All about Folates

Studies Confirm Folates Role in Preventing Depression

Two new studies provides further evidence that low folate levels may have a role to play in depression. In one study, high folate levels were linked to a greater success rate in treating depressed geriatric patients. In the other study, patients with depression had a more difficult time metabolizing folate.

Researchers undertook the one study because previous findings suggested that lower folate levels are associated with reduced responsiveness to treatment with selective serotonin reuptake inhibitors (SSRI). In addition, depressed geriatric patients have lower levels of folate than controls, and folate supplements have been found to reduce death related to depression.

In the current small, randomized, observational study, researchers with New York University Medical Center gave 12 geriatric patients the SSRI sertraline, while 10 patients received the SSRI nortriptyline for 12 weeks. The researchers measured folate levels at the beginning of the study and after treatment. At the beginning of the study, all patients had folate levels within the normal range. However, patients who had higher folate levels at the beginning of the study achieved greater improvement after treatment.

The reason why folate may help reduce depression is because deficiency of this important B vitamin causes brain levels of mood-boosting serotonin to fall. In addition, low levels of folate triggers a reduction of 5-hydroxyindoleacetic acid, which may contribute to depression in individuals with a predisposition to depression. Folate also plays a role in the metabolism of S-adenosylmethionine (SAMe), an action which also contributes to proper levels of brain serotonin. The researchers called for additional research to evaluate the role of folate supplements in patients with folate levels in the low normal

range.

In the other study, researchers studied folate levels and levels of the amino acid homocysteine in 5,948 subjects aged 46 to 49 years and 70 to 74 years. The study authors found that subjects who had relatively high levels of homocysteine in their blood were almost twice as likely to be depressed, compared to people with the lowest homocysteine blood levels. Depressed subjects also were more likely to have impaired folate metabolism. Homocysteine is an amino acid implicated in heart disease. Folate is known to lower levels of homocysteine. The study authors concluded that folic acid supplements may help prevent depression.

In the middle-aged subjects, low levels of folate also were linked to depression. But even though markers of folate metabolism were altered in depression, actual levels of folate in the blood did not differ between the elderly subjects with and without depression. Researchers suggested that this may be because measuring folate in the blood may not be an accurate indication of how much folate actually resides in cells.

Biosynthesis of Folates

Tetrahydrofolate and its derivatives (folates) are essential cofactors of one-carbon metabolism which are required for the biosyntheses of purines, thymidylate, serine and methionine in a wide variety of organisms; they are also required for the formylation of methionyl-tRNA in eubacteria. Whereas plants and many microorganisms obtain folate coenzymes by de novo synthesis, vertebrates depend on nutritional sources. Insufficient supply of the vitamin is conducive to anemia in adults and to neural tube malformation in human embryos. Similar to bacteria and yeasts, plants make folates de novo from pterin, p-aminobenzoate (PABA), and glutamate moieties. In contrast, humans and other mammals lack a complete folate-synthesis pathway and thus need dietary folate. Because plant foods

are major folate sources, and folate deficiency is a global health problem, enhancing plant folate content is a prime target for metabolic engineering. This engineering demands knowledge of the biosynthetic pathway.

The plant folate-synthesis pathway is not understood fully, but is most probably similar to that in bacteria. The pterin hydroxymethyldihydropteroate is formed from GTP and PABA from chorismate. The pterin and PABA units are condensed, glutamylated, and reduced to give tetrahydrofolate, and a polyglutamyl tail is added.

The biosynthesis of tetrahydrofolate has been studied in some detail. The first committed step catalysed by GTP cyclohydrolase I (I) is a mechanistically complex ring expansion reaction affording dihydroneopterin triphosphate (2).
The first step of pterin synthesis is of special interest, because it commits GTP (1) to pterin production and is considered to control flux into the pathway.

A pyrophosphatase (II) and a phosphatase (III) have been proposed to convert dihydroneopterin triphosphate to 7,8-dihydroneopterin (3) in two consecutive steps, but the details are still incompletely understood. The conversion of 7,8-dihydroneopterin into 6-hydroxymethyl-7,8-dihydroneopterin (5) and glycolaldehyde (4) is catalysed by 7,8-dihydroneopterin aldolase (IV). The enzyme product is converted into dihydrofolate by the consecutive action of 6-hydroxymethyldihydroneopterine pyrophosphokinase (V), dihydropteroate synthase (VI), dihydrofolate synthetase (VII) and dihydrofolate reductase (VIII). These two final steps of tetrahydrofolate (6) biosynthesis are common chemotherapeutic targets for antibacterial and antiparasitic agents. It could be observed that dihydropteroate synthase can be inhibited by sulfonamides, the first synthetic antimicrobial and antiparasitic drugs with broad action spectrum. Dihydrofolate reductase can be inhibited by trimethoprim, which acts against a variety of bacterial pathogens. However, effective inhibitors of the early steps of folate biosynthesis could not

been found.

