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SHORT COMMUNICATION

Research goals for folate and related B vitamin in Europe

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In the past decade, the understanding of folate bioavailability, metabolism and related health issues has increased, but several problems remain, including the difficulty of delivering the available knowledge to the populations at risk. Owing to the low compliance of taking folic acid supplements, for example, among women of child-bearing age who could lower the risk of having a baby with a neural tube defect, food-based strategies aimed at increasing the intake of folate and other B-group vitamins should be a priority for future research. These should include the development of a combined strategy of supplemental folate (possibly with vitamin B_{12}), biofortification using engineered plant-derived foods and micro-organisms and food fortification for increasing folate intakes in the general population. Currently, the most effective population-based strategy to reduce NTDs remains folic acid fortification. However, the possible adverse effect of high intakes of folic acid on neurologic functioning among elderly persons with vitamin B_{12} deficiency needs urgent investigation. The results of ongoing randomized controlled studies aimed at reducing the prevalence of hyperhomocysteinemia and related morbidity must be available before food-based total population approaches for treatment of hyperhomocysteinemia can be recommended. Further research is required on quantitative assessment of folate intake and bioavailability, along with a more thorough understanding of physiological, biochemical and genetic processes involved in folate absorption and metabolism.
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Introduction

Many issues relating to folate (the prevention of neural tube defects, the reduction of hyperhomocysteinemia, the minimization of cancer risk, the bioavailability from foods and potential strategies to increase dietary folate intakes) were discussed in February 2004 in Warsaw during the First International Conference on Folates: Analysis, Bioavailability and Health (EuroFoodFolate). Over 160 delegates from over 18 countries attended the meeting, including world known researchers, young scientists and students, not only from Europe but also from the USA and Australia. The conference was organized with support from the European Commission, and featured the latest results and findings from the ongoing European Union framework programme

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This work is dedicated to Professor Clive West (deceased August 27, 2004; Division of Human Nutrition, Wageningen University, The Netherlands), who was an outstanding researcher in the field of vitamin bioavailability and status, and contributed to the Conference.
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project 'Folate: from food to functionality and optimal health' (FolateFuncHealth; QLRT-1999–00576). FolateFuncHealth is indicated as 'the EU project' in this paper and was a 4-year collaboration bringing together both consumer and commercial interests with an overall aim to provide folate-rich and enriched foods with specified and scientifically verified consumer benefits for optimal bioavailability, function and health. The main deliverables were assessment of the accuracy and reliability of current food folate data in European food composition tables, and implications for intakes; development of new and refined processing techniques for foods with enhanced folate content, bioavailability and functionality; dvelopment of rapid *in vitro* models for predicting folate availability for absorption *in vivo*, and of *in vivo* models for predicting major factors that influence the rate and limit of folate absorption; critical evaluation of efficacy of different dietary folate strategies on lowering plasma homocysteine in the general population and more effective use of dietary versus supplemental strategies for population-based prevention of chronic diseases.

The aim of this paper is to present the consensus view from the conference on the current knowledge on folates and health and the research agenda and policy goals that need to be addressed in Europe. It also includes key published literature in the folate field published since the conference took place in 2004.

Neural tube defects

Evidence that periconceptional supplementation with folic acid in women can prevent a significant proportion of neural tube defects, such as spina bifida, was established in a series of intervention trials. The amount of folic acid tested in these studies ranged from 0.4 to 4.0 mg/day. Although the two large randomized intervention trials that conclusively demonstrated its efficacy used multivitamins containing either 4.0 mg of folic acid per day to evaluate recurrence (MRC Vitamin Research Group, 1991) or multivitamins containing 800 μg of folic acid per day to examine occurrence (Czeizel and Dudas, 1992), another intervention trial using just folic acid alone indicated that 400 μg/day would be effective (Berry *et al*., 1999). This knowledge has led most governments around the world to recommend that women of child-bearing age should consume 400 μg of folic acid per day for prevention of NTDs occurrence. Daly *et al*. (1995) demonstrated an inverse dose relationship between folate status and risk of NTDs. An important aspect of this study was that risk was graded throughout the entire normal range for the vitamin, suggesting that any improvement in folate status would ameliorate risk to some extent. A subsequent placebo-controlled intervention trial in young women showed that considerable protection might be afforded by intakes of 400 μg/day but even lesser amounts, taken over time in a sustained programme, would provide some protection and dramatically reduce the population prevalence of these defects (Daly *et al*., 1997).

