Girls	
9 through 13 years	2 mg/day
14 through 18 years	3 mg/day
Males	
19 and over	4 mg/day
Females	
19 and over	3 mg/day
Pregnancy	
14 through 18 years	3 mg/day
19 through 50 years	3 mg/day
Lactation	
14 through 18 years	3 mg/day
19 through 50 years	3 mg/day

The Food and Nutrition Board of the Institute of Medicine has recommended the following tolerable upper limits (UL) for fluoride.

Infants	(UL)
0 through 6 months	0.7 mg/day
7 through 12 months	0.9 mg/day
Children	
1 through 3 years	1.3 mg/day
4 through 8 years	2.2 mg/day
Children and Adults	
More than 8 years	10 mg/day
Pregnancy and Lactation	
14 through 50 years	10 mg/day

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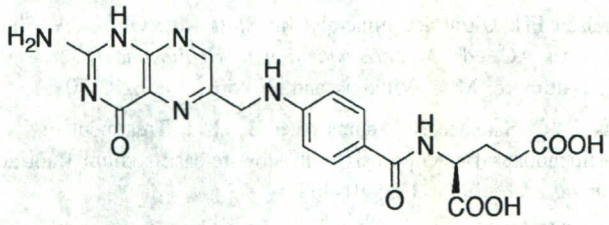
Folate

DESCRIPTION

The term folate is used in two different ways. Folate, a member of the B-vitamin family, is a collective term for a number of chemical forms which are structurally related and which have similar biological activity to folic acid. Folate is also the term which is used for the anionic form of folic acid. Folic acid or pteroylglutamic acid (PGA) is comprised of *para*-aminobenzoic acid linked at one end to a pteridine ring and at the other end to glutamic acid. The pteridine-*para*-aminobenzoic acid portion of the molecule is called the pteroyl group. Folic acid is a synthetic folate form which is used for food fortification and nutritional supplements. It is not one of the principal naturally occurring forms of folate, used in the collective sense.

The naturally occurring forms of folate differ in the extent of the reduction state of the pteroyl group, the nature of the substituents on the pteridine ring and the number of glutamyl residues attached to the pteroyl group. The naturally occurring folates, include 5-methyltetrahydrofolate (5-MTHF), 5-formyltetrahydrofolate (5-formyl-THF), 10-formyltetrahydrofolate (10-formyl-THF), 5,10-methylenetetrahydrofolate (5,10-methylene-THF), 5,10-methenyltetrahydrofolate (5,10-methenyl-THF), 5-formiminotetra-

hydrofolate (5-formimino-THF), 5,6,7,8-tetrahydrofolate (THF) and dihydrofolate (DHF). Most naturally occurring folates are pteroylpolyglutamates, containing two to seven glutamates joined in amide (peptide) linkages to the gamma-carboxyl of glutamate. The principal intracellular folates are pteroylpentaglutamates, while the principal extracellular folates are pteroylmonoglutamates. Pteroylpolyglutamates with up to 11 glutamic acid residues exist naturally. Folate is represented by the following chemical structure.



Folic Acid

Folate participates in several key biological processes, including the synthesis of DNA, RNA and proteins. It is necessary for DNA replication and repair, the maintenance of the integrity of the genome, and is involved in the regulation of gene expression, among other things. Folate deficiency leads to an anemia, called megaloblastic anemia, which is very similar to that caused by vitamin B_{12}. However, folate deficiency does not result in the neurological symptoms and signs that occur with vitamin B_{12} deficiency. Other symptoms and signs of folate deficiency, include weakness, fatigue, irritability, headache, difficulty concentrating, cramps, palpitations, shortness of breath and atrophic glossitis. Laboratory findings of folate deficiency, include decreased serum folate and erythrocyte folate concentrations, elevated serum homocysteine concentration, hypersegmentation of the neutrophils, decreased hemoglobin and erythrocyte concentrations, decreased hematocrit and macrocytic, hyperchromic erythrocytes. Marginal folate deficiency appears to increase the risk of cardiovascular disease, certain types of cancer, Alzheimer's disease and depression. Marginal folate deficiency in pregnant women results in an increased incidence of neural tube defects, including meningomyelocele (e.g., spina bifida), anencephaly, meningocele and craniorachischisis, in their neonates.

A number of conditions can lead to folate deficiency. Chronic alcohol users can become deficient in the vitamin secondary to inadequate intake as well as to ethanol's impairment of folate absorption and hepatobiliary metabolism, as well as to increased renal folate excretion caused by ethanol. Malabsorption syndromes, including Crohn's disease, lymphoma or amyloidosis of the small intestine, diabetic enteropathy, tropical sprue and non-tropical sprue (gluten-sensitive enteropathy), can result in folate deficiency secondary to inadequate absorption of the vitamin, as can small intestinal resections or diversions for the same reason. Some conditions or situations, such as chronic hemolytic anemias (e.g., sickle cell disease), chronic hemodialysis or peritoneal dialysis, chronic exfoliative skin disorders and pregnancy, cause increased demand for folate and folate deficiency will result if the increased demand is not met. Certain drugs, e.g., methotrexate, trimethoprim, pyrimethamine, sulfasalazine and phenytoin, interfere with folate metabolism and may cause functional folate deficiencies. In fact, the mechanism of action of certain antimetabolites, including methotrexate, 5-fluoruracil and the newer multitargeted antifolates, depends on their creating a functional folate deficiency. Some genetic disorders result in folate deficiency and are responsive to folate treatment. Folate-induced remission has been reported in aplastic anemia with familial defect of cellular folate uptake.

