ethnicity-race, and sex as postabsorptive bioavailability modifiers; and *3*) discusses the adequacy of the US folate Recommended Dietary Allowance in achieving folate sufficiency in select subpopulations. *Am J Clin Nutr* 2010;91(suppl):1455S–60S.

INTRODUCTION

Folate is a generic descriptor for a group of chemically heterogeneous vitamers that differ in one-carbon substitutions, the state of oxidation, and the number of conjugated glutamates (1, 2). Folate functions in the transfer of one-carbon moieties in a metabolic network commonly referred to as one-carbon metabolism (1, 2). The folate-derived one-carbon units may be used for interconversions of serine and glycine, nucleotide biosynthesis (thymidine and purines), or methionine production from homocysteine (1). On activation of methionine to *S*-adenosylmethionine, the methyl group formerly associated with folate may be transferred to numerous acceptor molecules, which yields a diverse array of products that includes neurotransmitters (adrenaline), phospholipids (phosphatidylcholine), energy sources (creatine), and methylated CpG dinucleotides with effects on genome expression and stability (3–5).

Folate insufficiency arising from inadequate dietary folate intake and/or certain genetic variants in one-carbon metabolic enzymes disrupts one-carbon metabolism as evidenced by hyperhomocysteinemia (6, 7). Overt folate deficiency is manifested as megaloblastic anemia due to inadequate nucleic acid synthesis and impaired cellular division. Over the past several decades, numerous chronic and developmental diseases, eg, neural tube defects (8–10), certain cancers (11, 12), stroke (13), osteoporosis (14), and cognitive impairment (15), have been linked to folate.

The wealth of evidence linking supplemental folic acid intake to lower neural tube defect risk led to mandated folic acid enrichment of grain products in the United States (16), Canada (17), and several other countries. In the United States, fortification of the food supply delivers an estimated 200 μg folic acid/d (18, 19) and has resulted in marked improvements in folate status across sex, age, and ethnic groups (20, 21). Substantial reductions in neural tube defect rate (19–54%) have also been reported in the United States and Canada (22–28) and in other countries with folic acid fortification programs (29, 30). However, because folic acid fortification of staple food items exposes entire populations to extra folate with potentially undesirable effects (31–37), it is not a strategy that has been embraced by all countries.

BIOAVAILABILITY INEQUITIES BETWEEN FOLATE FORMS

In food, naturally occurring folate is present primarily in the reduced, more labile, polyglutamated form with methyl or formyl as the one-carbon substitution (38, 39). Fortified foods and supplements contain folic acid, the nonnatural, synthetic, and fully oxidized monoglutamate form of folate. Once absorbed, folic acid requires reduction to tetrahydrofolate to enable its participation in metabolic reactions. Relative to folic acid, naturally occurring food folate has a lower bioavailability, which is defined as the proportion of folate that is absorbed and available for metabolic reactions and/or storage. Several luminal factors may hinder the absorption of natural food folate; these factors include partial release from the food matrix (incomplete liberation from cellular structures), destruction within the gastrointestinal tract, and incomplete hydrolysis of glutamates in excess of one (possibly due to partial inhibition of deconjugation by

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other dietary constituents such as organic acids) (40–47). On the contrary, such factors are negligible in the case of added folic acid that does not require release from cellular structures, is the oxidized form and is less susceptible to destruction within the lumen, and exists as a monoglutamate. Folate bioavailability (both naturally occurring and as folic acid) is also modified by postabsorptive factors that are specific to the individual and include pool sizes of folate and other relevant nutrients (vitamin C, vitamin B-12, and vitamin B-6, niacin, riboflavin, and choline), variations in genetics and homeostatic mechanisms, sex, ethnicity/race, and other biological/physiologic factors (45, 46, 48–52).

Although it is widely recognized that food folate bioavailability is lower than that of folic acid, there is much uncertainty as to their bioequivalence, particularly as part of a whole diet (53). Imprecise estimates of folate bioavailability impede policy development efforts aimed at optimizing folate status to improve health outcomes and hinder revisions of dietary recommendations in countries expressing targeted intake amounts as Dietary Folate Equivalents (DFEs) (46).