The crucial message is that women need to be consuming folic acid before they become pregnant. By the time most pregnancies are diagnosed, the critical period of 28 days following conception is passed and it is too late to start supplementation in order to reach protective folate levels in the embryonic tissues (Wald, 2004). As in the UK (Wild *et al*., 1997), surveys in the US (March of Dimes Birth Defects Foundation, 2001) and other countries (Miller *et al*., 2000; De La Vega *et al*., 2002) indicate that only 30% of women aged 18–45 years use folic acid correctly, that is, periconceptionally. Among younger women, and among less-educated and socioeconomically disadvantaged women, its use is even less. Some campaigns promoting the use of folic acid supplements (e.g. the Health Education Campaign in the UK) have been partially effective (Buttriss, 2005), but many women remain unaware and many pregnancies are still unplanned. Overall, educational campaigns in many European countries appear to have had a limited impact in the primary target group (Botto *et al*., 2005).

While long-term high vegetable intake by vegetarians may favourably affect folate status (Koebnick *et al*., 2001), the effect of dietary folate intervention in increasing red cell folate is generally unclear (Cuskelly *et al*., 1996), and little is known of folate bioavailability from diets (Gregory, 1997). On balance, fortification seems to be a more effective way of increasing folate intake at the population level without changing behaviour. Several countries, most notably the USA, Canada and Chile, have already accepted the ineffectiveness of the health awareness strategy and have opted for a population-based mandatory fortification policy. It is now clear that significant decreases have occurred in the incidence of NTDs in the USA, Canada and Chile since fortification began (Honein *et al*., 2001; Persad *et al*., 2002; Ray *et al*., 2002; Lopez-Camelo *et al*., 2005). Furthermore, the fact that there was no significant decrease in prevalence in the USA and Canada between 1995 and 1997, when health awareness campaigns were actively encouraging women to increase their folate intake (Honein *et al*., 2001; Persad *et al*., 2002), emphasizes the futility of alternative strategies.

There is no substantive evidence that folic acid is toxic even when consumed in very large doses. Despite concerns that chronic high intakes may have negative consequences in specific subgroups such as those with undiagnosed vitamin B_{12} deficiency, a condition that is relatively common in the elderly, a mandatory folic acid fortification policy does not appear to have caused a major increase in the masking of vitamin B_{12} deficiency anaemia (Mills *et al*., 2003). Nor does it appear to have had an effect on anticonvulsant drug treatment (Ray *et al*., 2005). However, there are suggestions that the efficacy of anti-folate treatment in ectopic pregnancy (Takacs and Rodriguez, 2005) and antimalarial therapy (Carter *et al*., 2005) may be reduced. These and other related issues have contributed to the fact that fortification remains a controversial issue in the

European Union. While it is important that potentially negative aspects of such programmes must be monitored at least as carefully as the intended outcomes, the incontrovertible evidence of the efficacy of even low levels of fortification in the USA and Canada (with an average intake approaching 200 μg/day (Choumenkovitch *et al*., 2002; Quinlivan and Gregory, 2003)) provides a strong argument against the present position of policy makers in European Union.

Goal 1: We recommend governments of countries in Europe implement policies of folic acid fortification of the diet in order to reduce the incidence of NTDs.