Recognizing the increased demand of folate during pregnancy and the increased risk of neural tube defects in neonates born to pregnant women with marginal folate status, the United States Food and Drug Administration (FDA) mandated that folic acid be added to all enriched cereal grains in order to prevent neural tube defects. The mandate became effective on January 1, 1998. The level of folic acid adopted for enriched cereal grain fortification was 140 micrograms per 100 grams. The U.S. Public Health Service recommends that all women of childbearing age in the U.S. consume 400 micrograms of folic acid daily to reduce their risk of having a baby affected with spina bifida or other neural tube defects. This is one of the few health claims allowed by the FDA for nutritional supplementation. The FDA determined 400 micrograms of folic acid daily to be an optimal dose for the prevention of neural tube defects. Those women who already have had a child with a neural tube defect require higher doses.

Natural folates are found in dark green leafy vegetables (spinach, kale, mustard greens, turnip greens, escarole, chard, arugula, beet greens, bok choy, dandelion green, mache, radicchio, rapini or broccoli de rabe, Swiss chard), oranges, lentils, pinto beans, garbanzo beans, asparagus, orange juice, broccoli, cauliflower, liver and brewer's yeast. The absorption efficiency of natural folates is approximately 50% that of folic acid (see Pharmacokinetics). Interestingly, folate was named because of its presence in green leafy vegetables (folium is Latin for leaf) and was originally isolated from four tons of spinach, such was the crudity of isolation techniques more than seven decades ago.

The principal biochemical function of folates is the mediation of one-carbon transfer reactions. 5-Methyltetrahydrofolate donates a methyl group to homocysteine, in the conversion of homocysteine to L-methionine. The enzyme

that catalyzes the reaction is methionine synthase. Vitamin B_{12} is a cofactor in the reaction. This reaction is of great importance in the regulation of serum homocysteine levels and is the only reaction in the body in which folate and vitamin B_{12} are coparticipants. (See Vitamin B_{12}). The L-methione produced in the reaction can participate in protein synthesis and is also a major source for the synthesis of S-adenosyl-L-methionine (SAMe). The methyl group that was donated by 5-methyltetrahydrofolate to homocysteine in the formation of L-methionine is used by SAMe in a number of transmethylation reactions involving nucleic acids, phospholipids and proteins, as well as for the synthesis of epinephrine, melatonin, creatine and other molecules (see S-Adenosyl-L-Methionine). Tetrahydrofolate is the folate product of the methionine synthase reaction. 5-Methyltetrahydrofolate can be generated in only one way: conversion of 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate via the enzyme methyleneterahydrofolate reductase (MTHFR). 5,10-Methylenetetrahydrofolate is regenerated from tetrahydrofolate via the enzyme serine hydroxymethyltransferase, a reaction, which in addition to producing 5,10-methylenetetrahydrofolate, yields glycine.

5,10-Methylenetetrahydrofolate, in addition to its role in the metabolism of homocysteine, supplies the one-carbon group for the methylation of deoxyuridylic acid to form the DNA precursor thymidylic acid. This reaction is catalyzed by thymidylate synthase and the folate product of the reaction is dihydrofolate. Dihydrofolate is converted to tetrahydrofolate via the enzyme dihydrofolate reductase.

Folates are also involved in reactions leading to *de novo* purine nucleotide synthesis, interconversion of serine and glycine, generation and utilization of formate, the metabolism of L-histidine to L-glutamic acid, the metabolism of dimethylglycine to sarcosine and the metabolism of sarcosine to glycine.

One of the natural folates, folinic acid, is used as a pharmaceutical agent. Folinic acid, also known as leucovorin, citrovorum factor and 5-formyltetrahydrofolate, is used as rescue therapy following high-dose methotrexate in the treatment of osteosarcoma. It is also used to diminish the toxicity of methotrexate. It is used in the treatment of megaloblastic anemia due to folate deficiency and in the prevention or treatment of the toxic side effects of trimetrexate and pyrimethamine. The combination of folinic acid and 5-fluorouracil has until recently been standard therapy for metastatic colorectal cancer. Folinic acid increases the affinity of flurouracil for thymidylate synthase. Folinic acid is available as a calcium salt for parenteral or oral administration. See *Physicians' Desk Reference* for further discussion of folinic acid.

In addition to being known as pteroylglutamic acid or PGA, folic acid is known chemically as *N*-[4-[[(2-amino-1,4-dihydro-4-oxo-6-pteridinyl)methyl]amino]benzoyl]-L-glutamic acid. Older names for folic acid are vitamin B_9, folicin, vitamin Bc and vitamin M. Its molecular formula is $C_{19}H_{19}N_7O_6$ and its molecular weight is 441.40 daltons. Folic acid forms yellowish-orange crystals. The color is imparted by the pteridine ring of folic acid. Pteridine also imparts color to butterfly wings.

ACTIONS AND PHARMACOLOGY

ACTIONS

Folic acid lowers the risk of neural tube defects and possibly other types of birth defects. It may also have antiatherogenic, anticarcinogenic, neuroprotective and antidepressant actions.

MECHANISM OF ACTION

Animal and epidemiologic studies have shown that folate deficiency is associated with defects of neural tube closure. Human studies have shown that folic acid, when taken by women planning to become pregnant, can greatly reduce the risk of bearing a child with spina bifida or other neural tube defects. The exact mechanism by which folic acid reduces the risk of neural tube defects and possibly other types of birth defects is not known. It is likely that this effect of folic acid is due to its role in nucleic acid synthesis and/or its role in the metabolism of homocysteine to methionine. Along a different line, it is hypothesized by some that folic acid may not prevent the occurrence of neural tube defects, but may instead selectively increase the abortion rate of affected fetuses.

