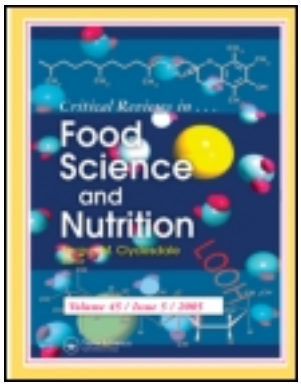

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EURRECA—Estimating Folate Requirements for Deriving Dietary Reference Values

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EURRECA—Estimating Folate Requirements for Deriving Dietary Reference Values

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In most countries, the dietary folate intake associated with adequate status of red cell folate and/or serum folate provides the basis for formulating reference values. One of the major challenges in setting dietary reference values for folate, however, is the need to account for the differences in bioavailability between the natural forms of the vitamin and the synthetic form, folic acid, albeit to date, few countries in Europe take bioavailability into consideration. A series of systematic reviews that included only those studies which used the most robust measures of both folate intake and folate status were carried out by the EURRECA Network of Excellence to examine the relationships between folate intake, status, and a number of health outcomes relevant to specific stages of the lifecycle. This review summarizes the available evidence and the issues to consider in the setting of dietary reference values for folate.

Keywords Folate, folic acid, systematic reviews, dietary recommendations, requirements, folate intake, folate status, folate bioavailability, health outcomes

ABSORPTION, METABOLISM, AND FUNCTION

Folate is a B-vitamin that occurs naturally in a wide range of plant and animal tissues, whereas folic acid is the synthetic form found in supplements and fortified foods. Folic acid is composed of a pteridine ring linked to a para-aminobenzoic acid molecule and a glutamic acid molecule. Food folates are predominantly polyglutamates, but are converted to monoglutamates prior to absorption in the proximal small intestine. Absorption is by a saturable, pH-dependent, active transport process, and once absorbed, folate is transported to the liver, where it is metabolized to polyglutamate forms prior to storage or reconverted to monoglutamates and released into the blood circulation predominantly in the form of 5-methyltetrahydrofolate (5-MTHF). Folate is also released into bile, where it is subsequently reabsorbed via enterohepatic circulation. The main circulating form of the vitamin must be reduced and reconjugated to the polyglutamate form before it is metabolically active. Folate is essential for the transfer and utilization of one-carbon units for amino acid metabolism, synthesis of purines and pyrimidines (precursors of DNA and RNA synthesis), and methylation reactions. Folate deficiency results in reduced DNA biosynthesis and thereby reduced cell division, leading to the major clinical manifestation of the deficiency, megaloblastic anemia. The deficiency also results in elevated plasma total homocysteine concentrations as functioning of the methylation cycle is impaired. Folate is required as a cofactor for methionine synthase, the vitamin-B12-dependent enzyme that converts homocysteine to methionine. Elevated plasma homocysteine and/or low folate status is associated with a number of chronic diseases such as stroke (Wang et al., 2007), cancer (Kim, 2006), and cognitive impairment (Durga et al., 2007).

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CURRENT DIETARY RECOMMENDATIONS

Nutrient recommendations can vary considerably from one country to the next, making comparisons difficult. In the case of folate, this comparison is further complicated by the fact that in some countries, including the United States, folate intake is expressed in micrograms of "dietary folate equivalents" (DFEs), the term used to account for the differences in bioavailability

between natural food folates and folic acid added to foods (Institute of Medicine, 1998). The DFE, where used, is calculated as the amount of natural food folate plus 1.7 times the amount of added folic acid in food. In most European countries however, this conversion factor is not applied and folate intakes and recommendations are expressed simply as total folate in μg/d, and thus disregarding the known differences in bioavailability between the natural food forms and folic acid.