Further research is needed to identify successful approaches for increasing folate intake in the population and also to determine the proportion of NTDs that can be attributed to inadequate folate status. Registration of national NTD incidence figures is an important tool to evaluate effects of ongoing recommendations and new programmes.

Goal 2: Longitudinal epidemiological data on NTD incidence in Europe is needed to evaluate the efficacy of food fortification and other strategies to reach the goal to increase folate status to levels protective of NTDs in offspring.

Hyperhomocysteinemia

Low folate status is an important cause of elevation of plasma concentrations of tHcy. Numerous retrospective and prospective studies have consistently found an independent, direct relationship between mild hyperhomocysteinemia and cardiovascular disease or all-cause mortality. The association of tHcy with cardiovascular disease is linear, but plasma tHcy levels >12 μmol/l are generally considered to be elevated. Such elevated levels are found in 5–10% of the general population and in up to 40% of patients with vascular disease. Additional risk factors (smoking, arterial hypertension, diabetes and hyperlipidemia) may additively or, by interacting with tHcy, synergistically increase overall risk. Hyperhomocysteinemia has also been associated with a higher risk of osteoporotic fractures in elderly persons (McLean *et al*., 2004; van Meurs *et al*., 2004). A significant fall in plasma Hcy has been noted as an additional potential benefit of the fortification strategy (Jacques *et al*., 1999; Pfeiffer *et al*., 2005).

Based on various models, reduction of elevated plasma tHcy concentrations may theoretically prevent up to 25% of cardiovascular events. A target plasma tHcy of <10 μmol/l is recommended (Ubbink, 2001). In prospective studies, worldwide about 50 000 patients have been randomized to tHcy-lowering B-vitamin (folic acid with or without vitamin B_{12} and B_6) treatment or placebo. Inadequate folate intake or marginal folate status is considered the most common cause of hyperhomocysteinemia. A supplement of 400 μg folic acid per day leads to maximal tHcy lowering (Van Oort *et al*., 2003). Results from two randomized, placebo-controlled, double-blind trials (one in North America, after introduction of folic acid fortification, and the CHAOS-2 study in the UK) have indicated that supplementation with folic acid alone or together with vitamins B_6 and B_{12} does not reduce the risk of ischaemic heart disease and stroke (Baker *et al*., 2002; Toole *et al*., 2004). However, it may be necessary to pool the outcomes of ongoing trials, as individual studies are often too small to exclude false negative results. Thus, definitive conclusions on the efficacy of folic acid to reduce the risk of cardiovascular disease may be pending until 2010 or later.

The effect of more folate from food on plasma tHcy levels was studied in the EU project. Nine studies were conducted in which a total of 241 healthy volunteers in six European countries received extra daily folate from folate-rich foods (change of dietary habits to include more foods with high folate content), or enriched foods in amounts of ca. 200 μg/day (de Meer *et al*., 2004). In these studies, the extra natural folate or folic acid from enriched food was fed for 1–3 months to free-living volunteers, and was compared with control treatment with foods without extra folate. A meta-analysis of the data from these studies showed that both food folate and fortified foods significantly increased plasma and red blood cell folate, and reduced plasma tHcy; the tHcy lowering was mainly confined to the quartile with the highest initial plasma tHcy. Thus, extra folate at a dose of 200 μg/day or more from folate-rich or folic acid-enriched foods increases folate status in healthy volunteers, but it appears that the decrease of plasma tHcy could remain confined to the highest initial plasma tHcy quartile.

As the effects of natural food folates on folate status may be lower as compared to the effect of folic acid supplements, and making use of Dietary Folate Equivalents (1.7 μg dietary folate equivalent with 1 μg folic acid, see below for comments), an intake of at least 680 μg/day of dietary folate would be needed to obtain the same effects as 400 μg/day of folic acid. That is very difficult to maintain with a balanced diet, and high-risk groups often find it impossible to meet such requirements. This is especially a problem in countries with no folic acid fortification of foods. Mandatory fortification or a combination of targeted supplementation and fortification strategies will be much more effective in population groups.

