Clinical review

Science, medicine, and the future

Is folic acid the ultimate functional food component for disease prevention?

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We are entering a new era in preventive medicine, which focuses on diet as a means to health. Folate has received much attention as a vitamin that can protect against many diseases, but do we know enough about the long term effects of supplementation?

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Summary points

- B vitamins, particularly folate, may give considerable protection against serious diseases such as cancer, heart disease, and birth defects
- The method of protection is by lowering homocysteine or through epigenetic mechanisms
- Common single nucleotide polymorphisms of several genes coding for folate dependent enzymes can modulate risk for several serious clinical conditions
- The level of risk or protection with some single nucleotide polymorphisms is further influenced by a person's nutritional folate status; this is the basis of a new subdiscipline within human nutrition—"nutrigenomics"
- Folate used in food fortification is not a natural coenzyme; we do not know the long term biological effects of exposure to unmodified synthetic folate
- Future trends will emphasise dietary practices that complement the genetic variation of an individual person

Mankind has been relatively unsuccessful in the search for the ultimate panacea for all ills; however, in the field of functional foods, few nutritional components have so many fundamental and diverse biological properties as folic acid and related B group vitamins. Moreover, few nutrients can claim to modulate, if not overtly benefit, such a wide array of clinical conditions.

Around 2500 years ago Hippocrates first espoused the "food as medicine" philosophy, which fell into obscurity by the 19th century. The first 50 years of the 20th century saw the discovery of the essential elements and vitamins, particularly in the context of deficiency diseases. Indeed, by 1912 Casimir Funk had put forward the "vitamine theory," proposing four different "vitamines" that would cure scurvy, pellagra, beri-beri, and rickets. During the 1970s the shift in emphasis from undernutrition to overnutrition and disease led to a flood of public health guidelines on optimising nutritional parameters. By the 1990s, with an ageing health conscious population, scientists from academia and the commercial world coalesced their thinking to create the trend we now know as functional foods.

Enrichment of flour as a US government programme to correct problems with nutrient deficiency was probably the first modern attempt to design a food for functional purposes related to nutritional outcome. The first consequence of this was the eradication of pellagra with niacin, and a current programme in the United States aims to do the same for neural tube defects through mandatory fortification of grain with folate at source.[1 2]

The ramifications of this mass use of folate as a functional food are likely to have even wider effects. Folate status and common variations in genes that code for folate dependent enzymes are linked to many types of cancer, vascular disease, birth defects, and complications of pregnancy.[3] This arises because several molecular mechanisms that underpin the genomic machinery are sensitive to B vitamin status and, in particular, are responsive to the interaction between folate nutrition and folate dependent enzyme polymorphisms (folate nutrigenomics). Mechanisms that may be affected include the maintenance of genomic CpG methylation patterns for regulated gene expression and the proficient synthesis of nucleotides to prevent DNA strand breakage.[4 5] The same nutrigenomics also influence plasma homocysteine status and thus risk for vascular disease.[3] These relations are shown in figure 1.

Worldwide interest in folate research

Clearly, the use of folate fortification has immense potential benefit. Interest in folate over the past decade

has rocketed in comparison with other nutrients, largely because scientists have recognised the importance of this vitamin in treating a broad range of both developmental and degenerative disorders that are sensitive to even marginal deficiencies in B vitamins.[6]

Although Lucy Wills's 1931 description of yeast extract being effective against the tropical macrocytic anaemia of late pregnancy in India represents the first record of folate being used for prevention of disease, folate as the critical factor involved was not isolated, nor was its structure elucidated, until later. Furthermore, it was not until more than half a century later that the significance of folate in preventive medicine was once again shown in a series of papers culminating in that by the Medical Research Council Vitamin Study Group in 1991, documenting how periconceptional folate prevents spina bifida.[7] This discovery was followed by a meta-analysis published in 1995, which presented data from 27 studies involving more than 4000 patients with occlusive vascular disease and a similar number of controls.[8] Data showed that homocysteine was an independent, graded risk factor for atherosclerotic disease in the coronary, cerebral, and peripheral vessels. This was of particular interest, as dietary folate lowers homocysteine through de novo biosynthesis of methionine,[9] and it opened new avenues for intervention with vitamins to prevent disease. Several single nucleotide polymorphisms that are related to folate and other B vitamins were also discovered in 1995. These affect the risk not only of birth defects and vascular disease but also of several cancers. The two most important cancers in this context are colorectal cancer and leukaemia.