The characterization of the intermediates, mechanisms and enzymes of the folate pathway from plants and microorganisms (yeast, eubacteria) by molecular and structural biology, biochemistry and NMR spectroscopy is one of the major focuses of the folate group.

Biosynthesis of Tetrahydrobiopterin (BH4)

The cofactor BH4 is synthesized by only three enzymes, namely GTP Cyclohydrolase I (CYHI), 6-Pyrovoyl-H4-pterin synthase (PTPS) and Sepiapterin reductase (SR). The biosynthetic pathway of BH4 includes minimally these three enzymes; the participation of a fourth enzyme, aldose reductase, is suggested but still controversial.

However, it was shown that aldose reductase is not important for BH4 biosynthesis in liver. The structures of Escherichia coli and human GTP cyclohydrolase I, rat liver 6-pyruvoyl tetrahydropterin synthase, and mouse sepiapterin reductase have been determined by x-ray crystallography. Besides the de novo biosynthesis of BH4, SR is also known to be involved in the pterin salvage pathway catalysing the conversion of sepiapterin to dihydrobiopterin (BH2) which is transformed by dihydrofolate reductase to BH4. Further more, a regeneration system for the cofactor is known involving pterin-4a-carbinolamine dehydratase (PCD) and dihydropteridine reductase (DHPR). The full complement of the three BH4-biosynthesizing enzymes can be found in significant amounts in many tissues of various species. The richest sources of SR are erythrocytes, liver and brain.

Tetrahydrobiopterin (BH4) is a multifunctional cofactor for phenylalanine, tyrosine and tryptophan hydroxylases, which catalyses the initial steps in phenylalanine degradation in the liver, and is the rate-limiting steps in the biosynthesis of the neurotransmitters, catechol amines and indole amines in the brain. Tetrahydrobiopterin

levels in mammalian cells are mainly determined by GTP-CH-I activity. Mutations in the GTPCH-I gene are responsible for severe diseases including dopa-responsive-dystonia and certain cases of atypical phenylketonuria. Mammalian GTP-CH-I is inhibited by tetrahydrobiopterin and stimulated by phenylalanine through complex formation with the GTP-CH-I feedback regulatory protein. A function to promote release of dopamine, serotonin and noradrenaline from the striatal and cortical nerve terminals has also been proposed for BH4. Serotonin (5-hydroxytryptamine; 5-HT) and its derivatives are neurotransmitters present in brain or pituitary gland, regulating a great number of physiological mechanisms such as sleep, appetite, thermoregulation, control of pituitary secretions and behaviour. BH4 has furthermore an essential role in the biosynthesis of nitric oxide (NO) as an allosteric activator of nitric oxide synthase (NOS) and seems to be necessary for catalytic turnover involving a redox-function of the cofactor. Recently, it was shown that an increase in BH4 biosynthesis in a pancreatic B-cell line (INS-1) is followed by enhanced NO production and subsequently, inhibition of insulin secretion. BH4 regulates human melanogenesis by forming a stable complex with the a-melanocyte stimulating hormone. Finally, BH4 is known as an essential co-factor for alkylglyceryl monooxygenases. Reduced levels of BH4 in the brain and cerebrospinal fluid are associated with several neuro-psychiatric diseases such as Parkinson's disease, Alzheimer's disease, depression and dystonia. In atypical phenylketonuria (PKU), BH4 deficiency results in neurological disorders as a result of decreased biosynthesis of brain catecholamines and serotonin. BH4 is involved in proliferation and growth regulation of erythroid cells. Partial depletion of BH4 in a murine erythroleukaemia cell line caused inhibition of cell growth.

GTP cyclohydrolase I (I; E.C. 3.5.4.16) catalyzes the conversion of GTP (1) to dihydroneopterin triphosphate (2; H2NTP, 6-D-threo-1´,2´,3´-hydroxypropyl-7,8-dihydroneopterin-3´-triphosphate).
In plants and certain microorganisms, the enzyme product serves as the first committed intermediate in the biosynthesis of

tetrahydrofolate. In animals, dihydroneopterintriphosphate is converted to tetrahydrobiopterin (8; BH4, [6R]-[L-erythro-1´,2´-dihydroxypropyl]-2-amino-4-hydroxy-5,6,7,8-tetrahydropteridine) by the sequential action of 6-pyruvoyl tetrahydropterin synthase (PTPS; E.C.4.6.1.10) and sepiapterin reductase (E.C. 1.1.1.153).