As part of the EURRECA Network of Excellence activities, a comparison of folate recommendations between some European and non-European countries was conducted by Doets et al. (2012). They reported that generally, dietary folate recommendations are set at similar levels of intake for both males and females (apart from pregnant and lactating women), and for younger and older adults. However, countries/organizations with more recently generated recommendations based on newer evidence estimate higher folate requirements than those countries (including the United Kingdom) with older recommendations still in place. In reports from all countries reviewed, except France, the average requirement (AR) was established based on the concentration of folate in blood or tissues that is associated with no deficiency symptoms. Red cell folate (RCF) and serum/plasma folate were the biomarkers of folate status, which were most often used as primary health indicators for establishing an AR, albeit cutoff values for adequacy varied across reports (cutoff values ranged from greater than 300 nmol/L to greater than 340 nmol/L for RCF, and from greater than 6.8 nmol/L to greater than 10 nmol/L for serum folate). In addition, whereas some countries also included plasma homocysteine concentrations as a folate-related health indicator (e.g., France, DACH countries, i.e., Germany, Austria, and Switzerland), others considered that folate requirements should not be based on homocysteine concentrations, given that the evidence for homocysteine as a cardiovascular disease (CVD) risk factor was inconclusive (Doets et al., 2012). Furthermore, upper intake levels for folic acid have been set for the Nordic and DACH countries, the United Kingdom, France, and the Netherlands. The online EURRECA resource "Nutri-RecQuest" collates folate recommendations across European and non-European countries (http://www.serbianfood.info/eurreca/; Cavelaars et al., 2010a).

CURRENT INTAKES/ADEQUACY

In most European countries, folate intakes are typically high enough to prevent overt clinical deficiency (i.e., megaloblastic anemia), but are insufficient to provide optimal folate intake to lower the risk of neural tube defects (NTDs) and possibly other chronic diseases (McNulty and Scott, 2008). There are relatively few foods that provide folate in rich supply and the risk of inadequate intake is common in those who have poor diets, such as low-income and immigrant groups, or during stages of the lifecycle where requirements are increased. Higher intakes are required during pregnancy and lactation for fetal growth and development as well as for maternal health. Elderly people, because of age-related changes in physiological function, poor dietary status, and/or polydrug use may be at risk of inadequate folate intake. The amount of folate available for ingestion is also affected by the method of food preparation and cooking, particularly in the case of green leafy vegetables (McKillop et al. 2002), which are often recommended as a rich dietary source of folate. Furthermore, while many European countries permit the addition of folic acid and other nutrients to foods on a voluntary basis (i.e., at the manufacturer's discretion), others prohibit fortification of any kind, creating great variability in intake across countries. The European Commission, however, is aiming in the near future to regulate in member states the minimum and maximum amounts of vitamins and minerals allowed to be added to foodstuffs (European Commission, 2006).

Several EURRECA research activities addressed folate intake and adequacy. A systematic review was carried out of the dietary methods used for assessing the adequacy of micronutrient intake (including folate) in European and non-European surveys and to compare adequacy for folate intake across surveys (Tabacchi et al., 2009). The percentage of adult women considered to have inadequate folate intake (based on a total of eight papers) varied considerably from 17.1 to 92.1%, depending on the reference value used to define adequacy. A further study investigated the prevalence of nutrient intake inadequacy in adult and elderly populations using European nutritional surveys. The Nordic nutritional recommendations and the estimated average requirement cutpoint were applied (Roman Viñas et al., 2011). This paper reported that folate inadequacy was common in both adult and elderly populations and in males and females, especially in countries such as the Netherlands and Finland (above 34%). The lowest reported prevalence of folate inadequacy was in countries such as Denmark and Ireland, but the prevalence was higher than 15% in all surveys and for both sexes, except for Italian males (10%). Examination of intake data from national surveys found folate intake to be inadequate for most life-stage groups (children and adolescents, adults, elderly, and pregnant and lactating women) in Europe, supporting the identification of folate as a priority nutrient for further investigation (Cavelaars et al., 2010b).

ESTABLISHING THE MOST ROBUST METHODOLOGY

Intake Assessment

A series of systematic reviews of dietary intake validation studies were carried out as part of EURRECA activities to analyze the utility of various dietary assessment methods, and in particular, the food frequency questionnaire (FFQ), for measuring micronutrient intake among specific population groups. Studies were grouped according to whether the reference method used reflected short-term intake (i.e., <7 days), long-term intake (i.e., ≥ 7 days), or a biomarker (i.e., serum folate/RCF). A scoring tool was developed whereby each study

Table 1 Validity of the food frequency questionnaire as a method to measure dietary folate or folic acid intake across specific population groups according to the reference method used

Life stage	Short-term intake (<7 d)	Long-term intake (≥7 d)	Biomarkers (SF/RCF)
		Infants/Children	
Folate	NA	NA	NA
Folic acid	NA	NA	NA
		Adolescents (13–18 y)	
Folate	Good	NA	Acceptable
Folic acid	NA	NA	NA
		Pregnancy	
Folate	Good	Acceptable	Poor
Folic acid	NA	Good	Good
		Elderly	
Folate	Acceptable	Good	Poor
Folic acid	NA	NA	NA

