Case Study: Folate Bioavailability\textsuperscript{1,2}

Jesse F. Gregory III\textsuperscript{3}

Food Science and Human Nutrition Department, University of Florida, Gainesville, Florida 32611-0370

ABSTRACT Folate nutritional status depends on intake from food and supplements as well as on the bioavailability of the various ingested forms of this vitamin. Although many advances in the understanding of folate bioavailability have occurred in recent years, many areas of uncertainty remain, especially with respect to naturally occurring dietary folate. This review includes a summary of factors that affect folate absorption and utilization, currently used and promising methods suitable for the assessment of bioavailability, significant findings on which current understanding is based and research needs. J. Nutr. 131: 1376S–1382S, 2001.

KEY WORDS: folate • bioavailability • fortification • absorption

"Folate" is a generic term for a vitamin that functions coenzymatically in the transfer and processing of one-carbon units needed for the remethylation of homocysteine to generate methionine, the synthesis of thymidylate and purines and the formation of methyl groups needed for many biological methylation reactions. Epidemiological studies have demonstrated inverse relationships between folate nutritional status and the risk of various forms of vascular disease, certain cancers and the occurrence of a neural tube defect in an infant should pregnancy occur. The primary ingested forms of folate include folic acid consumed in supplements and added to foods and the main naturally occurring folates that exist as tetrahydrofolate species, often in polyglutamyl form (Fig. 1).

The bioavailability of folate has been a topic of active investigation for many years. Much of the interest in this area originated from reports by Tamura and Stokstad (1973) and Babu and Srikanthia (1976) that showed a wide range of bioavailability of endogenous folate in a wide variety of common foods. Additional impetus for research in this area was provided in a series of studies by Colman and associates (1982) that showed efficacy but apparent incomplete bioavailability of folic acid added to native cereal foods consumed in South Africa. Folate bioavailability has been considered in many previous comprehensive reviews (Anderson and Talbot 1981, Gregory 1989, 1995, Rodriquez 1978). A summary/critique of folate bioavailability and related methodology also was published (Gregory 1997).

Folate bioavailability in large part is governed by the extent of intestinal absorption. Polyglutamyl folates, which constitute much of naturally occurring food folate, must undergo enzymatic deconjugation in the small intestine before absorption. This reaction is catalyzed primarily by a pteroyl polyglutamate hydrolase associated with the jejunal brush border membrane (Halsted 1990), with possibly some contribution by hydrolase activity from pancreatic secretion (Bhandari et al. 1990).

Absorption of monoglutamyl folates occurs via a saturable transport process with acidic pH optimum (K_m \approx 1–3 \mu mol/L), and an apparently nonsaturable absorption mechanism also functions when folate concentrations in intestinal contents exceed 5–10 \mu mol/L (Mason 1990). Because of the existence of two absorption processes, findings regarding folate bioavailability at a certain dosage level may not be predictive of bioavailability at a substantially higher or lower intake. The pH optimum for folate transport is \textasciitilde 5 (Mason 1990), whereas the pH optimum of enzymatic deconjugation is \textasciitilde 6–7 (Halsted 1989). As discussed later, physiological conditions or medications that alter the pH of the upper small intestine could impair folate absorption. In addition, the administration of excessively large doses of acidic foods in bioavailability protocols, as in certain previous studies, could also yield results that do not reflect the bioavailability of more normal doses.

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\textsuperscript{3} To whom correspondence should be addressed at P.O. Box 110370, Newell Drive, Food Science and Human Nutrition Department, Gainesville, FL 32611-0370. E-mail: jfgy@ufl.edu

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in human nutrition has not been established. In addition, it is likely that conclusions reached from rat or chick bioassays regarding the utilization of dietary polyglutamyl folates would not be predictive of bioavailability in humans because of the well-documented differences in the mechanism of intestinal folate deconjugation among rats, chickens, and humans, as reviewed previously (Gregory 1995, Halsted 1990). In addition, several other conceptual and practical characteristics of animal bioassays limit their relevance to human nutrition, including: 1) the need to administer test doses in dry powdered form blended into basal diets, which differs markedly from the form of food consumed by humans, 2) basal diets used in rat bioassays contain an oral antibiotic to suppress intestinal microflora and their endogenous folate production and 3) certain foods or dietary supplements intended for human use cannot be tested in an animal model (e.g., the absorption of folic acid from a commercial vitamin pill cannot be evaluated in a rat or chick).