The recently available natural folate stereoisomer, [6*S*]-5-methyltetrahydrofolate, as a potential fortificant or supplement, is a possible alternate to folic acid in some situations (Pentieva *et al*., 2004). Within the EU project it was demonstrated that it has similar effects on folate status and plasma concentrations of tHcy in humans as folic acid (Lamers *et al*., 2004) and on theoretical grounds could be used in the elderly as it would not mask vitamin B_{12} deficiency. Another major finding of the EU project was that even during middle-age folate absorption appears to be diminished (de Meer *et al*., 2005). However, we would not advocate the use of [6*S*]-5-methyltetrahydrofolate as an alternate to women of child-bearing age without further

epidemiological data supporting the equivalence of this form to folic acid in the prevention of NTDs.

Goal 3: In order to establish whether there is a role for folate in the prevention of ischaemic heart disease and stroke, further research is needed, especially clinical trials with hard end points or validated intermediate end points.

Cancer

There has been intense interest in the possible role of folate in protecting against cancers at various sites. Most interest has been directed towards colorectal cancer and cervical cancer, although epidemiological studies raise the possibility of an involvement of folate in cancer of the lungs and pancreas, oesophagus, stomach and breast (Choi and Mason, 2000; Giovannucci, 2002). Plausible biological mechanisms have been raised to support these findings, with a particular focus on the well-established role of folate in DNA synthesis and methylation (Johanning *et al*., 2002). There has been a report of reduction in the incidence of some childhood cancers since folic acid fortification began in the USA (French *et al*., 2003).

Evidence linking folate intake with colorectal cancer risk comes mainly from animal studies or case–control studies in humans, often hospital based (Giovannucci, 2002). Furthermore, human case–conrol studies have generally examined preinvasive lesions as an outcome (adenomatous polyps) rather than invasive cancer. Results are reasonably consistant and therefore moderately encouraging, but for causation to be established there is a need for robust intervention trials with invasive cancer as an outcome. The situation is less satisfactory for cervical cancer in that although there have been several randomized controlled trials as well as case–control studies, there has been a general failure to take account of the presence of human papilloma virus (HPV) in these studies (Rampersaud *et al*., 2002), and this HPV infection is now considered to be a necessary factor for the occurrence of cervical cancer. However, despite the protective role for folate observed in many human studies of cancer risk, there is a small body of evidence that suggests that high intakes of folate may enhance the progression of existing precancerous lesions (Kim, 2004). While such findings certainly need confirmation in further studies, they should inform the debate on mandatory fortification.

Goal 4: In order to establish whether there is a role for folate in the prevention of malignancy, further research is needed, especially clinical trials with hard end points or validated intermediate end points, such as polyps.

Vitamin B_{12} and potential impact of folic acid fortification strategies

In developed countries, low intake of folate and other B vitamins, and low vitamin B_{12} status due to gastric changes in the elderly, are common. The public health problems arising from this are expected to increase in prevalence as the elderly population grows. In addition to the anemia associated with these deficiencies, there is concern with respect to impaired cardiovascular health and cognitive function in this population group.

Up to 30% of the elderly have food-bound B_{12} malabsorption due to such factors as a decrease in gastric acidity, the presence of atrophic gastritis, and of bacterial overgrowth (Institute of Medicine, 2000). Serum vitamin B_{12} concentration alone is not a sensitive parameter for the assessment of vitamin B_{12} status. More sensitive functional indices for vitamin B_{12} such as plasma methylmalonic acid and holotranscobalamin are needed to reassess the prevalence of suboptimal B_{12} status in this population group.