SF = serum folate, RCF = red cell folate, NA = no available studies; Good (0.51–0.70), acceptable (0.30–0.50), and poor (<0.30) (Ortiz-Andrellucchi et al., 2009a, 2009b, 2009c; Serra-Majem et al., 2009; Roman-Viñas et al., 2010).

was given a quality score and then the correlation coefficient was weighted according to the quality score. The validation studies were then classified as very good (≥0.7), good (0.5–0.69), acceptable (0.3–0.49), and poor (<0.3) on the basis of the mean-weighted correlation coefficients (Serra-Majem et al., 2009). Table 1 shows that no or few studies were identified for infant, children, and adolescent population groups (Roman-Viñas et al., 2010). In pregnancy and in adolescents, the FFQ was found to be a good method of measuring folate intake when short-term intake was used as the reference method and for measuring long-term intake in elderly people. Furthermore, in pregnancy, the FFQ was a good measure of folic acid intake when both long-term intake and biomarkers were used. In contrast, the FFQ was a poor method of measuring natural folate intake when biomarkers were used as the reference method (Ortiz-Andrellucchi et al., 2009a, 2009b, 2009c). However, apart from studies in pregnancy and in elderly that validated the FFQ against reference methods reflecting short-term folate intake, the other classifications were based on one or two studies only and therefore must be interpreted with caution. One approach that may help to clarify true intakes of folate in future studies assessing dietary intake would be to measure folate intake as DFEs rather than μg/d folate. Application of the adjustment factor (i.e. 1.7) would account for the lower bioavailability of food folates compared with folic acid, a factor not considered in previous validation studies.

In addition to the well-documented issues affecting the accuracy of nutrient intake data, obtaining robust information on folate intake is further complicated by differences in stability and bioavailability between the chemical forms of the vitamin and in the method used to measure food folate. The validity of using a conversion factor of 1.7 is debated as its estimation relies heavily on just two bioavailability studies (Sauberlich et al., 1987; Pfeiffer et al., 1997). Despite this limitation, the value of 1.7 is considered preferable to measuring folate intake and setting recommendations simply as micrograms of total folate per day, with no adjustment for the known difference in bioavailability between the natural and the synthetic vitamin forms. However, DFEs are not in current use by European-based bodies responsible for setting nutrient recommendations except for the DACH countries. We showed that folic acid was a much more important determinant of RCF concentration than natural food folate, and that folate intakes were much more strongly correlated with biomarker status when expressed as DFEs than as micrograms of total folate (Hoey et al., 2007); results that support the approach of expressing folate intake as DFEs.

Another issue of relevance to assessing folate intake is that different analytical procedures for measuring food folate content can lead to inaccuracies in reported food folate values. Food composition databases are often based on traditional methods of extracting folate from the food matrix compared with the newer tri-enzyme treatment method that results in substantial increases in measurable folate and thus more accurate folate intake data (Tamura, 1998). In addition, published food tables often do not take into account fortified foods or do not use the most up-to-date information on micronutrient values in fortified foods. These inaccuracies in food composition data will in turn have major implications for the calculation of dietary folate intake, and consequently, for setting folate recommendations.

Status Assessment

EURRECA partners along with invited international folate experts formed a 'Biomarkers of Status Working Party' at a 2008 workshop in Norwich, United Kingdom. The available biomarkers used to assess folate status were discussed and eminence-based assessment reported RCF and serum/plasma folate to be the most sensitive and robust biomarkers to reflect status. RCF measures body stores, hence long-term dietary intake; while serum/plasma folate is considered to represent recent dietary intake. Additionally, plasma total homocysteine concentration was considered a good functional marker of folate status (McNulty and Scott, 2008; Table of Biomarkers of Status/Exposure, www.eurreca.org/everyone/8647/5/0/32). It is however a non-specific marker, as it is influenced by nutritional status of other B-vitamins and nonnutritional factors (e.g., age, sex, ethnicity, pregnancy, renal impairment and lifestyle (Refsum et al., 2004)). Although robust biomarkers are available, variability is common between different folate assay methodologies and in the same methodology across laboratories, leading to a lack of universally accepted cutoff values for folate. The microbiological assay is widely considered to be more sensitive and accurate than the newer protein-binding assays for measuring serum/plasma folate. Liquid chromatography–mass spectrometry/mass spectrometry (LCMS/MS) techniques are also available but they are not considered to be as accurate or reliable as the microbiological assay (Pfeiffer et al., 2010; Expert workshop, Leiden, the Netherlands, 2011).