In order to examine research trends in this area, I did a systematic key word search of the PubMed National Library of Medicine for the years 1992-2002. I noted the number of citations for folate in association with a range of developmental and degenerative conditions for each year. Figure 2 shows how interest in this vitamin has changed over the past 10 years. It is clear that all areas of research related to folate and disease have expanded, but interest in folate in relation to cancer has increased in particular (fig 2 (top)). However, when the data are expressed as a percentage of all papers on folate, only research in relation to vascular disease has expanded year on year in real terms (fig 2 (bottom)), presumably owing to worldwide interest in the beneficial effects on vasculotoxic homocysteine. Although not shown, numbers of publications on folate in general far exceed those relating to other similar micronutrients. To provide a limited illustration of how popular the field of folate-homocysteine interrelations has become, figure 3 shows how folate research and vitamin C research had similar outputs until the mid to late 1990s, when interest in folate began to surge. However, publications on homocysteine were fewer than those on either folate or vitamin C up to 1996, but at the time that research on folate surged (around 1997) homocysteine research substantially outstripped the number of publications dealing with either of the other vitamins. The antioxidant nature of vitamin C makes it a popular

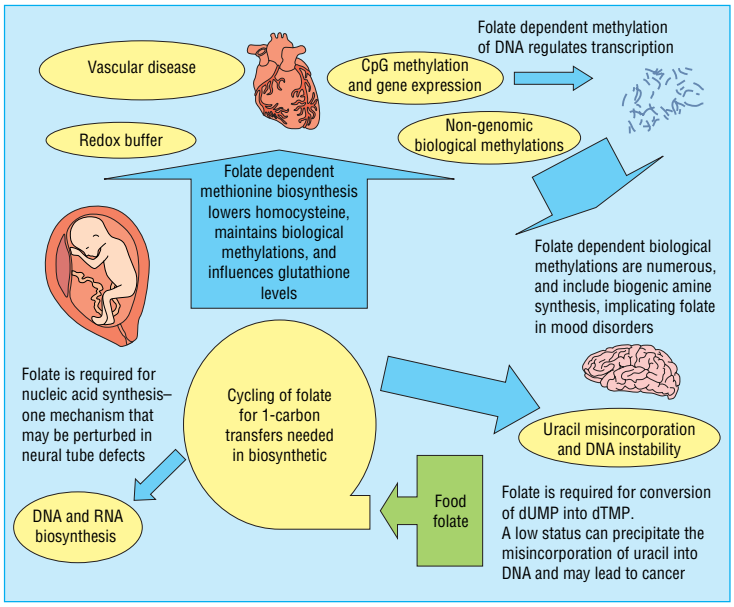

Fig 1 Molecular mechanisms affected by dietary folate

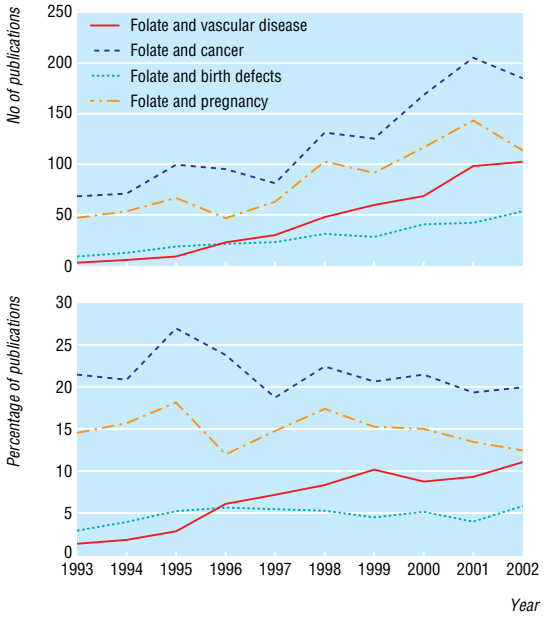

Fig 2 Number of publications on folate and specific diseases (top); publications on folate and specific diseases as a percentage of all publications on folate (bottom)

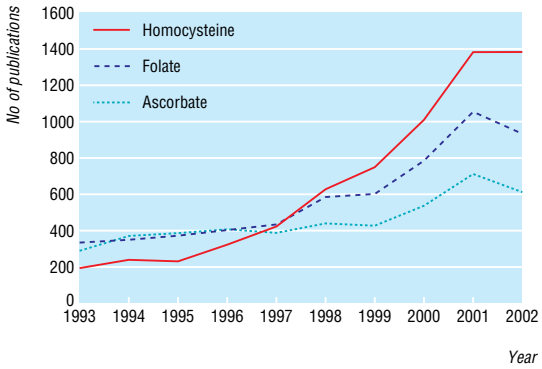

Fig 3 Interest in folate-homocysteine inter-relations relative to interest in vitamin C

vitamin for biomedical studies, so it provides a fair baseline for comparison.