Pteridine and folate research has long been recognized as important for many biological processes, such as amino acid metabolism, nucleic acid synthesis, neurotransmitter synthesis, cancer, cardiovascular function etc.

Vitamin B Rich Folates Significantly Reduce Alzheimer's Disease Risk

Beats antioxidants, like vitamin E, and other nutrients for health of aging brain in study of senior citizens

Aug. 12, 2005- A study of senior citizens says those who eat the daily recommended allowance of folates – B vitamin nutrients found in oranges, legumes, leafy green vegetables and folic acid supplements – "significantly reduce" their risk of developing Alzheimer's disease.

The study, a long-term look at diet and brain aging by the National Institute on Aging, also found that folates appear to have more impact on reducing Alzheimer's risk than vitamin E, a noted antioxidant, and other nutrients considered for their effect as a brain-aging deterrent.

Ultimately, 57 of the original 579 participants in the study developed Alzheimer's disease. But the researchers found that those with higher intake of folates, vitamin E and vitamin B6 shared lower comparative rates of the disease. And when the three

vitamins were analyzed together, only folates were associated with a significantly decreased risk.

In turn, no association was found between vitamin C, carotenoids (such as beta-carotene) or vitamin B-12 intake and decreased Alzheimer's risk.

Although folates appear to be more beneficial than other nutrients, the primary message should be that overall healthy diets seem to have an impact on limiting Alzheimer's disease risk, said Dr. Corrada.

Scientists compared the food nutrient and supplement intake of those who later developed Alzheimer's disease to the intake of those who did not develop the disease. It is the largest study to date to report on the association between folate intake and Alzheimer's risk and to analyze antioxidants and B vitamins simultaneously.

The researchers used data from the Baltimore Longitudinal Study of Aging to identify the relationship between dietary factors and Alzheimer's disease risk. Between 1984 and 1991, study volunteers provided detailed dietary diaries, which included supplement intake and calorie amounts, for a typical seven-day period.

The participants who had intakes at or above the 400-microgram recommended dietary allowance of folates had a 55-percent reduction in risk of developing Alzheimer's, said Dr. Corrada, an assistant professor of neurology. "But most people who reached that level did so by taking folic acid supplements, which suggests that many people do not get the recommended amounts of folates in their diets."

Folates have already been proven to reduce birth defects, and research suggests that they are beneficial to warding off heart disease and strokes.

Although folates are abundant in foods such as liver, kidneys, yeast, fruits (like bananas and oranges), leafy vegetables, whole-wheat bread, lima beans, eggs and milk, they are often destroyed by cooking or processing.

Recent research is beginning to show relationships between folates and brain aging.

Earlier this year, Dutch scientists showed that adults who took 800 micrograms of folic acid daily had significant improved memory test scores, giving evidence that folates can slow cognitive decline.

Given the observational nature of this study, it is still possible that other unmeasured factors also may be responsible for this reduction in risk, said Dr. Kawas. People with a high intake of one nutrient are likely to have a high intake of several other nutrients and may generally have a healthy lifestyle.

Another study begun in 1958 by the NIA, the Baltimore Longitudinal Study of Aging is America's longest-running scientific study of human aging. BLSA scientists are learning what happens as people age and how to sort out changes due to aging from those due to disease or other causes. More than 1,400 men and women are study volunteers.

]+

Folate: What is it?

Folate is a water-soluble B vitamin that occurs naturally in food. Folic acid is the synthetic form of folate that is found in supplements and added to fortified foods.

Folate gets its name from the Latin word "folium" for leaf. A key observation of researcher Lucy Wills nearly 70 years ago led to the identification of folate as the nutrient needed to prevent the anemia of pregnancy. Dr. Wills demonstrated that the anemia could be corrected by a yeast extract. Folate was identified as the corrective substance in yeast extract in the late 1930s, and was extracted from spinach leaves in 1941.
Folate helps produce and maintain new cells. This is especially important during periods of rapid cell division and growth such as infancy and pregnancy. Folate is needed to make DNA and RNA, the building blocks of cells. It also helps prevent changes to DNA that may lead to cancer. Both adults and children need folate to make normal red blood cells and prevent anemia. Folate is also essential for the metabolism of homocysteine, and helps maintain normal levels of this amino acid.