Relevant Health Outcomes

An overview of available evidence from public health reports and the scientific literature has associated folate with a number of health outcomes (Cavelaars et al., 2010b). It is universally acknowledged that folate is protective against NTDs (MRC Vitamin Study Research Group, 1991; Czeizel and Dudas, 1992) and there is emerging evidence to suggest other beneficial effects of folate during pregnancy (Fekete et al., 2010) and against a number of chronic age-related diseases, such as stroke (Wang et al., 2007; Saposnik et al., 2009), cognitive decline (deLau et al., 2007; Durga et al., 2007), cancer (Kim, 2006), and osteoporosis (Sato et al., 2005). The priority health outcomes identified for each specific population group were summarized by Matthys et al. (2011).

Population Groups

Worldwide, women are recommended to take an additional 400 μg/d folic acid periconceptionally to protect against first occurrence of NTDs (Centers for Disease Control and Prevention, 1992; Busby et al., 2005), but compliance with these recommendations is low (McNulty et al., 2011). Most women therefore have to rely on dietary intake to meet their increased folate requirements, yet dietary folate from natural food sources is widely acknowledged to be considerably less bioavailable than the synthetic vitamin folic acid. Mandatory folic acid fortification of cereal grains was implemented in the United States and elsewhere to reduce the number of pregnancies affected by NTDs. Despite evidence to show the success of this policy, mandatory fortification with folic acid remains virtually nonexistent in Europe because of ongoing safety concerns regarding chronic exposure to folic acid. Such concerns include the potential of high-dose folic acid to mask the hematological symptoms of vitamin B12 deficiency in the elderly while allowing the associated irreversible neurological damage to progress (Dickinson, 1995). A greater concern is that high-dose folic acid may potentially promote colorectal tumorigenesis in patients with preexisting lesions (Cole et al., 2007; Mason, 2011). It is therefore important to consider not only the health protective benefits of folic acid but also any potential adverse effects that may occur, especially in populations already exposed to high folic acid intakes (through fortification and supplementation).

COLLATING, SUMMARIZING, AND INTERPRETING SOURCES OF EVIDENCE

The available evidence relating to folate was examined in two separate population groups: the "young cluster" and the "old cluster." The former was made up of infants, children, adolescents, and pregnant and lactating women, the latter of adults and elderly people. The methodology for each review was based on the standard methodology developed for systematic reviews and meta-analysis (Hooper et al., 2009), with revisions applied as necessary according to the specific folate relationship under investigation and the population group studied.

The best practice guidelines for both intake and status of folate were adopted in the systematic reviews (EURRECA Consensus document/report on dietary survey methodology; Table of Biomarkers of Status/Exposure, www.eurreca.org/everyone/8647/5/0/32).

Factorial/Bioavailability Approach

The factorial approach was considered to be inappropriate for establishing dietary recommendations for folate as there are insufficient data available. Indeed, a systematic review of the evidence on maintenance of folate body stores and additional requirements for specific stages of the lifecycle concluded that meta-analyses were not possible owing to the limited number of studies available and the large differences in methodologies, folate doses, outcome measures, and ways of presenting the results. Folate bioavailability is an important consideration, but to date, bioavailability studies have predominantly been conducted in adults with little evidence available for the "young cluster."

Compared with supplemental folic acid in the free form, the bioavailability of natural food folates is assumed to be about 50% (Sauberlich et al., 1987) and that of folic acid added to foods, 85% (Pfeiffer et al., 1997)—estimates that were combined to generate the DFE value of 1.7. The factors that potentially contribute to bioavailability of different folate forms were extensively reviewed by McNulty and Pentieva (2010). Folate bioavailability is also affected by other factors specific to the individual such as age, sex, ethnicity, genetics, nutritional status, and physiological state (e.g., stage of maturation of the gastrointestinal tract in infants, pubertal status, pregnancy, and lactation). An overview of folate bioavailability is provided in a recent article by Caudill (2010) following a EURRECA workshop to discuss current knowledge on bioavailability of priority micronutrients (including folate) and consideration of future research priorities.