A central feature of fetal development is widespread and sustained cell division. Folate plays a central role in the formation of nucleic acid precursors, such as thymidylic acid and purine nucleotides, which are essential for nucleic acid synthesis and cell division. The requirement for folate increases during times of rapid tissue growth. The teratogenic effect of folate deficiency may be a result of an insufficient supply of nucleic acid precursors in the rapidly dividing embryonic cells. Increasing folate tissue concentrations might overcome a metabolic deficiency of the vitamin in the production of nucleic acids, and possibly also proteins, at the time of neural tube closure, which typically occurs 24 to 28 days after conception. An insufficient supply of nucleic acid precursors, however, might be expected to cause more general birth defects than the highly specific and predictable nature of the congenital defects caused by folate deficiency. Although derivatives of the neural ectoderm are affected more than other tissues by folate deficiency, all of the embryonic tissues are dividing rapidly during the susceptible developmental period. An insufficient supply of nucleic acid precursors may play some role in the mechanism of folate

deficiency-induced neural tube defects, but it is not a sufficient explanation for these congenital disorders.

Some studies have found that homocysteine levels in pregnant women who subsequently gave birth to children with neural tube defects, were significantly higher than those of pregnant women who gave birth to normal children. This would be expected to occur in pregnant women with low folate status. The enzyme that metabolizes homocysteine to methionine, methionine synthase, uses 5-methyltetrahydrofolate, as well as vitamin B_{12}, as a cofactor. There is some evidence that pregnant women with elevated homocysteine levels have a defect in the methionine synthase enzyme. A defect in the enzyme would lead to decreased production of methionine and S-adenosylmethione (SAMe). SAMe is involved in a number of transmethylation reactions, including reactions involved in the formation of myelin. Further, increased homocysteine levels could result in increased oxidative stress which might be contributory to a teratogenic effect. Homocysteine has been found to be teratogenic in avian embryos. Avian embryos treated directly with D,L-homocysteine or with L-homocysteine thiolactone showed neural tube defects which were prevented with folic acid, indicating that homocysteine *per se* can cause dysmorphogenesis of the neural tube.

Hyperhomocysteinemia is associated with cardiovascular disease, cerebrovascular disease and carotid artery stenosis in adults. Folic acid can lower homocysteine blood levels by converting homocysteine to methionine. A high intake of folate has been associated with a lower risk of coronary events. There is evidence that hyperhomocysteinemia is a risk factor for coronary heart disease independent of other known risk factors (hypercholesterolemia, hypertension, diabetes, smoking). Folic acid, as mentioned above, can lower homocysteine blood levels, but the mechanism by which hyperhomocysteinemia might increase the risk of vascular disease is unclear. A number of hypotheses have been proposed. Homocysteine may promote atherogenesis through endothelial dysfunction and oxidative stress. Elevated homocysteine levels may result in increased oxidation of low-density lipoprotein cholesterol (LDL-C). Oxidized LDL-C is thought to be a major etiological factor in atherogenesis. Homocysteine can promote the growth of smooth muscle cells and increase platelet adhesiveness and affect several factors in the coagulation cascade. Thus, homocysteine can be thrombogenic.

Approximately 10% of the population have a defective folate metabolizing enzyme called methylenetetrahydrofolate reductase (MTHFR). MTHFR catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. 5-Methyltetrahydrofolate transfers its methyl group to homocysteine in the formation of methionine, in a reaction catalyzed by methionine synthase. Flavin adenine dinucleotide (FAD), derived from riboflavin (vitamin B_2) and reduced nicotinamide adenine dinucleotide phosphate (NADPH), derived from niacin, are cofactors in the reaction. Deficiency of MTHFR is the most common inborn error of folate metabolism and is a major genetic cause of hyperhomocysteinemia. The polymorphism A222V (alanine to valine substitution at residue 222 of the enzyme), in which there is a base change at position 677 of its gene, of cytosine to thymine, is homozygous in about 10% of the population. Those homozygous for the T677 allele are found to have elevated homocysteine levels which appear to be associated with an increased risk of vascular disease. The mutant enzyme is thermolabile and does not bind as tightly to its cofactor FAD as does the normal enzyme. Addition of folate to the mutant enzyme stabilizes its binding to FAD and also stabilizes the enzyme against heat inactivation.

Folic acid may have antiatherogenic mechanisms other than that of lowering homocysteine levels. Impaired availability of endothelium-derived nitric oxide (NO), produced by the enzyme endothelial nitric oxide synthase (eNOS), has been identified as a mediator of atherosclerosis. Folic acid and 5-methyltetrahydrofolate have been demonstrated to restore impaired NO status in hypercholesterolemic subjects. In cultured endothelial cells, 5-methyltetrahydrofolate was found to enhance the enzymatic activity of partially tetrahydrobiopterin (BH_4)-repleted eNOS, enhancing NO formation. BH_4 is the cofactor for eNOS. The enhancement of eNOS activity may be another mechanism for the possible antiatherogenic activity of folic acid.

5-Methyltetrahydrofolate has been found to directly scavenge superoxide radicals *in vitro*. Activated eNOS also decreases the production of superoxide. Uncoupling of eNOS, which occurs under conditions of hypercholesterolemia, results in decreased production of NO and increased production of superoxide. Folate appears to restore impaired NO availability by an ameliorative effect on eNOS uncoupling.

Epidemiologic studies have shown that diminished folate status is associated with colorectal, lung, esophageal, brain, cervical and breast cancers. Data supporting the effect of folate status on carcinogenesis are most compelling for colorectal cancer. The mechanism of the possible anticarcinogenic activity of folate is not well understood. Folate deficiency may induce DNA hypomethylation and gene "unsilencing." Folate is critical for the synthesis of the transmethylating agent S-adenosylmethione (SAMe). SAMe methylates certain bases in DNA leading to gene silencing. Gene "unsilencing" alters gene expression and can disrupt the integrity of the genome. DNA hypomethylation appears to be an early, and consistent event in carcinogenesis,

including that of colorectal cancer. Folate deficiency may lead to increased uracil incorporation in DNA. Folate is critical for the formation of thymidylic acid from deoxyuridylic acid. Increased uracil incorporation in DNA can lead to disruption of the integrity of DNA. Folate deficiency may also result in diminished DNA repair, impaired natural killer cell surveillance, secondary choline deficiency, decreased stimulation of T lymphocytes by phytohemagglutinin and activation of tumorigenic viruses. A recent report demonstrated that folate deficiency produced progressive DNA strand breaks in the highly conserved region of the p53 tumor-suppressor gene in rat colon.