Although food fortification in the US does not appear to cause a major increase in the masking of vitamin B12 deficiency anaemia (Mills *et al*., 2003), high folate intake has unexpectedly been associated with a faster rate of cognitive decline in the elderly (Morris *et al*., 2005). This begs the unresolved question of whether, in the absence of clinical signs of anaemia, folic acid may exacerbate the neurological symptoms associated with functional vitamin B_{12} deficiency in the elderly. If a direct causal link were to be proved, this would be a critical unwanted side effect of fortification programmes.

In Europe, the intake of additional synthetic folic acid has been assigned an upper tolerance level of 1000 μg/day by policy makers. Apart from the concern expressed above, there are no data to support the reasoning that folic acid at this intake level is detrimental for health in the general population. However, in countries such as the Netherlands, present law prohibits the addition of any extra amount of folic acid to foods.

We take the standpoint that combined fortification of folic acid and vitamin B_{12} can partly resolve the above concerns (apart from loss of intrinsic factor in the elderly) associated with mandatory folic fortification. The low vitamin B_{12} status in the elderly population is a public health problem that should be addressed independently of the need for adequate folate intake. Since increasing folate and B_{12} intakes are required for optimal health during reproduction and at increasing age, there is a need to address the efficacy of combinations of folic acid and vitamin B_{12} supplements.

Goal 5: There is an urgent need to address the efficacy of combinations of folic acid and vitamin B_{12} supplements in women of child-bearing age and in elderly persons.

Bioavailability and potential strategies to increase dietary folate intakes

Bioavailability and dietary equivalence of folates, and genetic interactions

The term bioavailability can be defined as 'the fraction of an ingested nutrient that is available for utilization in normal

physiologic functions and for storage' (Jackson, 1997). It should not be confused with the newer term 'functional bioefficacy', which is defined as 'the fraction that has a positive effect on a functional parameter' (e.g. tHcy in the case of folate) (Brouwer *et al*., 2001).

The bioavailability of folate in a wide variety of foods is generally incomplete and highly variable and it can be a major determinant of folate status. The bioavailability of folic acid (both supplemental and in fortified foods) is almost always higher than the net bioavailability of naturally occurring folates in food. Several studies have shown a bioavailability in the range of 50–80% for polyglutamyl folates in humans using different methods and protocols. However, one study has reported 65–75% relative bioavailability for polyglutamated folate compared to supplemental folic acid, but similar homocysteine-lowering effects (Brouwer *et al*., 2001).

A clear understanding of the relationship between dietary recommendations and optimal health will require reliable estimates of both folate intake and bioavailability. To compensate daily intake for the differences in the bioavailability of folates in foods, adjustment is needed. The concept of Dietary Folate Equivalents was introduced for this reason. Dietary folate equivalents converts all forms of dietary folate, including folic acid in fortified foods, to an amount that is equivalent to food folate and are defined as: DFE = μg natural food folate + 1.7 $\times$ μg synthetic folic acid. The 1.7 multiplier for converting folic acid to DFE was based on the assumption that added folic acid (consumed with a meal as a supplement or fortificant) is $\sim$85% available and food folate is $\sim$50% available; thus the ratio 85/50 yields the multiplier of 1.7 in the DFE calculation. The 85/50 ratio also infers that the bioavailability of food folate when consumed as part of a mixed diet is $\sim$60% (50/85 $\times$ 100) relative to added folic acid. Much of the uncertainty in the 85/50 ratio resides in the imprecision and variability of the denominator.

The 50% estimate for food folate was based on a single study that was not designed to assess food folate bioavailability quantitatively (Sauberlich *et al*., 1987). A new study using US volunteers (Yang *et al*., 2005) was designed to examine the dietary folate equivalency of added folic acid. This study was a long-term controlled dietary folate feeding protocol which provided either 400 or 800 μg DFE/day derived from various combinations of food folate and folic acid. Folic acid was converted to DFE utilizing the 1.7 multiplier from the DFE calculation and was consumed with a meal throughout the treatment period. Folate status response to the different dietary treatments was assessed using multiple folate status indicies. The fact that the folate status response to the different combinations within the 400 and 800 μg DFE/day groups was not different supports the basis of the equivalency conversion (1.7).