What foods provide folate?

Leafy green vegetables (like spinach and turnip greens), fruits (like citrus fruits and juices), and dried beans and peas are all natural sources of folate.

The following table suggests a variety of dietary sources of folate.

Table 1: Selected Food Sources of Folate and Folic Acid [5]

Food	Micrograms (µg)	% DV^
*Breakfast cereals fortified with 100% of the DV, ¾ cup	400	100

Beef liver, cooked, braised, 3 ounces	185	45
Cowpeas (blackeyes), immature, cooked, boiled, ½ cup	105	25
*Breakfast cereals, fortified with 25% of the DV, ¾ cup	100	25
Spinach, frozen, cooked, boiled, ½ cup	100	25
Great Northern beans, boiled, ½ cup	90	20
Asparagus, boiled, 4 spears	85	20
*Rice, white, long-grain, parboiled, enriched, cooked, ½ cup	65	15
Vegetarian baked beans, canned, 1 cup	60	15
Spinach, raw, 1 cup	60	15
Green peas, frozen, boiled, ½ cup	50	15
Broccoli, chopped, frozen, cooked, ½ cup	50	15
*Egg noodles, cooked, enriched, ½ cup	50	15
Broccoli, raw, 2 spears (each 5 inches long)	45	10
Avocado, raw, all varieties, sliced, ½ cup sliced	45	10
Peanuts, all types, dry roasted, 1 ounce	40	10
Lettuce, Romaine, shredded, ½ cup	40	10
Wheat germ, crude, 2 Tablespoons	40	10
Tomato Juice, canned, 6 ounces	35	10
Orange juice, chilled, includes concentrate, ¾ cup	35	10
Turnip greens, frozen, cooked, boiled, ½ cup	30	8
Orange, all commercial varieties, fresh, 1 small	30	8
*Bread, white, 1 slice	25	6
*Bread, whole wheat, 1 slice	25	6

Egg, whole, raw, fresh, 1 large	25	6
Cantaloupe, raw, ¼ medium	25	6
Papaya, raw, ½ cup cubes	25	6
Banana, raw, 1 medium	20	6

* Items marked with an asterisk (*) are fortified with folic acid as part of the Folate Fortification Program.

^ DV = Daily Value. DVs are reference numbers developed by the Food and Drug Administration (FDA) to help consumers determine if a food contains a lot or a little of a specific nutrient. The DV for folate is 400 micrograms (µg). Most food labels do not list a food's magnesium content. The percent DV (%DV) listed on the table indicates the percentage of the DV provided in one serving. A food providing 5% of the DV or less is a low source while a food that provides 10-19% of the DV is a good source. A food that provides 20% or more of the DV is high in that nutrient. It is important to remember that foods that provide lower percentages of the DV also contribute to a healthful diet. For foods not listed in this table, please refer to the U.S. Department of Agriculture's Nutrient Database Web site: http://www.nal.usda.gov/fnic/cgi-bin/nut_search.pl.

What are the Dietary Reference Intakes for folate?

Recommendations for folate are given in the Dietary Reference Intakes (DRIs) developed by the Institute of Medicine of the National Academy of Sciences. *Dietary Reference Intakes* is the general term for a set of reference values used for planning and assessing nutrient intake for healthy people. Three important types of reference values included in the DRIs are *Recommended Dietary Allowances* (RDA), *Adequate Intakes* (AI), and *Tolerable Upper Intake Levels* (UL). The RDA recommends the average daily intake that is sufficient to meet the nutrient requirements of nearly all (97-98%) healthy individuals in each age and gender group. An AI is set when there is insufficient scientific data available to establish a RDA. AIs meet or exceed the

amount needed to maintain a nutritional state of adequacy in nearly all members of a specific age and gender group. The UL, on the other hand, is the maximum daily intake unlikely to result in adverse health effects.

The RDAs for folate are expressed in a term called the *Dietary Folate Equivalent*. The Dietary Folate Equivalent (DFE) was developed to help account for the differences in absorption of naturally occurring dietary folate and the more bio-available synthetic folic acid. Table 2 lists the RDAs for folate, expressed in micrograms (µg) of DFE, for children and adults.

Table 2: Recommended Dietary Allowances for Folate for Children and Adults.

Age (years)	Males and Females (µg/day)	Pregnancy (µg/day)	Lactation (µg/day)
1-3	150	N/A	N/A
4-8	200	N/A	N/A
9-13	300	N/A	N/A
14-18	400	600	500
19+	400	600	500

*1 DFE = 1 µg food folate = 0.6 µg folic acid from supplements and fortified foods.