Low concentrations of folate in the blood have been associated with poor cognitive function, dementia and Alzheimer's disease-related neurodegeneration of the brain. A recent report from the "Nun Study" showed that low serum folate was strongly associated with atrophy of the cerebral cortex. The mechanism of the neuroprotective effect of folate is not well understood. It has been suggested that elevated homocysteine levels secondary to folate deficiency may account, in large part, for neurodegeneration via increased oxidative stress and endothelial dysfunction, among other things. Elevated serum homocysteine levels were associated with progressive atrophy of the medial temporal lobe in subjects with Alzheimer's disease, in one study.

Folate deficiency has been associated with depression and other psychiatric symptoms. Consistent findings in major depression have been low plasma and low erythrocyte folate levels which have been linked to poor response to antidepressants. Subjects with low plasma folate levels responded less well to the antidepressant fluoxetine than did those with normal folate levels. A recent study reported that folic acid enhanced the antidepressant action of fluoxetine in subjects who did not appear to be folate deficient. The mechanism by which folate affects brain functions is unclear. It is thought that the most likely explanation is folate's role in the synthesis of S-adenosylmethione (SAMe). 5-Methyltetrahydrofolate is the methyl donor in the formation of methionine from homocysteine. It is thought that this reaction is important for the maintenance of the SAMe pool (SAMe is formed from methionine). SAMe is the methylating agent in the formation of the catecholamine neurotransmitters in the brain. These neurotransmitters are important in maintaining the affective state. SAMe itself has been found to have mood-modulating activity (see SAMe).

PHARMACOKINETICS

Folic acid or pteroylglutamic acid (PGA) is the form of folate used in food fortification and the principal form of folate found in nutritional supplements. Natural food folates are pteroylpolyglutamate derivatives. Pteroylpolyglutamate derivatives are hydrolyzed to pteroylmonoglutamate forms prior to absorption from the small intestine. The enzyme that catalyzes the cleavage is called folate conjugase or gamma-glutamylhydrolase. The monoglutamate forms of folate, including folic acid, are transported across the proximal small intestine via a saturable pH-dependent process. Higher doses of the pteroylmonoglutamates, including folic acid, are absorbed via a nonsaturable passive diffusion process. The efficiency of absorption of the pteroylmonoglutamates is greater than that of the pteroylpolyglutamates.

Because of the difference in absorption efficiency between natural food folate and folic acid, the concept of dietary folate equivalents (DFEs) has been introduced. Folic acid taken on an empty stomach is twice as available as food folate. Folic acid taken with food is 1.7 times as available as food folate. For example, 400 micrograms of folic acid taken on an empty stomach is equivalent to 470 micrograms of folic acid taken with food and is equivalent to 800 micrograms of food folate. DFEs can be calculated as follows:

1 microgram of DFEs = 1 microgram of food folate = 0.5 micrograms of folic acid taken on an empty stomach = 0.6 micrograms of folic acid taken with meals.

Following absorption of physiological amounts of folic acid into the enterocytes, a certain percentage undergoes reduction. Reduced folate is transported to the liver via the portal circulation. Much of a pharmacological dose of folic acid is transported to the liver as such, without first undergoing metabolism in the enterocytes. The various natural pteroylmonoglutamate forms undergo some metabolism in the enterocytes to pteroylpolyglutamate forms, but for the most part are also transported as their unmetabolized forms via the portal circulation to the liver. The folates are taken up by the liver and metabolized to polyglutamate derivatives (principally pteroylpentaglutamates), via the action of folylpolyglutamate synthase. Folates are stored in tissue in their polyglutamate forms. Folate is metabolized to its various metabolic forms in the liver. The various pteroylpolyglutamate forms are the active cellular cofactor forms of folate. Folate polyglutamates are released from the liver to the systemic circulation and to the bile. When released from the liver into the circulation, the polyglutamate forms are hydrolyzed by gamma-glutamylhydrolase and reconverted to the monoglutamate forms.

The principal folate in the plasma is 5-methyltetrahydrofolate in its monoglutate form. 5-Methyltetrahydrofolate circulates in erythrocytes in its polyglutamate form. Approximately two-thirds of folate in plasma is protein bound. All tissue forms of folate are polyglutamates, while circulating forms of folate are monoglutamates. When

pharmacological doses of folic acid are administered, a significant amount of unchanged folic acid is found in the plasma. The liver contains approximately 50% of the body stores of folate, or about 6 to 14 milligrams. The total body store of folate is about 12 to 28 milligrams.

Folate is excreted in the urine as folate cleavage products. Intact folate enters the glomerulus and is reabsorbed into the proximal renal tubule. Very little intact folate is excreted in the urine. Folate is excreted in the bile and much of it is reabsorbed via the enterohepatic circulation.

INDICATIONS AND USAGE

Folic acid is indicated for the prevention of some birth defects. Its widely heralded usefulness in cardiovascular disease has recently been called into question by results from some large, long-term studies. Its use in cancer has now also become controversial, with some data suggesting that it may actually be tumorigenic in some circumstances. While available evidence suggests significant preventive/protective effects against various cancers, the use of folic acid as an interventive treatment for cancer is too premature to recommend due to some preliminary findings that it might promote growth of some established cancers. There is very preliminary evidence that folic acid might be helpful in reducing the symptoms of some psychiatric disorders. It has been hypothesized that folic acid supplementation might help prevent Alzheimer's disease and recurrent spontaneous early pregnancy loss, but the research that might confirm or refute these suggestions has not been performed. There is some recent data suggesting that supplemental folate might help preserve hearing function in the aged.

RESEARCH SUMMARY

So strong are the data indicating that folic acid can protect against neural tube birth defects that the Food and Drug Administration issued a regulation that became effective in 1998 requiring fortification of all uncooked cereal grain products and all flour with folic acid.