To assess folate bioavailability, both acute and chronic study protocols are used but both have limitations that may confound interpretation of the findings. Many of the available acute studies are of insufficient duration to accurately quantify the full biological response, and chronic studies (although potentially more meaningful) provide major challenges. Folate intake must be strictly controlled throughout the intervention period, and food folate and folic acid must be provided at equivalent doses; a problematic task owing to poor stability of reduced folates to cooking methods and the fact that most food composition databases underestimate food folate levels because of methodological issues. Few studies are available that have considered all of these factors.

A systematic review is underway of studies on bioavailability in adults that used the following inclusion criteria: (1) participants were apparently healthy humans with no use of

medications known to influence folate metabolism; (2) included studies were randomized controlled trials (RCTs; crossover or parallel) or non-RCTs with a placebo or control group; (3) the study duration was ≥8 hour for blood folate response and ≥3 days for urinary folate response (acute studies), and ≥4 weeks for chronic studies; (4) the study administered natural food folates (whole diets/mixed meals/single meal), or different folate forms that typically occur in food (usually 5-MTHF) as a supplement; (5) the study included commercial preparations (e.g., folic acid supplement or folic acid-fortified food) as a reference against which the bioavailability of food folate was compared; and (6) the study measured peak values and the area under the curve (AUC) for serum/plasma/urinary folate (acute studies), and serum/plasma folate or RCF alone or in combination with plasma homocysteine response (chronic studies). Studies using both labelled and unlabelled folates were included but were analyzed separately within the review. Exclusion criteria were as follows: (1) baseline folate status was not reported and (2) RCF response was measured before 12 weeks. The current paper provides a systematic review of the existing evidence, together with a clear justification for adopting the current DFE factor of 1.7, or at least the need to generate an independent factor with which to adjust for the incomplete bioavailability of food folate relative to folic acid.

Intake–Status–Health Relationships

In order to quantitatively assess dose–response relationships between folate intake, status, and relevant health outcomes in adults, stringent inclusion criteria were applied as follows: (1) the participants in the study were apparently healthy humans aged ≥18 years. Patient groups with preexisting disease were excluded from intake–status relationships on the basis that the biomarker responses may not be representative of the general population or the disease state may confound the outcomes. However, relevant high-risk groups at baseline were also included when examining the relationships with health outcomes; and (2) the study provided folic acid as an oral supplement on a daily basis for at least 2 weeks. Only studies that provided the vitamin as folic acid were included in order to quantify the biomarker response that reliably reflects a given folate intake. Studies that provided the vitamin using natural food folates or as folic acid added to foods were investigated separately to determine the bioavailability of folates from food relative to folic acid. Furthermore, studies in which folic acid was administered in combination with other nutrients or another intervention were excluded. For relationships with health outcomes, studies using multivitamins were excluded as the independent effects of folate on the specific health outcome could not be addressed, albeit studies that provided folate along with other metabolically related B-vitamins were included. Studies that supplemented with folic acid at intermittent time points (i.e., weekly, alternate days) were also excluded; (3) only studies designed to provide the strongest available evidence were included. To assess intake–status relationships, only RCTs with a parallel placebo group were included; however, to investigate relationships with health outcomes, observational studies (i.e., prospective cohort studies and nested case–control studies) were also included. Cross-sectional and case–control studies were excluded; (4) only studies reporting robust, specific, and sensitive measures of biomarker response (i.e., RCF or serum/plasma folate) were included. Studies using plasma homocysteine as the response indicator were included only if this metabolite was measured in addition to RCF or serum/plasma folate. Studies that reported homocysteine response alone were excluded as homocysteine is a nonspecific marker of folate status. In the case of RCF, the minimum duration was set at 12 weeks to ensure that the change in intake was accurately reflected in RCF status; (5) the study reported intake data for the intervention used or used a validated FFQ/diet history, a 24-hour recall/food record/diary measure for at least 3 days or if for <3 days, adjusted for intraindividual variability to measure folate intake; (6) the study reported health outcomes considered to be the most relevant to adult populations; and (7) the study reported confounders (e.g., baseline folate intake and status). A summary of the available evidence for the "young cluster" and "old cluster" is provided below.

Infants/Children and Adolescents

There are no or few RCTs available on healthy infants, children, and adolescents, and there is a lack of research that has focused solely on the effect of folate intake or status on health outcomes, especially cancer (DNA synthesis), and cognitive functions and psychomotor development. Therefore, insufficient data were available to conduct meta-analyses on intake, status, and health relationships and thus further studies are required.