**Human subjects: short-term protocols.** The bioavailability of folate from dietary sources and supplements often can be determined with short-term protocols based on measurement of the change in plasma or serum folate and/or urinary folate excretion. A limitation of such methods is their relative insensitivity, which makes these protocols suitable only for foods that are relatively high in folate (at least ~300 µg/dose). It is important that a sufficient number of blood samples be taken to allow reliable estimation of the area under the curve (AUC). Several previous studies of folate bioavailability are probably flawed because responses to test and reference doses were compared only on the basis of change in plasma folate 1–2 h after the oral doses. It is likely that the AUC for plasma/serum folate for a given folate dose would be influenced by the folate status of the subjects. Thus, subjects should be screened to ensure that they are in a similar state of folate nutriture. When using urinary folate excretion as a response indicator, it is important to recognize that urinary excretion constitutes "renal reabsorption (Cooperman et al. 1970, O'Keefe et al. 1995). To improve uniformity among subjects and enhance folate excretion, Tamura and Stokstad (1973) devised a "saturation" protocol in which subjects consumed 2 mg folic acid every other day before and between trials of bioavailability studies. Saturation in this context does improve the precision of bioavailability estimates; however, assessments of bioavailability based on urinary excretion are still quite imprecise due to a high degree of variability among subjects (Tamura and Stokstad 1973).

**Human subjects: long-term protocols.** Long-term protocols of typically 3–6 wk duration are suitable for comparative studies of diets that differ in the source of folate. Consequently, if the total folate contents of test diets were equivalent, then any difference in final folate status of subjects would be due to a difference in folate bioavailability. In such proto-

4 Abbreviation used: AUC, area under the curve; NTD, neural tube defect.
cols, serum folate is the most responsive indicator, whereas plasma homocysteine reflects differences in folate status within several weeks of intake. Erythrocyte folate changes in response to folate intake more slowly because <1% of circulating erythrocytes is replaced daily, and most of erythrocyte folate deposition occurs during erythropoiesis. A conceptual advantage of long-term protocols is the fact that the response is based on the aggregate utilization of folate in all meals consumed. Although such protocols are more lengthy and expensive than short-term procedures, they also permit assessment of bioavailability in diets that may not be compatible with AUC measurements.

Several important examples illustrate the use of long-term protocols in studies of folate bioavailability (Brouwer et al. 1999, Colman 1982, Cuskelly et al. 1996, Malinow et al. 1998, Sauberlich et al. 1987). Ideally, such a protocol involves all of the subjects consuming the same basal diet, with various treatment groups chronically consuming either a source of supplemental folic acid (or other chronic reference) dose or a test food material. In the case of the study of Brouwer et al. (1999), subjects consumed either supplemental folic acid or various fruits and vegetables, and plasma folate and homocysteine concentrations were monitored. This protocol indicated ~60–90% bioavailability of the folate from fruits and vegetables, relative to folic acid. In contrast, the study by Cuskelly et al. (1996), whereas not designed as a bioavailability study per se, compared the efficacy of a folic acid supplement, fortified foods and high folate foods. On the basis of erythrocyte folate response during the 3-mo trial, the fortified foods were similar to the supplement in folate bioavailability, both of which greatly exceeded the response observed with the high folate foods that were examined.

Isotopic methods

The use of isotopic labeling in studies of folate bioavailability has the main advantage of specificity and clarity of interpretation. For example, labeled folate molecules appearing in plasma or urine could only be derived from the labeled dose administered. Radiolabeled folates have been used extensively in both humans and animals for the investigation of folate absorption, metabolism and in vivo kinetics. At present, such traditional applications of radioisotopic methods for the study of folate bioavailability have declined due to the current trend to lessen the use of radioisotopes and to reduce the exposure of human subjects and in part because of the development of alternative procedures using stable isotopic labeling. It should be noted that 14C-labeled folates in trace amounts may be suitable for use in human studies of folate bioavailability because of the application of accelerator mass spectrometry methods (Buchholtz et al. 1999), although such data have not yet been reported.