There is insufficient information on folate to establish an RDA for infants. An Adequate Intake (AI) has been established that is based on the amount of folate consumed by healthy infants who are fed breast milk. Table 3 lists the Adequate Intake for folate, in micrograms (µg), for infants.

Table 3: Adequate Intake for folate for infants

Age	Males and Females

(months)	(µg/day)
0 to 6	65
7 to 12	80

The National Health and Nutrition Examination Survey (NHANES III 1988-94) and the Continuing Survey of Food Intakes by Individuals (1994-96 CSFII) indicated that most individuals surveyed did not consume adequate folate. However, the folic acid fortification program, which was initiated in 1998, has increased folic acid content of commonly eaten foods such as cereals and grains, and as a result most diets in the United States (US) now provide recommended amounts of folate equivalents.

When can folate deficiency occur?

A deficiency of folate can occur when an increased need for folate is not matched by an increased intake, when dietary folate intake does not meet recommended needs, and when folate excretion increases. Medications that interfere with the metabolism of folate may also increase the need for this vitamin and risk of deficiency.

Medical conditions that increase the need for folate or result in increased excretion of folate include:

- pregnancy and lactation (breastfeeding)
- alcohol abuse
- malabsorption
- kidney dialysis
- liver disease
- certain anemias

Medications that interfere with folate utilization include:

- anti-convulsant medications (such as dilantin, phenytoin and primidone)

- metformin (sometimes prescribed to control blood sugar in type 2 diabetes)
- sulfasalazine (used to control inflammation associated with Crohn's disease and ulcerative colitis)
- triamterene (a diuretic)
- methotrexate (used for cancer and other diseases such as rheumatoid arthritis)
- barbiturates (used as sedatives)

What are some common signs and symptoms of folate deficiency?

 Folate deficient women who become pregnant are at greater risk of giving birth to low birth weight, premature, and/or infants with neural tube defects.
 In infants and children, folate deficiency can slow overall growth rate.
 In adults, a particular type of anemia can result from long term folate deficiency.
 other signs of folate deficiency are often subtle. Digestive disorders such as diarrhea, loss of appetite, and weight loss can occur, as can weakness, sore tongue, headaches, heart palpitations, irritability, forgetfulness, and behavioral disorders. An elevated level of homocysteine in the blood, a risk factor for cardiovascular disease, also can result from folate deficiency.

Many of these subtle symptoms are general and can also result from a variety of medical conditions other than folate deficiency. It is important to evaluate these symptoms so that appropriate medical care can be given.

Do women of childbearing age and pregnant women have a special need for folate?

Folic acid is very important for all women who may become pregnant. Adequate folate intake during the pre-conceptual period,

the time just before and just after a woman becomes pregnant, protects against neural tube defects. Neural tube defects result in malformations of the spine (spina bifida), skull, and brain (anencephaly). The risk of neural tube defects is significantly reduced when supplemental folic acid is consumed in addition to a healthful diet prior to and during the first month following conception. Since January 1, 1998, when the folate food fortification program took effect, data suggest that there has been a significant reduction in neural tube birth defects. Women who could become pregnant are advised to eat foods fortified with folic acid or take a folic acid supplement in addition to eating folate-rich foods to reduce the risk of some serious birth defects. For this population, researchers recommend a daily intake of 400 μg of synthetic folic acid per day from fortified foods and/or dietary supplements.

Who else may need extra folic acid to prevent a deficiency?

People who abuse alcohol, those taking medications that may interfere with the action of folate (including, but not limited to those listed above), individuals diagnosed with anemia from folate deficiency, and those with malabsorption, liver disease, or who are receiving kidney dialysis treatment may benefit from a folic acid supplement.

Folate deficiency has been observed in alcoholics. A 1997 review of the nutritional status of chronic alcoholics found low folate status in more than 50% of those surveyed. Alcohol interferes with the absorption of folate and increases excretion of folate by the kidney. In addition, many people who abuse alcohol have poor quality diets that do not provide the recommended intake of folate. Increasing folate intake through diet, or folic acid intake through fortified foods or supplements, may be beneficial to the health of alcoholics.

Anti-convulsant medications such as Dilantin increase the need for folate. Anyone taking anti-convulsants and other medications that interfere with the body's ability to use folate should consult with a medical doctor about the need to take a folic acid supplement.