Pregnancy/Lactation

Similar inclusion/exclusion criteria to those applied in the systematic review in adults were used with the exception that studies which provided folic acid as supplements or fortified foods were included, as were studies that provided the main cofactor form found in food as a supplement (i.e., [6S]-5-MTHF). Of the 4067 papers identified from the searches, 15 studies were included and these showed that supplementation with folic acid (via supplements or fortified foods) at doses of up to 1 mg/d for periods of 4–24 weeks, in addition to dietary folate intake, significantly increased serum/plasma folate and RCF and significantly lowered plasma homocysteine concentrations. The relationship between folate intake and blood folate status appeared to be stronger when folate supplements were provided in the form of 5-MTHF compared with folic acid. Although relationships between folate intake and folate status were weaker in pregnant and lactating women compared with women of childbearing age, the majority of studies ($n = 10$) were carried out on the latter group, with only three studies on pregnant women and two studies on lactating women. Heterogeneity was high and

this was not explained by dose, duration of supplementation, or population subgroup. Furthermore, the majority of included studies were of small sample size and found to have a moderate to high risk of bias. Further research, therefore, is required to assess the dose–response relationship between folate intake and status in pregnant and lactating women.

A lack of data on maternal health outcomes was reported. Data that are available focus on periconceptional supplementation and fetal malformations, but few RCTs exist because of ethical concerns relating to such trials. Therefore, insufficient data from RCTs were available to undertake meta-analyses on intake, status, and health relationships. It was concluded that further research is required to investigate the role of folate throughout pregnancy.

Adults

After the removal of duplicates, a total of 3200 papers were identified from the searches and expert consultation. Of these, 29 RCTs (reported in 19 publications) were identified and these examined the relationship between folate intake and status and fulfilled the inclusion criteria. All but two studies reported serum/plasma folate response to folic acid supplementation, 12 reported RCF response, and all studies reported plasma homocysteine response. The number of participants in each treatment arm was small and the study duration was <12 weeks in most cases. Only one study had a large sample size ($n = 818$) and was for a duration of 3 years. Half of the studies administered folic acid at physiological doses ($\leq$400 μg/d), whereas in the other studies, pharmacological doses of up to 5000 μg/d were administered and therefore were of questionable relevance with respect to dietary folate recommendations. Folic acid supplementation significantly increased serum/plasma folate and RCF concentrations and significantly lowered plasma homocysteine concentrations. For each biomarker measured, the effect was stronger and heterogeneity was lower in the studies that used physiological doses of folic acid than in the studies that used doses of 500 μg/d and above (presumably because these doses exceed the renal threshold for reabsorption, so a large proportion of the administered dose would be excreted in urine). The response of serum/plasma folate and RCF to folic acid supplementation was then examined according to the analytical method used. In studies that used the microbiological assay, the response was greater and heterogeneity lower compared with studies that used other assays showing that folate response was dependent on the assay methodology used to measure status. The main focus of the paper (Duffy et al., submitted) is on those studies that examined folate response using physiological doses of folic acid (i.e. $\leq$400 μg/d) as this is the level of folate intake that is achievable by dietary means.

Three systematic reviews are underway to examine the relationships between folate intake/status and cognitive function, CVD, or cancer. A total of 4522 papers were identified from the searches. For the cognitive review, four RCTs of longer than 1-year duration and 10 prospective cohort studies, the majority of which looked at the relationship between folate status and cognition, fulfilled the inclusion criteria and were included in the review. A large degree of heterogeneity was present between studies in population characteristics and, in particular, in the cognitive outcomes measured. A variety of cognitive tests were used and these measured either global cognitive function or specific cognitive domains (e.g., memory, attention, information-processing speed), and in some studies, a mean global cognitive score was calculated from the scores of the individual tests administered. For this reason, the potential for meaningful meta-analysis was limited. Of the 10 prospective cohort studies included, eight reported a positive association between low baseline folate status or intake and cognitive decline or incident dementia. However, all but one RCT reported no effect of homocysteine-lowering therapy (i.e., folic acid doses of between 0.8 to 5 mg/d, vitamin B12, and vitamin B6) on cognitive function in normal and cognitively impaired older adults. The positive trial supplemented with folic acid alone had the largest sample size, was of the longest treatment duration, and was conducted in a cognitively normal population that was not exposed to voluntary or mandatory folic acid fortification of foods. Currently, there is insufficient evidence available to use cognitive function as a health indicator for establishing dietary reference values (DRVs) for folate.