For any isotopic study, especially when two or more labeled tracers are used, validation studies must be conducted to establish that: 1) analytical methods provide accurate measurement of the labeled tracer(s) and metabolite(s), 2) there is no loss of label during metabolism, analytical preparation, purification steps and quantification (e.g., gas chromatography-mass spectrometry procedures) and 3) there is equivalent metabolic behavior among various tracer forms and the unlabeled vitamin species. When deuterium or tritium labeling is used, in which bond energies and rates of dissociation and/or exchange may differ from unlabeled hydrogen (1H), it is essential that labeling be in metabolically and chemically inert components of the molecule. Often, bioavailability studies involve a dual-tracer design, so equivalent metabolic and chemical behavior of tracers at all stages of metabolism and analysis is critical. In this regard, equivalent behavior of [3, 5’-2H2], [glutamate-H4] and [glutamate-13C5] forms of stable isotope-labeled folic acid has been demonstrated under conditions of use in short-term bioavailability protocols (Gregory et al. 1990, Rogers et al. 1997).

Since the late 1980s, studies using dual-tracer protocols with stable isotope-labeled folates have permitted investigation of many aspects of folate bioavailability in humans. These generally have involved short-term studies in which relative bioavailability between tracers or among experimental groups is assessed using urinary excretion of labeled folates for 24–48 h after dosing. Analyses of urinary folates were initially performed in our studies because gas chromatography-mass spectrometry methods of the mid-1980s were only marginally suitable to measure labeling of plasma folate. Urinary excretion of folate in normal renal function is primarily a function of plasma folate concentration. The short-term urinary excretion of a folate tracer depends in large part on the folate status of the subjects; it is a small and variable fraction of the dose at typical folate intakes of 200–400 µg. A saturation protocol that is a modification of that devised by Tamura and Stokstad (1973) increases the excretion of labeled folate and reduces between-subject variability. If subjects are in approximately the same folate status, it is possible to conduct bioavailability studies without the saturation protocol (Pfeiffer et al. 1997). This eliminates any question regarding an influence of elevated folate status on bioavailability (Gregory 1997), although there is consequentially reduced precision.

In an effort to improve the precision by normalizing for variation in urinary excretion, an injected reference dose has been used in several isotopic bioavailability protocols. A typical bioavailability protocol using this approach would involve oral administration of [13C5]folic acid and a bolus intravenous injection of [2H4]folic acid. In such a protocol, the response variable used is a normalized excretion, i.e., excretion of labeled folate derived from the oral dose divided by excretion derived from the injected dose (Gregory et al. 1991 and 1992, Pfeiffer et al. 1997).

In typical pharmacological studies of drug or nutrient bioavailability, a comparison of the plasma AUC derived from oral and injected doses allows the determination of absolute bioavailability. Rogers et al. (1997) tested this approach for moderate doses of folate: 400 µg oral [13C5]folic acid and 100 µg [2H4]folic acid. The AUC response for labeling of plasma folate, when adjusted for the difference in dose, was ~15 times greater for intravenous folates than for oral folates. This suggests that absolute bioavailability cannot be readily determined by this conventional protocol because of the extensive hepatic uptake and enterohepatic recycling of absorbed folates derived from the oral dose. These findings are in marked contrast to those typically found using much larger oral and injected doses of nonlabeled folates (e.g., DeVito et al. 1993). In view of this limitation of the determination of plasma AUC and in view of the relative ease of urine collection and analysis, it appears that analysis of urinary excretion of labeled folates in a well-designed protocol is the best approach in many applications of stable isotopic techniques.