Folate nutrigenomics

Much of the current interest in folate stems from the discovery of several single nucleotide polymorphisms that modulate risk for a range of important diseases associated with considerable morbidity and mortality.[5] Box 1 gives some examples of these. Of even greater importance is the fact that dietary folate can interact with the proteins that are encoded by these variant genes and ameliorate risk to the extent that an overt protection against the disease is conferred. Box 2 shows the functional consequence of the C677T single nucleotide polymorphism of 5,10-methylenetetrahydrofolate reductase, in terms of dTMP nucleotide biosynthesis, DNA methylation, and homocysteine metabolism, and relates these to the pathology of cancer, developmental disorders, and vascular disease.

How much and what type of folate do we need in our diet?

Despite the heightened interest and clear benefits of folate supplements, caution is needed. The form of folate in supplements and in fortified foods is pteroylmonoglutamate (PGA), a form that does not occur in nature. It is both cheap and stable unlike most native forms of the vitamin. The body metabolises PGA into methylfolate, the normal form of the vitamin transported in plasma. However, research shows that this absorption and biotransformation process is saturated at doses in the region of 400 µg PGA or less.[23 24] Thus at doses at or just below 400 µg PGA all this synthetic form of folate is converted into biologically active methylfolate during absorption. At higher doses synthetic PGA is also transported into the blood in a manner that is directly proportional to dose. This raises the possibility of a lifetime's exposure to unmetabolised PGA where mandatory fortification is undertaken. Although such exposure may present no health risk at all, we cannot know this for certain. Also, what of the increasing tendency among clinicians to give 5 mg doses of PGA (more than 10 times that needed to give maximal methylfolate concentrations)? These doses are not uncommon in patients with vascu-

Box 1: Some important single nucleotide polymorphisms of B vitamin genes associated with various clinical conditions

- C677T variant of 5,10-methylenetetrahydrofolate reductase gene:
  - Colon cancer[10 11]
  - Elevated homocysteine, a risk for vascular disease[12 13]
  - Spina bifida[14]
  - Down's syndrome[15]
  - Oral cleft[16]
  - Adult acute lymphocytic leukaemia[17]
  - Complications of pregnancy (pre-eclampsia, recurrent early pregnancy loss, fetal growth restriction)[3]
- A1298C variant of 5,10-methylenetetrahydrofolate reductase gene:
  - Spina bifida[18]
  - Adult acute lymphocytic leukaemia[17]
- A2756G variant of methionine synthase gene:
  - Thromboembolic vascular disease[19]
  - Influences homocysteine response to diet
- A66G variant of methionine synthase reductase gene:
  - Spina bifida[20]
  - Down's syndrome[21]
- C1420T variant of serinehydroxymethyl transferase gene:
  - Adult acute lymphocytic leukaemia[22]
- 2R3R variant of thymidylate synthase gene:
  - Adult acute lymphocytic leukaemia[22]
- C1561T variant of glutamate carboxypeptidase (folate deconjugase) gene:
  - May affect cardiovascular disease

(In many of the above examples, protection against as well as susceptibility to disease is conferred)

Box 2: Functional consequence of C677T variant of 5,10-methylenetetrahydrofolate reductase gene[3 5]

Direct biochemical effect
Folate stabilises the polymorphic enzyme encoded by the C677T variant gene by preventing it from relinquishing its flavin cofactor. As 5,10-methylenetetrahydrofolate reductase is a flavin protein, several authors have suggested that people with the TT recessive genotype may respond more rapidly to riboflavin (vitamin B_2) supplements as well as folate to lower homocysteine

Nucleotide biosynthesis
dTMP used for DNA is synthesised by thymidylate synthase from dUMP and requires the one-carbon unit of 5,10-methylenetetrahydrofolate. If folate levels are low, uracil misincorporation occurs, leading to breaks in the DNA strand, which predispose to cancer. The polymorphic enzyme encoded by the C677T variant gene can enhance synthesis of dTMP nucleotide if folate status is good (bottleneck in one-carbon flux into methionine favours diversion to nucleotides), and this is thought to afford protection against cancer of the colon and leukaemia. If folate status is poor, the single nucleotide polymorphism may confer risk rather than protection