For the CVD review, heterogeneity was high, with diverse outcomes such as stroke, coronary heart disease (CHD), myocardial infarction, bypass surgery, death, thrombosis, and angina measured, thereby limiting the potential to combine the data in a single meta-analysis. Folate is one of several metabolically related B-vitamins required for homocysteine metabolism, and as the meta-analysis described briefly above shows, homocysteine concentrations can be lowered by supplementation with folic acid. In the past, elevated plasma homocysteine was considered to be an independent risk factor for CVD, but in more recent years, this viewpoint has become controversial, with many believing that elevated homocysteine per se is unlikely to be causatively implicated in CVD but is merely a functional marker of suboptimal B-vitamin status. Despite evidence from observational studies to suggest that low folate status and/or elevated homocysteine is associated with an increased risk of CVD, especially stroke (Homocysteine Studies Collaboration, 2002; Wald et al., 2002), the evidence from RCTs often fails to confirm this contention. Since 2004, the results of seven secondary prevention trials in at-risk patients showed that combined supplementation with folic acid, vitamin B12, and vitamin B6 had no benefit on CVD events generally. These trials were of long duration (2–7.3 years) and all used high-dose B-vitamin supplementation, including folic acid at a dose between 0.8 and 2.5 mg/d (reviewed by McNulty et al., 2012). However, some argue that these trials were not sufficiently powered to detect a significant effect for the predicted magnitude of association between homocysteine and CVD risk. Results from two recent meta-analyses of RCTs showed that homocysteine lowering by folic acid was associated with greater reductions in stroke in trials of more than 3-year duration, with a homocysteine-lowering

effect of 20% or greater, and with nonsecondary prevention of stroke (Wang et al., 2007; Lee et al., 2010). The evidence for improved folate status and/or homocysteine lowering appears to be stronger for stroke prevention than for heart disease, and perhaps strongest for the role of folic acid in the primary prevention of stroke. Currently, there is insufficient evidence available to use CVD as a health indicator for deriving DRVs for folate.

For the cancer review, the study designs, which were included, were nested case–control studies, prospective cohort studies, and RCTs. No association between folate status and risk of colon, rectal, prostate, pancreatic, lung, or breast cancer was observed in the nested case–control studies. As only one or two studies were included and these studies investigated site-specific cancer, the data were not subjected to meta-analysis. Prospective cohort studies examined a number of different types of cancer. No associations were observed between higher intake of folate and reduced risk of ovarian, stomach, bladder, or rectal cancer, albeit the number of studies available was small in each case. Higher folate intakes appeared to be associated with a lower risk of colon and breast cancer but the data were conflicting, and in a few studies, an increased risk of breast and lung cancer was observed with increasing total folate intakes (generally attributable to supplemental folic acid). Three RCTs, which supplemented with doses of folic acid ranging from 0.5 to 5 mg/d for durations of at least 3 years, examined colorectal cancer using adenoma recurrence as the outcome measure. Results were equivocal, with one study reporting a decreased risk, one no effect, and the other a potential increased risk of colorectal neoplasia with folic acid supplementation. Outcomes, which were measured in the other RCTs, were diverse and included DNA methylation, cell proliferation, and effects on other molecular markers of cancer. As there is evidence to suggest that folic acid at doses of >1 mg/d may promote colorectal tumorigenesis in patients with preexisting lesions (Cole et al., 2007) or increase cancer risk generally (Figueiredo et al., 2009), it is important that policy-makers consider not only the potential for health protective benefits of folate but also any potential adverse effects of long-term exposure to high-dose folic acid.

Multiple Micronutrients (Interactions)

Natural food folates are predominantly polyglutamates that must be hydrolyzed to the monoglutamate form before absorption. This process is controlled by the intestinal brush-border enzyme glutamate carboxypeptidase II (GCPII). The activity of this enzyme is reported to be inhibited by organic acid ions (citrate, malate, ascorbate, and phytate; reviewed by McNulty and Pentieva, 2010). Furthermore, the enzyme is dependent upon zinc; hence, zinc deficiency could impair folate absorption, but further evidence is required (Sandström, 2001). Folate metabolism is also dependent upon adequate status of vitamins B12 and B6, riboflavin, and choline (Caudill, 2010) and deficiency of any one of these nutrients can modify folate bioavailability.