In vitro methods

The development of in vitro methods has long been a research objective in many areas of research concerning nutrient bioavailability to allow screening of test materials more rapidly and easily than could be achieved with in vivo methods. An initial hypothesis in the author’s laboratory was that
the components of foods would reduce the bioavailability of polyglutamyl folates by inhibiting the action of intestinal brush border pteroylglutamate hydrolase (conjugase). We further hypothesized that as a measurement of the extent of conjugase inhibition by food extracts in a standardized assay of conjugase activity in brush border membrane vesicles with a synthetic polyglutamyl folate as substrate, one could classify or rank foods that predict the probably bioavailability of their naturally occurring polyglutamyl folates. Indeed, studies of conjugase inhibition by extracts of a wide variety of foods indicated that inhibition was common and that there was a wide range of inhibitory potency when tested using brush border membrane vesicles from either human or pig small intestine (Bhandari and Gregory 1990). Subsequent studies showed that the predominant conjugase inhibitors were anionic species of organic acids, including citrate, malate and ascorbate, all of which acted as competitive inhibitors (Wei and Gregory 1998). The importance of these in vitro studies is that they show the potential for incomplete bioavailability of polyglutamyl folates in foods but do not definitively predict incomplete bioavailability. This relationship was evaluated in a series of in vivo stable isotopic studies involving the addition of monoglumyl and polyglutamyl folate tracers to orange juice, canned tomatoes, lima beans, a citrate buffer equivalent to citrate in orange juice and water controls (Wei et al. 1996). Although all of these substances yielded moderate-to-substantial inhibition of intestinal conjugase in vitro, only orange juice caused inhibition of the bioavailability of the polyglutamyl folate tracer (i.e., ~33%). Despite this significantly incomplete bioavailability of the polyglutamyl tracer added to orange juice, orange juice is still a good source of available folate because of the high concentration of folate that has reasonably high bioavailability (Wei et al. 1996).

Seyoum and Selhub (1999) devised an alternative approach to the in vitro estimation of folate bioavailability. In this method, a food extract containing naturally occurring folates is incubated under conditions designed to simulate gastric acid, followed by neutralization and exposure to intestinal conjugase activity. A “bioavailability index” is calculated on the basis of both in vitro retention (i.e., stability) and extent of deconjugation. Of interest was the fact that significant correlation was obtained between the in vitro bioavailability index and apparent in vivo bioavailability derived from the human studies of Tamura and Stokstad (1973). Further work is needed to assess and confirm the merits of in vitro prediction of folate bioavailability.

Population-based methods

Some important information regarding folate bioavailability may be inferred for a well-characterized population group through the analysis of relationships among total folate intake, consumption patterns of foods and dietary supplements and biochemical indicators of folate status. For example, Tucker et al. (1996) examined data describing 885 elderly subjects of the Framingham Heart Study and found significant independent dose-response relationships between the consumption of breakfast cereals and of fruits and vegetables and folate status as indicated by plasma folate and homocysteine concentrations. These results indicate that each of these classes of food represents a source of available folate. Similarly, Rimm et al. (1998) examined relationships between vitamin B-6 and folate intake from foods and supplements and cardiovascular disease incidence in the Nurses’ Health Study. This study provided clear evidence of an impact on health of both dietary and supplemental forms of folate, but no attempts to quantify relative effects were reported. Although such methods provide important information regarding comparative bioavailability of dietary folate sources, their absolute accuracy may be limited by the currently imprecise values in food composition databases.

Key findings that shape modern views of folate bioavailability

Chemical form of folate. Monoglutamyl folates. As reviewed previously (Gregory 1995), several studies have reported differences in the apparent bioavailability of synthetic forms of individual folates with inconsistent results. An isotopic investigation showed ~30% lower response to the biologically active isomer of tetrahydrofolate than that of three other reduced folates (Gregory et al. 1992). However, urinary excretion of labeled folates derived from [7H]folic acid exceeded that of all reduced folates. This is now interpreted as indicating lesser in vivo retention of folic acids when consumed by folate-saturated subjects at moderately high doses. It is likely that differences among monoglumyl folates reported previously for moderately high doses (at least several hundred micrograms) are due to differences in hepatic uptake and enterohepatic circulation, tissue distribution and urinary reabsorption. Lower doses of radiolabeled folic acid, 5-methyltetrahydrofolate and 5-formyl-tetrahydrofolate in rats showed essentially complete absorption, similar short-term distribution and metabolism and in vivo kinetics (Bhandari and Gregory 1992). Thus, it appears that there is little inherent difference among the various folate species when consumed at low doses.