Anemia is a condition that occurs when there is insufficient hemoglobin in red blood cells to carry enough oxygen to cells and tissues. It can result from a wide variety of medical problems, including folate deficiency. With folate deficiency, your body may make large red blood cells that do not contain adequate hemoglobin, the substance in red blood cells that carries oxygen to your body's cells. Determine whether an anemia is associated with folate deficiency and whether supplemental folic acid is indicated.

Several medical conditions increase the risk of folic acid deficiency. Liver disease and kidney dialysis increase excretion (loss) of folic acid. Malabsorption can prevent your body from using folate in food. Evaluate the need for a folic acid supplement.

What are some current issues and controversies about folate?

Folic Acid and Cardiovascular Disease
Cardiovascular disease involves any disorder of the heart and blood vessels that make up the cardiovascular system. Coronary heart disease occurs when blood vessels which supply the heart become clogged or blocked, increasing the risk of a heart attack. Vascular damage can also occur to blood vessels supplying the brain, and can result in a stroke.

Cardiovascular disease is the most common cause of death in industrialized countries such as the US, and is on the rise in developing countries. The National Heart, Lung, and Blood Institute of the National Institutes of Health has identified many risk factors for cardiovascular disease, including an elevated LDL-cholesterol level, high blood pressure, a low HDL-cholesterol level, obesity, and diabetes. In recent years, researchers have identified another risk factor for cardiovascular disease, an elevated homocysteine level. Homocysteine is an amino acid normally found in blood, but elevated levels have been linked with coronary heart disease and stroke. Elevated homocysteine levels may impair endothelial vasomotor

function, which determines how easily blood flows through blood vessels. High levels of homocysteine also may damage coronary arteries and make it easier for blood clotting cells called platelets to clump together and form a clot, which may lead to a heart attack. A deficiency of folate, vitamin B_{12} or vitamin B_6 may increase blood levels of homocysteine, and folate supplementation has been shown to decrease homocysteine levels and to improve endothelial function. At least one study has linked low dietary folate intake with an increased risk of coronary events. The folic acid fortification program in the U. S. has decreased the prevalence of low levels of folate and high levels of homocysteine in the blood in middle-aged and older adults. Daily consumption of folic-acid fortified breakfast cereal and the use of folic acid supplements have shown to be an effective strategy for reducing homocysteine concentrations.

Evidence supports a role for supplemental folic acid for lowering homocysteine levels; however this does not mean that folic acid supplements will decrease the risk of cardiovascular disease. Clinical intervention trials are underway to determine whether supplementation with folic acid, vitamin B_{12}, and vitamin B_6 can lower risk of coronary heart disease. It is premature to recommend folic acid supplementation for the prevention of heart disease until results of ongoing randomized, controlled clinical trials positively link increased folic acid intake with decreased homocysteine levels and decreased risk of cardiovascular disease.

Folic Acid and Cancer

Some evidence associates low blood levels of folate with a greater risk of cancer. Folate is involved in the synthesis, repair, and function of DNA, our genetic map, and there is some evidence that a deficiency of folate can cause damage to DNA that may lead to cancer. Several studies have associated diets low in folate with increased risk of breast, pancreatic, and colon cancer. Over 88,000 women enrolled in the Nurses' Health Study who were free of cancer in 1980 were followed from 1980 through 1994. Researchers found that women ages 55 to 69 years in this study who took multivitamins

containing folic acid for more than 15 years had a markedly lower risk of developing colon cancer. Findings from over 14,000 subjects followed for 20 years suggest that men who do not consume alcohol and whose diets provide the recommended intake of folate are less likely to develop colon cancer. However, associations between diet and disease do not indicate a direct cause. Researchers are continuing to investigate whether enhanced folate intake from foods or folic acid supplements may reduce the risk of cancer. Until results from such clinical trials are available, folic acid supplements should not be recommended to reduce the risk of cancer.

Folic Acid and Methotrexate for Cancer
Folate is important for cells and tissues that rapidly divide. Cancer cells divide rapidly, and drugs that interfere with folate metabolism are used to treat cancer. Methotrexate is a drug often used to treat cancer because it limits the activity of enzymes that need folate.

Unfortunately, methotrexate can be toxic, producing side effects such as inflammation in the digestive tract that may make it difficult to eat normally. Leucovorin is a form of folate that can help "rescue" or reverse the toxic effects of methotrexate. There are many studies underway to determine if folic acid supplements can help control the side effects of methotrexate without decreasing its effectiveness in chemotherapy. It is important for anyone receiving methotrexate to follow a medical doctor's advice on the use of folic acid supplements.