DIETARY FOLATE EQUIVALENTS

Introduced in 1998, DFEs are units that attempt to adjust for bioequivalence inequities between folic acid and naturally occurring food folate (54, 55). On the basis of data available at the time of derivation, it was estimated that food folate bioavailability was 50% and that folic acid bioavailability was 85% when taken with food (either as a food fortificant or as a supplement) or 100% when taken on an empty stomach with water (55). Thus, folate derived from fortified foods or from supplements was either 1.7 (85/50) or 2.0 (100/50) times, respectively, as bioavailable as naturally occurring food folate. Expressed differently, the bioavailability of naturally occurring food folate is 60% that of folic acid when consumed with a meal (50/85 × 100). The bioavailability estimates of folic acid were supported by a well-designed study employing stable isotopes (56) and are consistent with previous anecdotal and empirical findings (47, 57–63). There is less confidence, however, in the precision of the bioavailability estimate for naturally occurring food folate (46).

CONTROLLED FEEDING STUDIES ASSESSING FOLATE BIOAVAILABILITY FROM WHOLE DIETS

At present, 4 controlled feeding studies using whole-diet approaches provide estimates of relative food folate bioavailability. Sauberlich et al (64) conducted a 92-d study of 10 women housed in a metabolic unit. After 28 d of folate depletion, all study participants consumed the basal diet, which provided 20 μg food folate/d plus increasing amounts of either folic acid or food folate (days 29–92). During the adequate repletion phase (days 71–92), the addition of 80 μg folic acid/d to the basal diet resulted in plasma folate concentrations that did not differ ($P > 0.05$) from the addition of 180 μg food folate (≈8 compared with 6 nmol/L, respectively; n = 2–4/group). Thus, the relative food folate bioavailability was ≈44% (80/180 × 100), which supports the authors' conclusion that, when compared with synthetic folic acid, dietary folate appeared to be no more than 50% available (64).

Brouwer et al (65) conducted a 4-wk study in 66 healthy adults (aged 18–45 y) in which extra folate was provided from fruits and vegetables (350 μg/d; n = 22) or from synthetic folic acid (500 μg given every other day as a tablet; n = 22). A control group in which extra folate was not provided was also included in this study (n = 22). On the basis of changes (week 4–week 0) in plasma folate, red blood cell (RBC) folate, and plasma homocysteine in response to the extra folate (ie, either 350 μg dietary folate/d or 250 μg synthetic folic acid/d), the percentage of relative bioavailability of natural folate was 60% with plasma homocysteine, 78% with plasma folate, and 98% with RBC folate. However, given that unmetabolized folic acid is observed in plasma with doses as low as 266 μg/d (66), it is likely that not all of the supplied folic acid was available for remethylation of homocysteine to methionine (and other reactions). In turn, the incomplete metabolism of the high folic acid dose administered in this study may have resulted in an overestimation of the relative bioavailability of dietary folate, which was recognized by the authors (65). Paradoxically, however, despite the reduced supply of a metabolically usable folate form, plasma homocysteine was more responsive to the administered folic acid than the other measured endpoints. This suggests that the form of folate may affect its metabolic fate as previously reported in a study employing isotopic tracer methodology (67).

Yang et al (68) conducted a 12-wk intervention study in premenopausal women (n = 45; aged 18–45 y) during which study participants were randomly assigned to receive 400 or 800 μg DFE/d derived from various combinations of folic acid and/or natural food folate (n = 6–9/group). The 1.7 multiplier from the DFE equation was used to convert folic acid to DFEs; the folic acid was consumed with breakfast. Foods used to increase folate included vegetables, legumes, fruits, nuts, seeds, and whole-wheat flour. RBC folate, serum folate, urinary folate excretion, and plasma homocysteine were the outcome measures used to monitor the response to the various treatments. At the end of the controlled treatment period, no differences ($P > 0.05$) were detected in serum folate or in RBC folate within the 400 DFE groups or within the 800 DFE groups. Specifically, in the 400 DFE group, serum folate concentrations were 23.1 and 22.4 nmol/L (10.2 and 9.9 ng/mL) among those consuming predominantly folic acid or exclusively food folate, respectively. Similar results were observed in the 800-DFE group with serum folate concentrations of 41.0 and 35.8 nmol/L (18.1 and 15.8 ng/mL) among those consuming predominantly folic acid or exclusively food folate, respectively (68). These data support the validity of the 1.7 multiplier (85/50) in the DFE equation and in turn suggest that the relative bioavailability of food folate is 60% (ie, 50/85 × 100) when consumed as part of a mixed diet. Direct calculations of relative bioavailability made by using an adapted equation published by Brouwer et al (65) yielded a relative mean food folate bioavailability of 52% (range: 44–59%) (68). Plasma homocysteine and urinary folate excretion could not be used as bioavailability estimates in this study because of the nonlinear relation between these variables and folate consumption (68). For example, at the end of the study, plasma homocysteine concentrations did not differ between the 400 and 800 DFE groups.