The data from this study also support the DFE estimation that the bioavailability of food folate as part of a mixed diet is $\sim$60% that of added folic acid. The importance of this study is that it was designed specifically to address the issues relative to the DFE calculation and the findings support the validity of using the 1.7 multiplier and therefore support the previously derived estimates of bioavailability on which the DFEs were based. However, since the multiplier may be affected by folate status or habitual folate consumption patterns, which are both higher in the US than European countries where folic acid fortification is not widespread, further studies are needed on European populations. Additionaly, further studies are needed to establish whether the overall use of a single factor is correct or needs to be replaced by conversion factors for individual foods or mixed diets. We also suggest that future folate requirements be expressed in terms of synthetic folic acid equivalents.

Thus, improvements in methods for determining folates in food, generation and validation of more accurate food database values for folates and a better understanding of folate bioavailability in representative mixed diets (rather than individual foods) are all future priorities (Sanderson *et al*., 2003). With respect to the factors determining bioavailability, release from the food matrix and deconjugation of folates present as polyglutamyl folates are important factors. Within the EU project, the use of a multicompartmental dynamic *in vitro* model of the gastrointestinal tract was evaluated as tool to study factors affecting bioavailability of folate. Using this model, folate-binding protein present in milk has been shown to inhibit the absorption of folic acid, and to a lesser extent of 5-methyltetrahydrofolic acid (Verwei *et al*., 2004). The results obtained with the model indicated that this type of model might be a useful tool to study bioaccessibility of new or modified food products.

Results from *in vivo* studies including those conducted in the EU project indicate that our currently held views on the absorption, metabolism and subsequent tissue distribution of folates need careful re-evaluation; as does the methodology in current use for estimating the 'relative absorption' of single folate test doses. The data and available *in vivo* models for folate bioavailability and bioefficacy have recently been reviewed (Gregory and Quinlivan, 2002; Melse-Boonstra *et al*., 2004b), and will not be further elaborated here. Studies using stable isotopically labelled folates and mathematical modelling techniques have demonstrated new insights into folate absorption and metabolism (Gregory *et al*., 2005; Wright *et al*., 2005). With the use of radioactive folate tracers and accelerator mass spectrometry, quantitative models of *in vivo* folate metabolism can be further developed and tested (Lin *et al*., 2004).

Research priorities are gaining a better understanding of the factors governing folate bioavailability and improving protocols for its determination in humans. Collection of data on the *in vivo* bioavailability of polyglutamated folates from foods will remain difficult, given the practical problems and cost to produce foods with endogenously enriched isotope-labelled reduced folates with varying glutamyl chain lengths.

Genetic factors also deserve attention for future research, because several enzyme polymorphisms may influence folate

bioavailability and metabolism (Finglas *et al*., 2003). This may give mechanistic insight into the molecular biology of folate bioavailability. A single polymorphism in the intestinal folate brush-border hydrolyase (folylpoly-γ-glutamate carboxypeptidase; GCP II) has been identified, which was reported to yield reduced folate status and lower bioavailability of polyglutamyl folates (Devlin *et al*., 2000). However, this has not been substantiated in later and larger studies (Vargas-Martinez *et al*., 2002; Afman *et al*., 2003; Melse-Boonstra *et al*., 2004a). The intestinal carrier-mediated uptake of folates has not been extensively studied, even though the reported prevalence in the population of 18% for the homozygous defect (RFC; A80G) is probably higher than the more common methyltetrahydrofolate reductase homozygous defect (MTHFR; 10–15%), and lower bioavailability might be expected. Results from the EU project using isotopically labelled folates have shown that there is no evidence that genetic polymorphism for MTHFR 677CT has any effect on modelled apparent absorption of supplemental folic acid or spinach folate. The observations from these studies need to be substantiated with larger subject numbers.