Biological methylations
Dietary methionine cannot provide all methyl groups needed for cellular methylation reactions, leading to a dependence on de novo synthesis of methionine through the folate one-carbon pool. S-adenosylmethionine (AdoMet) levels regulate protein, lipid, biogenic amine, and DNA methylation. AdoMet dependent DNA methylation of specific CpG sites regulates gene expression and plays a critical role in the developmental process. Methylation of clusters of CpG sites associated with promoter regions tends to silence gene expression. A deficiency of methyl groups may therefore alter the normal control of proto-oncogene expression. The polymorphic enzyme encoded by the C677T variant gene may reduce availability of de novo methyl groups for these important reactions

Homocysteine metabolism
Polymorphic 5,10-methylenetetrahydrofolate reductase reduces one-carbon flux to methylfolate, the donor molecule for conversion of homocysteine into methionine. This single nucleotide polymorphism may thus elevate homocysteine, which is an independent risk factor in heart disease. Homocysteine is atherogenic and undergoes redox cycling in the presence of transition metal ions, forming radicals that cause oxidative damage to low density lipoprotein. Homocysteine may also react with cysteine SH groups and modify apolipoproteins. It is also a hypertensive compound, reacting with endothelium-derived relaxation factor to form S-nitrosohomocysteine and superoxide. This causes a loss of vasodilation action. It also inhibits or downregulates anticoagulants, including prostacyclin synthesis, activation of protein C, thrombomodulin expression, heparin sulphate expression, and fibrinolysis. In addition, it activates procoagulants such as factor V and tissue clotting factor. Some other effects include proliferation of vascular smooth muscle and increased platelet coagulability. Its final effect is to chelate copper and inhibit lysyl oxidase, which impairs cross linking of collagen and elastin and leads to connective tissue abnormalities

lar problems or with an elevated homocysteine. In one recent study we examined B vitamins and polymorphic markers in thrombotic vascular disease.[19] The serum folate measurements were usually in the range 3-30 ng/ml but were interrupted by several exceedingly high values (100-200 ng/ml). These values were real, as they were mirrored by the corresponding red cell folate values, which were also particularly high (by high pressure liquid chromatography and routine haematological assay). When we subsequently examined some of these high serum folates by high pressure liquid chromatography, we found that only around half of the serum folate measured by routine haematological assay was methylfolate. The assumption was that the remainder was unmodified PGA, as only this and other partially oxidised folates do not fluoresce and thus are not directly measurable by high pressure liquid chromatography.[25]

Nobody yet knows what, if any, the long term biological effect of mandatory fortification of grain products would be at a national level. In a recent report on the situation in the United States, more people than expected were subjected to the established tolerable upper limit of exposure to PGA.[26] Folate metabolism is extraordinarily complex, underpinning several pathways crucial for life. Notwithstanding this fact, unmetabolised non-native PGA should be assessed for its in vivo effects on folate dependent enzymes. It is metabolised by dihydrofolate reductase, for which it has a higher Km (lower affinity) than has dihydrofolate itself—could this have an antifolate effect through competitive interaction? Could similar phenomena occur at other enzymes, particularly allosteric enzymes where structurally similar dihydrofolate plays a regulatory role?[27] In vitro studies do show that PGA derivatives act to inhibit certain enzymes,[28] including those associated with nucleotide biosynthesis.[29] Although such in vitro interactions remain hypothetical to the in vivo situation, research to test for their existence in humans seems only prudent.

With some people receiving more than 600 μg/day of folate, largely as PGA, and clinicians regularly prescribing 5 mg/day of PGA, we need to know what the consequences of long term exposure to PGA are (no matter how subtle). Perhaps we should also be assessing supplementation with isomer specific native folates that represent the natural food forms of the vitamin, as well as simple ways to preserve native folate activity during the food preparation process.

One thing is for sure—folate is of great interest and of great clinical value, a veritable panacea among functional foods. Given the fundamental importance of B vitamin nutrigenomics, and the pace of development in molecular diagnostics, it is not hard to envisage a new era in preventive medicine that has even greater emphasis on diet as a means to a long and healthy life—indeed, a return to Hippocrates' famous "Let food be thy medicine and medicine be thy food" philosophy. As they say, "what goes round comes round," the only difference today being the remarkable use of polymerase chain reaction technology.

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