Polymorphisms

The 677C→T variant in the gene encoding the folate-metabolizing enzyme methylenetetrahydrofolate reductase (MTHFR) is the most widely researched polymorphism affecting folate metabolism and one for which there is a clear and consistent phenotype that impacts folate status. The homozygous mutant TT genotype for this MTHFR polymorphism affects about 10% of people worldwide, but can be considerably higher in some European areas (e.g., 26% in Southern Italy; Wilcken et al., 2003). Individuals with the TT genotype have reduced MTHFR activity, resulting in impaired folate metabolism and elevated plasma homocysteine concentrations (Frosst et al., 1995), and are considered to have increased dietary folate requirements on the basis that they have lower RCF compared with those without this genetic variant (Molloy et al., 1997). Despite this effect on folate requirements, it was considered unnecessary to take the MTHFR polymorphism into account when deriving DRVs for folate in the United States. A recent study (Robitaille et al., 2009), also from the United States, concluded that modifications to current folate recommendations to account for genetic variation data are not warranted at this time as the dietary requirement for folate differs little between different racial and ethnic groups. At the Leiden workshop (in 2011) that involved EURRECA partners and invited experts, it was considered important to note genetic variability in future folate studies, but at this point in time, MTHFR and other folate polymorphisms should not be included when deriving DRVs.

Integrating the Evidence

Countries/organizations that have set DRVs for folate in the past typically used the level of intake associated with adequate concentrations of RCF or serum/plasma folate, both of which are robust markers of folate status. The current evidence available does not justify a change in this approach as evidence linking folate intake or status and relevant health outcomes, particularly at typical dietary exposure levels, is lacking. However, emerging dietary folate recommendations should consider bioavailability. At this time, there is no evidence available to justify setting folate requirements based on genetic variability.

Research Gaps and Priorities

Gaps in current knowledge and priorities for future research were identified at two workshops involving EURRECA partners and invited experts (Leiden, the Netherlands, 2011, and Brussels, Belgium, 2012). These were:

(1) The need for representative data at the national level on food composition and folate intake, taking into account intakes of both natural food folate and folic acid obtained through

supplements and fortified foods, and corresponding biomarker status.
(2) More studies that quantify the recognized lower bioavailability of natural food folate (from various food folate forms) relative to folic acid.
(3) Well-designed studies using physiological doses of the vitamin and of suitable duration and that examine the relationships between intake and status of folate and health outcomes across the lifecycle and account for exposure from dietary sources.
(4) Studies that examine folate interactions with other nutrients such as the metabolically related B-vitamins, choline, and zinc.
(5) Monitoring potential beneficial, as well as any adverse effects, in populations exposed to food fortification and comparison of the data with unfortified populations.

SUMMARY AND CONCLUSIONS

Serum/plasma folate and RCF are the biomarkers of folate status that are most often used as primary health indicators for establishing an AR, albeit some countries also include plasma homocysteine concentrations, as it was considered in the past to be an independent risk factor for CVD. Preliminary data from the series of systematic reviews undertaken within EURRECA suggest that currently, there is a paucity of data evaluating the relationships between folate intake and status in specific population groups such as infants, children, adolescents, pregnant, and lactating women, and for relevant health outcomes in all population groups. Where sufficient studies are available, they often differ in study design and characteristics, greatly limiting the ability to conduct meaningful meta-analyses. Insufficient evidence is therefore available to base DRVs on health outcomes at this time. In addition to considering folate intakes necessary to achieve "optimal status," adverse effects associated with high intake of folic acid must be considered. The practice of voluntary fortification of foods with folic acid is highly variable across Europe. Policy in this area is unregulated and difficult to monitor. The outputs generated from the EURRECA research activities will form a foundation upon which additional data from further high-quality studies will be added to create a valuable resource that will be of use to international bodies tasked with setting DRVs for folate for "optimal health" in the future.

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ABBREVIATIONS

5-MTHF	5-methyltetrahydrofolate
AR	Average requirement
DFEs	Dietary folate equivalents
DRVs	Dietary reference values
FFQ	Food frequency questionnaire
MTHFR	Methylenetetrahydrofolate reductase
NTDs	Neural tube defects
RCF	Red cell folate
RCTs	Randomized controlled trials

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