Improving the production and preservation of folate in the food chain

Recommendations for increasing the folate content of natural food folates indicate that an increase of 2–3-fold might be possible for increasing the consumption of natural folates in the European diet from around 200 to 400 μg/day folate, and several studies support this approach. Examples taken from the EU project include (1) combinations of yeast fermentation, malting/germination and *Lactobaccilus casei* starter cultures have potential for further optimization for folate enhancement in breads, beer, dairy products and vegetables; (2) high pressure treatments are better for folate retention in vegetables, fruit juices, soups and beverages than the more traditional sterilization processes and (3) the improved selection of raw materials and ingredients rich in natural folates may increase folate content by two-fold in rye breads, fermented milks and other dairy foods. It would seem possible to increase folate bioavailability from foods by processing. For example, by breaking open the matrix, or by deconjugating glutamate residues from the polyglutamate chain, or by developing new varieties of plants with increased bioavailability, and this is an important area for future research.

Goal 6: Future priorities include improvements in methods for determining folates in food, generation and validation of more accurate food database values for folates, increasing understanding of folate bioavailability in representative mixed diets and interactions with the human genome. Also, future folate requirements should be expressed in terms of synthetic folic acid equivalents.

Biotechnology

Microorganisms (e.g. *Lactobacillus reuteri*) have been selected and developed that can be used to increase the folate and B vitamin (e.g. B_2 and B_{12}) content of fermented milk products. In some cases, production is so high that with an average serving of the product, the RDA for the vitamin would be met, or exceeded (Sybesma *et al*., 2003).

The increase of folate contents in staple food through genetic modification, so-called biofortification, can offer an alternative or at least complement the current methods to enhance intakes of natural food folates. Such an approach is being developed with respect to provitamin A, iron and zinc for major crops in developing countries within the HarvestPlus Programme (see http://www.harvestplus.org). If possible, marker-assisted breeding is preferred but it may be necessary to genetically modify suitable plants, be they staple foods or vegetables.

With respect to genetically enhanced crops, the situation is different for developing countries as compared to the developed world. Biofortified crops offer potential advantages over supplementation or fortification strategies as seeds can be re-sown every year from the saved harvest. Thus, they will be particularly valuable in developing countries where other methods of folate fortification can be hampered because of, for example, the cost of quality control requirements. Recent advances in genomics of the model plant Arabidopsis (The Arabidopsis Genome Initiative, 2000) and rice (Yu *et al*., 2002), combined with accumulated knowledge of biochemistry of folate biosynthesis (Scott *et al*., 2000), make folate enhancement by biofortification feasible.

In view of enhancing folate levels in crop plants, the present approach is to first gain solid knowledge about the limiting steps in the folate biosynthesis pathway in model systems as Arabidopsis and rice. In addition, genes of C1 metabolism possibly affecting the pathway should be identified. These findings can then be tested in other cereals that are a potential target for folate enhancement (e.g. wheat and barley). At a second stage, the feasibility of genetic enhancement of folates by gene modification can be evaluated. On the other hand, the potential applicability of genetic enhancement by marker-assisted breeding should be explored. To that end, the natural variation in folate contents and forms of folate in target cereals should be analysed. If successful, marker-assisted breeding programs could be initiated.

The biotechnological research priorities should be set in parallel with farmer and consumer acceptance studies in order to determine the acceptability of transgenic cereals with enhanced folate levels. This should be done both for the developing and the developed world. A cost–benefit analysis in relation to the degree of acceptance, compared to available strategies to fight folate deficiency and strategic implementation, is essential to determine the economic feasibility of biofortified crops on the market. Bioavailability

studies will be a final but equally essential step in this process prior to marketing high folate crops.

Goal 7: Development of biofortified staple and vegetable crops with enhanced folate contents and their implementation in developing countries is urgently required. This research should be accompanied by economic feasibility studies that compare biofortification to conventional supplementation and fortification strategies.

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