Winkels et al (69) investigated the relative food folate bioavailability at the end of a 4-wk dietary intervention study involving 72 healthy adults. The food folate group consumed 296 μg extra folate/d, which was derived primarily from vegetables, fruit, and liver paste. The folic acid groups (n = 3) consumed

additional folic acid from capsules providing 92, 191, and 289 μg/d. In addition, all 4 groups consumed 58 μg [$^{13}C_{11}$]-folic acid/d. On the basis of the percentage of labeled folate in plasma and/or the change in serum folate, the relative food folate bioavailability was either 70% or 80% depending on whether food folate was analyzed microbiologically or by HPLC, respectively. However, as noted by the authors (69), HPLC analysis of food folate underestimates dietary folate, which results in an overestimation of relative food folate bioavailability. Thus, the 70% relative bioavailability estimate is likely a more accurate bioavailability estimate.

Of the markers used in these studies, serum folate is likely the best indicator of bioavailability because it is responsive to a wide range of folate intake, is specific to folate intake/status, and, as the circulating form, reflects the folate available to tissues. Compilation of the data derived from these controlled feeding studies shows that the percentage of relative bioavailability of food folate ranges from ≈44–80 with a median of 65% when based on serum folate. Assuming that the folic acid supplement was taken with food in these studies, the 65% estimate approximates the 60% value obtained by using the DFE equation when expressed as the bioavailability of food folate relative to folic acid consumed with a meal (50/85 × 100). The wide range in food folate bioavailability estimates likely arises from the fraction administered as folic acid, the different foods used to manipulate folate intake, the presence/absence of genetic factors, and/or the variations in existing pool sizes of folate and other nutrients that may affect folate absorption/metabolism.

GENETIC VARIATION AS A POSTABSORPTIVE MODIFIER OF FOLATE BIOAVAILABILITY

The 677C→T single nucleotide polymorphism in the methylenetetrahydrofolate reductase (*MTHFR*) gene is the best example to date of a genetic variant with robust effects on folate metabolism particularly when folate status is compromised (50, 70–73). The MTHFR protein catalyzes the unidirectional reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the major form of folate found in circulation and the subtype used for cellular methylation. Individuals with the *MTHFR* 677TT genotype [≈11% of the US population with differences among ethnic groups (74)] frequently have lower serum folate concentrations, higher plasma homocysteine concentrations, and increased risk of chronic/developmental abnormalities relative to those with the 677CC genotype. The adverse effect of the 677TT genotype on MTHFR activity appears to be countered with higher folate intakes possibly by increasing the affinity of the MTHFR protein for its flavin adenine dinucleotide coenzyme (75, 76). Under conditions of folate inadequacy, additional nutrients, which include riboflavin, interact with the *MTHFR* 677TT genotype to affect biomarkers of folate status (77–79).