Several studies have shown that women with low plasma folate and vitamin B_{12} concentrations are at significantly increased risk of giving birth to babies with neural tube defects, as well as some other birth defects. Moreover, several double-blind, placebo-controlled studies have demonstrated that neural tube defects can be significantly prevented when women take folic acid supplements during the periconceptional period. A dose of 400 micrograms of folic acid daily is now widely recommended for women of reproductive age.

Additional studies have shown that multivitamin supplements containing folic acid also significantly reduce the incidence of neural tube defects. There is some preliminary indication that periconceptional supplementation with these preparations may reduce the incidence of orofacial clefts, limb defects and cardiovascular anomalies. For some time, folic acid and various B vitamins have been credited with having significant impact in the prevention of cardiovascular disease. The American Heart Association went so far as to declare that these vitamins were playing a significant role in preventing 17,000 deaths from coronary causes each year in this country. Recently, however, a succession of large, long-term studies have failed to find benefit from B vitamins in heart disease. The older positive data are discussed first and then are followed by a discussion of the more recent and more negative findings.

In a meta-analysis of studies conducted some years ago of the relationship between folic acid intakes and plasma homocysteine levels and between the incidence of coronary heart disease, cerebrovascular and peripheral vascular disease and homocysteine levels, these conclusions were reached: The association between elevated homocysteine and these diseases is "consistent and very strong." Folic acid intakes up to 600 micrograms daily significantly reduce homocysteine levels in a dose-dependent fashion.

Based upon findings from this updated meta-analysis, it has been estimated that, for every 50 microgram daily increase in average food folate intake, 4,000 to 18,000 deaths due to cardiovascular disease could be prevented annually. It was further estimated that with population-wide consumption of 400 micrograms daily of supplemental folic acid, 3,000 to 23,000 deaths from cardiovascular disease could be prevented each year. Another group of researchers has estimated that ten percent of all U.S. heart disease is attributable to elevated homocysteine levels.

It was demonstrated in a prospective, randomized, placebo-controlled trial that folic acid can help prevent some of the deleterious effects of triglyceride-rich lipoproteins on endothelium-dependent vasodilation in healthy volunteers challenged with an acute oral fat load. These protective effects were achieved with oral doses of folic acid (10 milligrams daily for two weeks). These same researchers had previously demonstrated that parenteral administration of folic acid could restore endothelial function *in vivo* in subjects with elevated LDL-cholesterol levels.

In a study of 45 subjects with established cardiovascular disease, folic acid intake was significantly inversely correlated with multiple indices of oxidized LDL-cholesterol. This correlation remained significant even when adjusted for potential confounding variables, including consumption of other vitamins and nutrients.

Evidence for a causal link between homocysteine and cardiovascular disease was further fortified in recent years via mendelian randomization studies showing that about

10% of the population has a genotype that leads to 25% higher homocysteine concentrations and also to higher cardiovascular disease risk. This and other evidence discussed above resulted in a statement a few years ago from the American Heart Association asserting that "the lowering of the population mean level of total homocysteine is estimated to have prevented 17,000 deaths from coronary causes each year." Some other authorities called for adding folic acid to a polypill designed to prevent heart disease and stroke.

Despite all of this, folic acid, and the B vitamins in general, are not winning universal praise from the medical establishment for their use in heart disease. Several large, randomized long-term studies are now failing to find benefit from B vitamin therapies in this context. These negative studies include the Vitamins Intervention for Stroke Prevention (VISP) trial and the Heart Outcomes Prevention Evaluation (HOPE-2) trial. Neither found any vitamin B benefit overall—with the exception of some significant protection against stroke seen in the HOPE-2 trial, but not in the VISP trial. The VISP trial, it should be noted, was designed specifically to look for a homocysteine effect on stroke, whereas the HOPE-2 trial was not. Overseas, several large trials have similarly failed to find benefit. These include the Cambridge Heart Antioxidant Study (CHAOS-2), Norwegian Vitamin Trial (NORVIT) and the Western Norway B-Vitamin Intervention Trial (WENBIT). More recently still, no benefit from vitamin B supplements was seen with respect to either coronary heart disease or stroke in the Women's Antioxidant and Folic Acid Cardiovascular Study (WAFACS). It had longer follow-up than other studies: 7.3 years.

In view of all these negative results, an editorial in *JAMA* asked: "Is there a role for additional trials or should researchers close yet another chapter, which seemed promising but has failed to deliver?" Possible reasons for the failure, the editorial continued, include, among other things, doses used in the various studies and even "potential unexpected proatherosclerotic effects of folic acid supplementation, which may have counteracted benefits associated with homocysteine lowering." A number of large trials are still ongoing, and many consider the jury still to be out. But as the *JAMA* editorial observed: "until further data become available it is important to remain fully grounded on the available evidence and to admit that once again experimental and observational data do not always translate into therapeutic benefits . . . In conclusion, vitamin B supplements cannot currently be recommended for the prevention of CVD events (with the exception of rare genetic disorders) and there is no role for routine screening for elevated homocysteine levels."

A recent editorial in *Circulation*, on the other hand, has urged researchers and the medical world, generally, not to "close the book" on folic acid and its use in cardiovascular disease. The editorialist noted that there are several reports in which folic acid improves endothelial function independent of homocysteine-lowering effects. In the same issue of *Circulation,* a report found a "dramatic" cardioprotective effect of folic acid that the editorial said "could potentially bring folic acid to the center stage in the management of ischemic heart disease." This was a rat study in which very high-dose folic acid was administered for one week. The rats were subjected to myocardial ischemia for 30 minutes. The hearts of the treated rats maintained cardiac function during ischemia and developed almost no infarct after reperfusion, while untreated rats suffered severe injury, including infarct in 60% of the area at risk. This high-dose treatment was said to be comparable to or exceeding ischemic preconditioning, "the most powerful protection against ischemic injury demonstrated so far." The study suggested that this result could also be achieved via acute intravenous delivery of folic acid 10 minutes after the onset of ischemia. The dose used was 400 times greater than the high recommended clinical dose. Evidence of antioxidant activity was noted, but the researchers said that this activity alone was unlikely to be responsible for the marked benefits observed. The researchers hypothesized that high concentrations of folate might increase purine synthesis and thus sustain ATP levels in the heart during ischemia. Loss of purines from ischemic myocardium has been noted for some time and is a major hallmark of ischemia. The editorialist observed that "identifying ways to prevent the loss of purines or to increase *de novo* purine synthesis is the holy grail of cardiac biochemistry."