Folic Acid and Methotrexate for Non-Cancerous Diseases
Low dose methotrexate is used to treat a wide variety of non-cancerous diseases such as rheumatoid arthritis, lupus, psoriasis, asthma, sarcoidoisis, primary biliary cirrhosis, and inflammatory bowel disease. Low doses of methotrexate can deplete folate stores and cause side effects that are similar to folate deficiency. Both high folate diets and supplemental folic acid may help reduce the toxic side effects of low dose methotrexate without decreasing its effectiveness. Anyone taking low dose methotrexate for the health

problems listed above should consult with a physician about the need for a folic acid supplement.

Caution about Folic Acid Supplements

Beware of the interaction between vitamin B_{12} and folic acid
Intake of supplemental folic acid should not exceed 1,000 micrograms (μg) per day to prevent folic acid from triggering symptoms of vitamin B_{12} deficiency. Folic acid supplements can correct the anemia associated with vitamin B_{12} deficiency. Unfortunately, folic acid will not correct changes in the nervous system that result from vitamin B_{12} deficiency. Permanent nerve damage can occur if vitamin B_{12} deficiency is not treated.

It is very important for older adults to be aware of the relationship between folic acid and vitamin B_{12} because they are at greater risk of having a vitamin B_{12} deficiency. If you are 50 years of age or older, check your B_{12} status before you take a supplement that contains folic acid. If you are taking a supplement containing folic acid, read the label to make sure it also contains B_{12} or speak with a physician about the need for a B_{12} supplement.

What is the health risk of too much folic acid?

Folate intake from food is not associated with any health risk. The risk of toxicity from folic acid intake from supplements and/or fortified foods is also low. It is a water soluble vitamin, so any excess intake is usually excreted in urine. There is some evidence that high levels of folic acid can provoke seizures in patients taking anti-convulsant medications. Anyone taking such medications should consult with a medical doctor before taking a folic acid supplement. The Institute of Medicine has established a tolerable upper intake level (UL) for folate from fortified foods or supplements (i.e. folic acid) for ages one and above. Intakes above this level increase the risk of adverse health effects. In adults, supplemental folic acid should not exceed the UL to prevent folic acid from triggering

symptoms of vitamin B_{12} deficiency. It is important to recognize that the UL refers to the amount of synthetic folate (i.e. folic acid) being consumed per day from fortified foods and/or supplements. There is no health risk, and no UL, for natural sources of folate found in food. Table 4 lists the Upper Intake Levels (UL) for folate, in micrograms (µg), for children and adults.

Table 4: Tolerable Upper Intake Levels for Folate for Children and Adults

Age (years)	Males and Females (µg/day)	Pregnancy (µg/day)	Lactation (µg/day)
1-3	300	N/A	N/A
4-8	400	N/A	N/A
9-13	600	N/A	N/A
14-18	800	800	800
19 +	1000	1000	1000

Selecting a healthful diet

As the 2000 *Dietary Guidelines for Americans* states, "Different foods contain different nutrients and other healthful substances. No single food can supply all the nutrients in the amounts you need". As indicated in Table 1, green leafy vegetables, dried beans and peas, and many other types of vegetables and fruits provide folate. In addition, fortified foods are a major source of folic acid. It is not unusual to find foods such as some ready-to-eat cereals fortified with 100% of the RDA for folate. The variety of fortified foods available has made it easier for women of childbearing age in the US to consume the recommended 400 mcg of folic acid per day from fortified foods and/or supplements. The large numbers of fortified foods on the market, however, also raises the risk of

exceeding the UL. This is especially important for anyone at risk of vitamin B_{12} deficiency, which can be triggered by too much folic acid. It is important for anyone who is considering taking a folic acid supplement to first consider whether their diet already includes adequate sources of dietary folate and fortified food sources of folic acid.

Plasma homocysteine has been identified as a risk factor for arterial disease, retinal artery and vein occlusions, and other common eye diseases. The value of treating an elevated plasma homocysteine with folic acid for preventing further vascular disease has not been proven. Although secondary prevention of coronary artery disease using this approach has been unsuccessful, trials on primary prevention of stroke and loss of cognitive function with folic acid supplementation appear to be successful. Further trial data are awaited. In patients with premature retinovascular disease, the measurement of plasma homocysteine is suggested and reduction of elevated homocysteine with folic acid for secondary prevention of retinal arterial and venous occlusion. Meanwhile, the debate on fortification of flour for primary prevention of neural tube defects, which has already taken place in North America, continues in European countries. Such fortification could have an impact on primary and secondary prevention of vascular disease.

Folates - The New Brain Food!