An additional aspect of dose-response behavior in folate bioavailability is now apparent, i.e., metabolic capacity. Single doses of folic acid of more than several hundred micrograms exceed the metabolic capacity for reduction and methylation (Kelly et al. 1997, Lucock et al. 1989).

Monoglumyl versus polyglutamyl folates. The bioavailability of polyglutamyl folates relative to monoglumyl folates forms has been extensively examined for many years and was reviewed by Gregory (1995). Long-chain (n = 5–7) polyglutamyl folates are available for absorption and metabolic utilization only to the extent that they undergo enzymatic deconjugation in the small intestine. Thus, incomplete bioavailability of doses of synthetic or purified naturally occurring polyglutamyl folates would be indicative of incomplete deconjugation. Of the many studies in which monoglumyl and polyglutamyl folates are compared, relative bioavailability values range from ~50 to ~100%, with an average of these studies of ~75% bioavailability for long-chain polyglutamyl species (Gregory 1995). The reason for this broad range is unclear; possibly there is a dose-dependent reflective of the kinetics of deconjugation, possibly inhibitors (e.g., ascorbate) are also involved and in some studies analytical error cannot be ruled out. In addition, studies based only on short-term changes in plasma folate concentrations may underestimate the bioavailability of polyglutamyl folates if absorption rate, rather than extent, differs. A number of well-conducted studies that indicate approximately equivalent bioavailability of monoglumyl and polyglutamyl folates strongly suggest that the human small intestine has sufficient pteroylglutamate hydrolase activity to deconjugate polyglutamyl folates fully in the absence of food materials. For example, as described later, a stable isotopic study that compared simultaneous doses of [7H]folic acid hexaglutamate with [7H]folic acid monoglutamate indicated equivalent bioavailability in five sequential trials (two control trials with aqueous folate solutions administered, along with those in which folates were administered after blending with tomatoes, lima
beans or a citrate buffer) in folate-saturated humans (Wei et al. 1996). A recently identified genetic polymorphism of intestinal pteroylglutamate hydrolase (glutamate carboxypeptidase II) is associated with impaired absorption of dietary folate and, hence, lower folate status in persons having the less active form of the enzyme (Devlin et al. 2000).

Bioavailability of naturally occurring and added folate in foods

It is widely recognized that for typical mixed diets, the bioavailability of naturally occurring folate is incomplete. The results of a long-term controlled dietary study with human subjects by Sauberlich et al. (1987) indicated that the bioavailability of folate in a typical mixed diet was no more than 50% relative to folic acid in a formula diet. In view of the small number of subjects and the variability of indices used, the value of \( \leq 50\% \) is quite imprecise. Cuskelly et al. (1996) conducted a similar study with free-living subjects and observed that fortified foods and folic acid in supplements were substantially more effective than high folate foods provided to the subjects. Whereas not a quantitative study of bioavailability, this study showed lower aggregate bioavailability for the high folate foods that were used.

It should be recognized that not all food sources of folate exhibit poor bioavailability. For example, Brouwer et al. (1999) found that an assortment of fruits and vegetables exhibited 60–90% bioavailability relative to folic acid. Moderately high bioavailability of spinach folate, although not quantified as a percentage, was reported by Prinz-Langenohl et al. (1999). Rhode et al. (1983) and Neuhouser et al. (1998) reported evidence of incomplete but reasonably high bioavailability of naturally occurring folate in orange juice.

The influence of diet composition and food selection on the overall bioavailability of dietary folate has not been determined and currently cannot be predicted. In addition, the reasons behind the often-incomplete bioavailability of individual foods are also unclear, although it can now be proposed to be a function of three main factors. First, it is likely that foods such as orange juice have partially incomplete bioavailability of polyglutamyl folates due to the presence of natural inhibitors (Wei et al. 1996, Wei and Gregory 1998). Second, in vitro evidence reported by Seyoum and Selhub (1998) strongly suggests that some chemical instability of reduced folate in the digestive system before absorption also accounts for some variation in bioavailability among foods. Third, it appears that entrapment of folates in the cellular structure of plant materials partially inhibits their absorption (Castenmiller et al. 2000, van het Hof et al. 1999).