There is precedent in other recent animal research for preservation of the total adenine nucleotide pool with a long-term supply of high doses of folate (in this case in combination with ribose in a rat model of right heart hypertrophy). The *Circulation* editorial, while stressing "the remarkable, almost magical, effect of folate on the ischemic myocardium" in the report contained in that journal, admitted that the new study raises as many questions as it answers and concluded: "As the effects demonstrated here are so powerful and as folic acid is highly affordable, further investigations are certainly warranted for both scientific and social causes."

Folic acid appears to protect against a number of cancers, particularly colorectal cancers. Some 20 epidemiologic studies suggest that those with the highest folate intake have an approximately 40% reduction in risk of this cancer. In the Nurses Health Study, involving 88,756 women, there was a 75% reduction in risk of colorectal cancer among those using

multivitamin supplements containing 400 or more micrograms of folic acid for 15 or more years, compared with those not using these supplements. This benefit was calculated after controlling for all relevant potential confounding factors.

Long-term folate supplementation was found, in another study, to reduce the incidence of colorectal neoplasia by 62% in subjects with extensive chronic ulcerative colitis. Such subjects, without this supplementation, typically have a 10-fold to 40-fold increased risk of developing colorectal neoplasia.

In a double-blind, placebo-controlled studies, administration of 5 to 10 milligrams of folic acid daily for six months to a year had several positive effects, as measured, for example, by a reduced biomarker of colorectal cancer development in patients with either colorectal cancer or adenomas. Another placebo-controlled study involved subjects who were given 1 milligram of folic acid daily following polypectomy. At two years post-surgery, the supplemented group had a recurrence rate half that of the placebo group. Larger multi-center, prospective studies are now in progress to test the effects of 1-5 milligrams of folic acid daily on recurrence of colorectal adenoma.

Effects of folic acid on uterine cervical cancer are in doubt. Earlier reports that folic acid supplementation can bring about reversal or regression of cervical dysplasia have not been confirmed in recent, well-controlled human trials using 5-10 milligrams of folic acid daily for three to six months.

Epidemiological studies hint at the possibility that folic acid might be helpful in preventing cancers of the brain, stomach and esophagus. Other such studies have recently shown considerably stronger evidence that, in both premenopausal and postmenopausal women who consume 15 grams of alcohol daily (the equivalent of one drink), total folate intake of at least 600 micrograms daily is significantly protective against breast cancer. The same protection is not evident in women who consume less than 15 grams of alcohol daily. Two of three case-control studies have also found evidence that folate might be protective against breast cancer in some women.

Another case-control study suggests that folate may be protective against pancreatic cancer in male smokers. Higher baseline serum folate concentrations were associated with a 55% reduction in pancreatic cancer risk, compared with those with lower baseline serum levels of folate.

There have been two studies showing that folic acid, in combination with vitamin B_{12}, can reverse a precursor of bronchial squamous cell cancer of the lung. In one of these randomized, placebo-controlled studies, both of which involved heavy smokers, 10 milligrams of folic acid daily, combined with 500 micrograms of vitamin B_{12} daily, resulted, after four months, in a significant reduction in the number of subjects exhibiting abnormal bronchial cells said to be cancer precursors.

One folate researcher, while observing that "folate appears to be an ideal candidate for chemoprevention given its proven safety and cost," nonetheless cautions that "the optimal dose, duration, and timing, as well as the appropriate target population, of folate chemoprevention need to be clearly defined." This researcher adds that "some animal studies have suggested that supraphysiologic levels of folate supplementation do not confer protection and, in some cases, may enhance carcinogenesis."

In a genetic murine model of colon cancer, for example, folate supplementation prevented development of tumors when administered prior to the presence of any microscopic neoplastic foci. But when supplementation began after the appearance of such foci, it promoted tumor development in this experiment. "Therefore," this researcher concluded, "it appears that folate supplementation should be implemented before the development of precursor lesions in the target organ or in individuals free of any evidence of neoplastic foci."

The controversy over folate use in cancer has continued in recent years. A recent editorial in *JAMA* emphasized that when it comes to this issue "timing is everything." It noted that "recent experiments have suggested that the time of folate administration during cancer progression can modify outcomes. Folate administered prior to the existence of preneoplastic lesions can prevent tumor development, whereas provision of folate once early lesions are established appears to increase tumorigenesis." The explanation for this may be that rapidly proliferating tissues, such as those that arise in tumors, have increased the need for nucleotides, which folate helps synthesize. Thus some antifolate drugs are efficacious in some cancer treatments. Some recent studies have provided further evidence that folate can increase colorectal neoplasia and cancer, when the agent is administered after lesions are present.

Recently a report was published in the *American Journal of Clinical Nutrition* demonstrating a strong inverse relationship between dietary intakes of folate and invasive breast cancer, with reductions of 40% and more in the highest quintile of intake compared with the lowest. An accompanying editorial, however, again cautioned that timing is of the essence in the use of folate in cancer treatment and that, at present, too little is known for the medically unsupervised use of this vitamin in cancer patients or in those at risk of cancer. Much more study is clearly needed and warranted.