IMPLICATIONS FOR ESTABLISHING RECOMMENDED INTAKES

Host–diet interactions will play a significant role in determining the amount of folate available for metabolic reactions. Taking such interactions into account is critical when establishing dietary recommendations aimed at meeting the needs of 97% of the target population. For example, controlled feeding studies conducted in premenopausal women have shown that, although the *MTHFR* 677TT genotype increases the requirement for folate, consumption of the US folate Recommended Dietary Allowance of 400 μg DFE/d (derived predominantly from folic acid or exclusively from food) is sufficient to achieve serum folate concentrations within the normal range (ie, ≥3 ng/mL) (50, 80). However, in Mexican American men, consumption of 438 μg DFE/d derived predominantly from food folate (326 μg/d) for 12 wk resulted in subnormal serum folate concentrations (ie, <3 ng/mL) in 14% of the men with the *MTHFR* 677CC genotype and in 34% of those with the 677TT genotype (71). In addition to showing that the US folate Recommended Dietary Allowance is not optimal for men with the *MTHFR* 677TT genotype, these data suggest that men require more folate than women, a finding that is consistent with recent reports that men need more folic acid than women to achieve comparable RBC folate concentrations (52).

Ethnicity-race may also be an important modifier of folate bioavailability and thus of requirements. Perry et al (49) conducted a 14-wk depletion repletion study involving African American women (n = 14), Hispanic American women (n = 14), and white American women (n = 14). Despite consuming equivalent intakes of folate, African American women had lower serum folate concentrations throughout depletion and throughout repletion with either 400 or 800 μg DFE/d. These findings are consistent with epidemiologic studies showing that African American women have lower serum folate concentrations compared with white American women pre- and post–folic acid fortification (81, 82). The reason(s) for the apparent higher folate requirements by African American women is unclear but likely biologically based (ie, genetic variation in a folate-metabolizing enzyme).

INCREASING FOOD FOLATE AS A STRATEGY FOR OPTIMIZING STATUS

Notably, the lower bioavailability of food folate does not imply that increasing food folate consumption is an ineffective strategy for optimizing folate status. On the contrary, the utility of increased consumption of food folate in improving folate status has been documented. In a 12-wk controlled feeding study involving premenopausal women with the *MTHFR* 677CC (n = 15) or TT (n = 17) genotype, consumption of 800 DFE/d derived exclusively from natural sources resulted in serum and RBC folate concentrations that were 67% (P = 0.005) and 33% (P = 0.001) higher, respectively, than consumption of 400 DFE/d (80). Importantly, women with the *MTHFR* 677TT genotype responded to additional food folate as well as those with the 677CC genotype (80). Likewise, other studies providing folate intakes that were ≈2.5 times the basal intake also reported serum folate increases ranging from 48% to 52% (65, 83, 84). Nonetheless, typical folate intakes in countries without mandated fortification (≈250 μg DFE/d) are not at the levels needed to optimize folate-related endpoints especially for certain genetic subgroups. In this regard, Ashfield-Watt et al (85) reported that a mean folate intake of 660 μg DFE/d that is derived mainly from fortified cereals was needed to achieve near-normal plasma homocysteine concentrations in European adults with the *MTHFR* 677TT genotype. This amount of intake approximates current consumption

amounts (≈700 μg DFE/d) among women residing in the United States (86).

RESEARCH PRIORITIES

Meta-analyses of dose-response data derived from controlled feeding studies (which have reliable measurements of folate intake) could be used to develop dietary folate recommendations that optimize folate status and to derive reasonably robust folate bioequivalency estimates. For example, serum folate concentrations are directly correlated with intake and thus may be used to ascertain intake levels needed to achieve desirable health outcomes (86, 87). Given the numerous pre- and postabsorptive factors affecting folate bioavailability, large-scale studies conducted in target populations are needed to more fully define the intake amount required to achieve folate sufficiency and to refine dietary folate bioequivalency values. Unfortunately, these types of studies await modernization of food folate composition tables (46) that use state of the art methodology (ie, trienzyme extraction followed by separation and measurements employing liquid chromatography mass spectrometry), which would enable measurements of naturally occurring food folate(s) as well as folic acid.

CONCLUSIONS

Bioavailability is a function of absorptive and postabsorptive processes that in turn are influenced by diet, individuality, and complex diet–host interactions. As such, it is unlikely that a single bioavailability figure will accurately reflect food folate bioavailability from every diet for every person. However, there is broad agreement that food folate is less bioavailable than folic acid with a median relative bioavailability of 65% (range: 44–80%), an estimate that approximates the 60% value derived from the DFE equation. As new data emerge, the precision of this estimate may be improved to better account for dietary and/or individual factors.

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