Several lines of evidence indicate higher bioavailability of added folic acid than naturally occurring folates in many foods. As stated previously, the study by Cuskelly et al. (1996) indicates similar bioavailability of folic acid in fortified foods and dietary supplements. In addition, labeled folate added to various cereal-grain foods also exhibited bioavailability similar to that of folic acid in aqueous solution (Pfeiffer et al. 1997), and folate in fortified breakfast cereal has been shown to be effective in raising folate status in humans (Malinow et al. 1998).

Bioavailability of folate in dietary supplements

Very little published data are available regarding the bioavailability of commercial dietary supplements or the influence of formulation on rate and extent of absorption. Despite concern that incomplete in vitro dissolution of certain commercial supplements may be indicative of incomplete bioavailability, no in vivo data have been published regarding this issue. In the context of long-term nutrition, minor differences in rate of absorption are of little or no consequence.

Another issue is the influence of food on the bioavailability of folic acid in supplements. In this regard, the influence of a light breakfast meal on the absorption of folic acid was examined in a stable isotopic study with humans (Pfeiffer et al. 1997). Consumption of the folate tracer with the light breakfast meal caused \( \sim 15\% \) lower bioavailability than that observed when consumed without food. The influence of food on the dissolution of various types of supplements has not been determined.

Dietary folate equivalents and assumptions regarding bioavailability

In the development of the 1998 Dietary Recommended Intakes for folate, the Institute of Medicine (1998) defined and initiated the use of “dietary folate equivalents.” This was done in an effort to place all sources of ingested folate on a comparable basis by adjusting for differences in their bioavailability, which is a major improvement in considering and comparing sources of folate. Dietary folate equivalents are defined as: naturally occurring food folate (\( \mu g \)) + 1.7 \times of synthetic folate (\( \mu g \)), which represents an attempt to adjust for the greater contribution of synthetic (added or supplemental) folate due to its generally greater bioavailability. The multiplier of 1.7 was derived as the ratio of the relative bioavailability of folic acid consumed with food of 85% (Pfeiffer et al. 1997) and the relative bioavailability of dietary folate, assumed to be 50% (Sauberlich et al. 1987). When one recognizes that each estimate, i.e., 85 and 50%, is a rather imprecise figure, then it is apparent that the uncertainty associated with this weighting factor in the calculation of dietary folate equivalents is even greater.

Physiological and pharmacological factors that affect folate bioavailability

The view of folate bioavailability becomes more complicated if one considers physiological and pathological variables associated with the host. The intestinal absorption of folate by intact intestinal tissue exhibits a broad pH-activity curve with a pH optimum of \( \sim 5.0 \) (Mason 1990), whereas folate deconjugation occurs maximally at pH 6–7 (Halsted 1990). Alterations in intestinal pH by achlorhydria or pancreatic insufficiency or drugs can alter the rate and extent of folate absorption (Russell et al. 1979 and 1986). Salicylosulphapyridine (Azulidine), a drug used in the treatment of inflammatory bowel disease, is a competitive inhibitor of folate absorption (Halsted 1990). Many commonly used anti-inflammatory drugs have been shown to have antifolate activity in in vitro models (Baggott et al. 1992), although in vivo effects are largely unknown. Although such drug effects may or may not be considered as true variables in many definitions of bioavailability, they should be considered as factors that affect folate utilization in the broad sense.