Since there is a reported high incidence of folate deficiency in some psychiatric patients, including those with depression, dementia and schizophrenia, some have suggested that supplemental folic acid might be beneficial in some of these individuals. Some open studies have shown some benefit from folic acid supplementation. Placebo-controlled studies have generally shown no therapeutic effect. These have often used very high doses (15-20 milligrams a day) with what one reviewer has called effects toxic enough to cause, rather than ameliorate, mental symptoms. Two placebo-controlled studies using smaller doses produced benefits in patients with psychopathology associated with folate deficiency and in another group of patients on long-term lithium therapy. The latter were treated with 200 micrograms of folic acid daily.

It has been hypothesized that, since folic acid deficiency is associated with reduced brain levels of S-adenosylmethionine and 5-hydroxytryptamine, supplemental folic acid might be helpful in some with depression. Studies have, in fact, confirmed that serum folate levels are often deficient in those suffering from depression. Whether supplemental folic acid would be of benefit remains unknown.

Similarly, an association has been made recently between low serum folate levels and the severity of atrophy of the neocortex in Alzheimer's disease subjects. But whether supplemental folic acid can be of any help in this disease has not been determined.

In a recent randomized, double-blind, placebo-controlled study, supplementation with 800 micrograms of folic acid daily for three years resulted in significantly improved cognitive function in men and women aged 50-70 years. Assessment measures used in this study included change in memory, information processing speed and sensorimotor speed. By all of these measures the supplemented group did significantly better than the placebo group.

Observational studies have suggested a relationship between age-related hearing loss and low folate levels. In one study, administration of 800 micrograms of folate daily helped reduce hearing loss over a three-year period in subjects thus supplemented, compared with those receiving placebo. Further research is needed to determine whether this observed effect can be replicated.

Finally, there has been a report that elevated homocysteine and reduced serum folate concentrations are significantly associated with recurrent spontaneous early pregnancy losses in humans. The suggestion has thus been made that folic acid supplementation might help prevent these recurrent early pregnancy losses. Again, this hypothesis has not yet been tested.

CONTRAINDICATIONS, PRECAUTIONS, ADVERSE REACTIONS

CONTRAINDICATIONS

Folic acid is contraindicated in those who are hypersensitive to any component of a folic acid-containing product.

PRECAUTIONS

Women of childbearing age, pregnant women and nursing mothers should ensure that their intake of folic acid from nutritional supplements and/or fortified food is 400 micrograms/day. A number of pre- and postnatal supplements deliver 1 milligram (1,000 micrograms) daily of folic acid. Doses higher than 1 milligam/day should only be used by the above groups if prescribed by their physicians.

The use of folic acid for the treatment of folate deficiency or for the treatment of any medical condition requires medical supervision.

The use of folic acid doses above 1 milligram/day may precipitate or exacerbate the neurological damage of vitamin B_{12} deficiency. Those who use folic acid doses above 1 milligram/day should only do so under medical supervision.

Those with undiagnosed anemia, should exercise caution in the use of supplementary folic acid. Doses of folic acid greater than 100 micrograms daily may result in hematologic improvement in those with vitamin B_{12} deficiency.

ADVERSE REACTIONS

Folic acid doses of up to 1 milligram daily are well tolerated. There are more than 100 reported cases in which vitamin B_{12}-deficient subjects who were receiving oral doses of folic acid of 5 milligrams daily or more experienced progression of neurological symptoms and signs. There are very few such reports in those receiving doses of folic acid less than 5 milligrams daily. There are rare reports of hypersensitivity reactions to oral folic acid. There is one report of a trial using oral doses of folic acid of 15 milligrams daily for one month in which some subjects experienced sleep disturbances, mental changes and gastrointestinal effects. Studies using comparable or higher doses, longer duration, or both, failed to confirm these findings.

INTERACTIONS

DRUGS

Anticonvulsants (carbamazepine, fosphenytoin, phenytoin, phenobarbital, primidone valproic acid): These first-generation anticonvulsants may cause decreased serum folate levels and increased serum homocysteine levels. High doses of folic acid may result in decreased serum levels of these drugs.

Cholestyramine: Concomitant use of cholestyramine and folic acid may cause decreased absorption of folic acid.

Colestipol: Concomitant use of colestipol and folic acid may cause decreased absorption of folic acid.

Colchicine: Colchicine is reported to depress blood folate levels.

Fluoxetine: The use of folic acid at a dose of 500 micrograms/day was found to enhance the antidepressant action of fluoxetine given at a dose of 20 milligrams daily in one study.

Lithium: The use of folic acid at a dose of 200 micrograms daily was found to improve the efficacy of maintenance lithium in one study.

Lometrexol: ("T64") Folic acid supplementation in mice was found to augment the therapeutic activity and ameliorate the adverse reactions of the experimental antifolate cancer chemotherapeutic agent lometrexol.

Metformin: Long-term use of metformin has been associated with elevated homocysteine levels which are reduced with folic acid administration.

Methotrexate: The use of folic acid at a dose of 1 milligram daily may significantly reduce the toxic side effects with no reduction in drug efficacy in those undergoing chronic methotrexate therapy for rheumatoid arthritis.

Nonsteroidal anti-inflammatory drugs (NSAIDS), including ibuprofen, indomethacin, naproxen, mefenamic acid, piroxicam, sulindac: When taken in large therapeutic doses, these NSAIDS may exert antifolate activity.

Phenytoin: Phenytoin may decrease serum folate levels and negatively affect folate status. High doses of folic acid may cause a decrease in serum phenytoin levels.

Pyrimethamine: The use of high doses of folic acid concomitantly with pyrimethamine to prevent bone marrow depression may cause a pharmacodynamic antagonism of the antiparasitic effect of pyrimethamine.

Sulfasalazine: Sulfasalazine may reduce the absorption of folic acid when used concomitantly.

NUTRITIONAL SUPPLEMENTS

Vitamin B_6: Vitamin B_6 may work synergistically with folic acid in lowering serum homocysteine levels.