The major impetus behind the recently implemented addition of folate to cereal grain foods in the United States is the finding that risk of neural tube defect (NTD) in pregnancy is reduced in proportion to folate nutritional status (Mollov et al. 1999, Scott et al. 1995). The reason for the protective effect of folate has not been identified. Several groups have investigated intestinal absorption is related to the risk of NTD. Neuhouser et al. (1998) examined short-term plasma response
in women who had a previous NTD-affected pregnancy and controls in response to either 400 µg folic acid or 0.95 L orange juice. Although the response of this study is ambiguous because of possible differences in folate status of these patients and controls, a statistically lower AUC response to the folic acid supplements was seen in the NTD case women. Davis et al. (1995) examined urinary excretion after an oral [1H4]folate dose to NTD case women and controls in a standardized folate-saturation protocol. A slightly but not significantly lower response was observed in NTD case women. This observation has been confirmed and extended in a recent study (Boddie et al. 2000). These results suggest the need for further examination of whether intestinal absorption of folate is somewhat impaired in women at risk of an NTD-affected pregnancy. One hypothesis is that this may be an indicator of impaired folate transport in other tissues at critical stages of development.

**Nutrient interactions that affect folate bioavailability**

Relatively little is known regarding nutritional interactions that affect folate bioavailability. In this regard, concern has been expressed regarding a proposed mutually inhibitory effect of zinc and folate. Milne et al. (1984) reported that folic acid supplementation increased the fecal excretion of zinc. Ghishan et al. (1986) showed evidence of a complexation between zinc and folic acid that reduced intestinal absorption of each in model absorption studies. However, further in vivo studies have shown that such an effect has little if any nutritional significance. Butterworth and Tamura (1989) reported that 5–15 mg/d supplementation with folic acid had no effect on long-term zinc status in women, and Keating et al. (1987) showed that the concurrent administration of folic acid and zinc did not reduce the short-term absorption of zinc in humans. Finally, Kauwell et al. (1995) examined the long-term (25 d) interactions of deuterium-labeled folic acid (800 µg/d) and two levels of dietary zinc. This high level of folic acid intake did not impair zinc status, and there was no relation between zinc intake and the bioavailability of the supplemental folate. Thus, concerns regarding this nutritional interaction are unfounded.

**Research needs**

As discussed in this review, major advances have been made in the development of research protocols and in the clarification of many issues regarding folate bioavailability. In part because of the growing understanding of the public health significance of this vitamin, folate has been the subject of considerable research effort. In certain respects, the bioavailability of folate is now understood more fully than that of many vitamins and minerals in foods and supplements. In addition, folate bioavailability is better understood than the bioavailability of most non-nutritive components of dietary supplements. However, there are many aspects of folate bioavailability that remain of high nutritional priority, including the following:

**Bioavailability of naturally occurring dietary folate.** A major research priority is attain an improved understanding of the bioavailability of naturally occurring folate and its relation to diet composition. This is particularly important in populations with little intake of fortified foods and dietary supplements.

**Influence of food preparation methods on bioavailability of naturally occurring folate.** Can food processing or preparation techniques significantly alter bioavailability by altering the physical properties (e.g., integrity of plant cell wall components)?

**Concept of dietary folate equivalents for expression of dietary allowances and description of food folate content.** Further quantification and fine tuning of assumptions regarding bioavailability of naturally occurring and synthetic folate are needed to improve the precision and utility of this approach to assessing folate intake.

**Health effects of various diets and forms of folate.** Continued research is needed to more fully assess the relative effect of diet composition variables, relative bioavailability of folate in such diets and relative impact on health of various foods and forms of supplemental folate.

**Analytical methodology.** The bioavailability of a nutrient cannot be assessed without accurate analytical data. Better analytical methods are needed for the measurement of total folate in diets, including naturally occurring folates and added folic acid. In addition, improvement in food composition data for folate is an urgent need.

**Bioavailability and health.** A number of questions should be addressed in which changes in folate bioavailability may be associated with direct health effects, including possible differences associated with populations at particular risk of NTD-affected pregnancy, segments of the population in whom digestive changes are common (e.g., achlorhydria in elderly persons) and the impact of commonly prescribed prescription drugs or over-the-counter medications.

**Dietary supplements.** Improved understanding of the relationships among formulation variables, rates of dissolution and solubilization in in vitro testing and in vivo bioavailability are high priorities.

**Population-based studies.** An examination of epidemiological and population-based data will provide an alternative and powerful means of examining the role of bioavailability as associated with many aspects of human health, food selection patterns, fortification methods and effects, disease effects on folate bioavailability and nutritional status and effects of common genetic polymorphisms.

**LITERATURE CITED**