Vitamin B_{12}: Vitamin B_{12} may work synergistically with folic acid in lowering homocysteine levels.

Zinc: Supplemental folic acid has been said to adversely affect the absorption of zinc. However, a review of the literature reveals no effect of folic acid supplementation on zinc nutriture.

FOODS

Administration of folic acid with food marginally decreases its availability.

OVERDOSAGE

There are no reports of folic acid overdosage in the literature.

DOSAGE AND ADMINISTRATION

The principal form of supplementary folate is folic acid. Folate triglutamate (pteroyltriglutamate) is also available. Folic acid is available in single ingredient and in combination products. A typical daily dose is 400 micrograms. Unit doses of one milligram or greater require a prescription.

The Food and Nutrition Board of the Institute of Medicine of the National Academy of Sciences has recommended the following Dietary Reference Intakes (RDI) for folate, expressed as dietary folate equivalents (1 microgram of dietary folate equivalents [DFEs]=1 microgram of food folate = 0.5 micrograms of folic acid taken on an empty stomach = 0.6 micrograms of folic acid with meals):

Infants	Adequate Intakes (AI)
0 through 6 months	65 micrograms/day ~ 9.4 micrograms/Kg
7 through 12 months	80 micrograms/day ~ 8.8 micrograms/Kg
Children	**Recommended Dietary Allowances (RDA)**
1 through 3 years	150 micrograms/day
4 through 8 years	200 micrograms/day
Boys	
9 through 13 years	300 micrograms/day
14 through 18 years	400 micrograms/day
Girls	
9 through 13 years	300 micrograms/day
14 through 18 years	400 micrograms/day
Men	
19 years and older	400 micrograms/day
Women	
19 years and older	400 micrograms/day
RDA for Pregnancy	
14 through 50 years	600 micrograms/day
Lactation	
14 through 50 years	500 micrograms/day

The DV (Daily Value) for folate, which is used for determining percentage of nutrient daily values on nutritional supplement and food labeling purposes, is 400 micrograms. This is based on the U.S. RDA for folate.

A Lowest-Observed-Adverse-Effect Level (LOAEL) for folate is set by the Food and Nutrition Board at 5 milligrams/day. Based on this LOAEL and assuming an uncertainty factor (UF) of 5, the Food and Nutrition Board has recommended the following Tolerable Upper Intake Levels (UL) for folate from fortified foods or supplements (folic acid):

Adults	(UL)
19 years and older	1,000 micrograms/day
Infants	
0 through 12 months	Not possible to establish for supplemental folic acid
Children	
1 through 3 years	300 micrograms/day
4 through 8 years	400 micrograms/day
9 through 13 years	600 micrograms/day
14 through 18 years	800 micrograms/day
Pregnancy	
14 through 18 years	800 micrograms/day
19 years and older	1,000 micrograms/day
Lactation	
14 through 18 years	800 micrograms/day
19 years and older	1,000 micrograms/day

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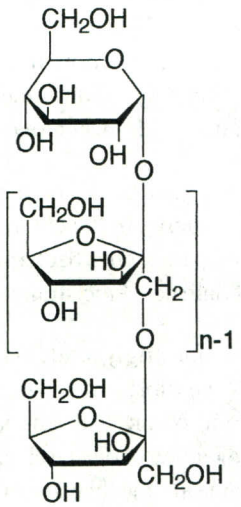
Fructo-oligosaccharides

DESCRIPTION

Fructo-oligosaccharides (FOS) typically refer to short-chain oligosaccharides comprised of D-fructose and D-glucose, containing from 3 to 5 monosaccharide units. Similar molecules are obtained by partial enzymatic hydrolysis of inulins (see Inulins). Those are called oligofructose. FOS, also called neosugar and short-chain FOS (scFOS), are produced on a commercial scale from sucrose using a fungal fructosyltransferase enzyme.

FOS are comprised of one molecule of D-glucose in the terminal position and from 2 to 4 D-fructose units. FOS containing 2 fructose residues are abbreviated GF_2 (G is for glucose, F, for fructose). Those with 3 fructoses are abbreviated GF_3, and those with 4 fructoses, GF_4. GF_2 is also called 1-kestose and GF_3 is called nystose. The linkage between fructose units in FOS is a beta-(2-1) glycosidic link. The structural formula is represented below.



Fructo-oligosaccharides
(The top sugar is glucose. n = 2-4 fructose residues)

FOS are resistant to digestion in the stomach and small intestine. The reason for this is the presence of the beta configuration of the anomeric C_2 in the D-fructose residues. The human digestive enzymes sucrase, maltase-isomaltase and alpha-glucosidase are specific for alpha-glycosidic linkages. FOS are considered nondigestible oligosaccharides. They are, however, fermented by a limited number of colonic bacteria. This could lead to changes in the colonic ecosystem in favor of some bacteria, such as bifidobacteria, which appear to be beneficial in some respects. FOS and other nondigestible oligosaccharides are referred to as bifidogenic factors.

Substances such as FOS that may promote the growth of beneficial bacteria in the colon are called prebiotics. Prebiotics are typically nondigestible oligosaccharides.

ACTIONS AND PHARMACOLOGY

ACTIONS

FOS may have anticarcinogenic, antimicrobial, hypolipidemic and hypoglycemic actions in some. They may also help improve mineral absorption and balance, and may have anti-osteoporotic and anti-osteopenic activities.

MECHANISM OF ACTION

The possible anticarcinogenic activity of FOS might be accounted for, in part, by the possible anticarcinogenic action of butyrate. Butyrate, along with other short-chain fatty acids, is produced by bacterial fermentation of FOS in the colon. Some studies suggest that butyrate may induce growth arrest and cell differentiation, and may also upregulate apoptosis, three activities that could be significant for antitumor activity. FOS may also aid in increasing the concentrations of calcium and magnesium in the colon. High