A recent study conducted at the University of California Irvine suggests that folates, the B-vitamin nutrients found in oranges, legumes, leafy green vegetables and folic acid supplements, are more effective in reducing the risk of Alzheimer's disease than antioxidants.

Maria Corrada and Dr. Claudia Kawas of UC Irvine's Institute for Brain Aging and Dementia looked at the diets of 579 healthy men and women over 60-years-of-age, using data from the Baltimore Longitudinal Study of Aging. They compared the dietary and

supplement intake of 57 participants, who later contracted Alzheimer's disease, to those that remained healthy. The researchers found that subjects who had a higher intake of folates, vitamin E and vitamin B6 has a lesser rate of contracting the disease. Among those three vitamins, only folates were associated with a significantly decreased risk.

Participants who took more than the 400 mcg recommended daily allowance of folates had a 55% reduction in risk of developing Alzheimer's. Most people reached that level through supplements rather than diet alone. The researchers note that although folates seem more beneficial than other nutrients, an overall healthy diet is recommended for limiting the risk of developing the disease.

Folates are rich in foods such as liver, kidneys, yeast, bananas, whole-wheat bread, beans, eggs and milk, but are often destroyed during cooking. Although folates have been added to U.S. grain products since 1998, many Americans are still deficient in this nutrient. Folates are also known to reduce birth defects and may help prevent heart disease and strokes.

This is the largest study to date on the relationship between folates and Alzheimer's disease, and the largest to analyze antioxidants and B vitamins together. Results are published in *Alzheimer's & Dementia: The Journal of the Alzheimer's Association.*

Folates and cardiovascular disease

It is increasingly recognized that folates may play a role in the prevention of cardiovascular disease. Over the last few years, several studies have reported beneficial effects of folates on endothelial function, a surrogate end point for cardiovascular risk. Consistently, observational studies have demonstrated an association between folate levels and cardiovascular morbidity and mortality. The exact mechanisms underlying the ameliorative effects of folates on the endothelium remain to be elucidated. Thus far, most studies have focused on the homocysteine-lowering effects of folates. However,

recently, benefits of folates independent of homocysteine lowering have also been reported. Potential mechanisms include antioxidant actions, effects on cofactor availability, or direct interactions with the enzyme endothelial NO synthase. Obviously, beneficial effects of folates on cardiovascular risk would have important clinical and dietary consequences. Folates function as a single carbon donor in the synthesis of serine from glycine, in the synthesis of nucleotides form purine precursors, indirectly in the synthesis of transfer RNA, and as a methyl donor to create methylcobalamin, which is used in the re-methylation of homocysteine to methionine. Oral folates are generally available in two supplemental forms, folic and folinic acid. Administration of folinic acid bypasses the de-conjugation and reduction steps required for folic acid. Folinic acid also appears to be a more metabolically active form of folate, capable of boosting levels of the coenzyme forms of the vitamin in circumstances where folic acid has little to no effect. Therapeutically, folic acid can reduce homocysteine levels and the occurrence of neural tube defects, might play a role in preventing cervical dysplasia and protecting against neoplasia in ulcerative colitis, appears to be a rational aspect of a nutritional protocol to treat vitiligo, and can increase the resistance of the gingiva to local irritants, leading to a reduction in inflammation. Reports also indicate that neuro-psychiatric diseases secondary to folate deficiency might include dementia, schizophrenia-like syndromes, insomnia, irritability, forgetfulness, endogenous depression, organic psychosis, peripheral neuropathy, myelopathy, and restless legs syndrome.

Folic acid

IUPAC name	N-[4(2-Amino-4-hydroxy pteridin-6-ylmethylamino) benzoyl]-L(+)-glutamic acid.
Other names	pteroyl-L-glutamic acid; Vitamin B_9, Vitamin M; Folacin
Identifiers	

CAS number	59-30-3
RTECS number	LP5425000
SMILES	C1=CC(=CC=C1C(=O)NC(CCC(=O)O)C(=O)O)NCC2=CN=C3C(=N2)C(=O)N=C(N3)N
Properties	
Molecular formula	$C_{19}H_{19}N_7O_6$
Molar mass	441.403 g/mol
Appearance	yellow-orange crystalline powder
Melting point	250 °C (523 K), decomp.
Solubility in water	8.5 g/100 ml (20 °C)
Acidity (pK_a)	1^{st}: 2.3, 2^{nd}: 8.3
Hazards	
Main hazards	non-toxic, non-flammable
Except where noted otherwise, data are given for materials in their standard state (at 25 °C, 100 kPa)	

Folic acid and **folate** (the anion form) are forms of the water-soluble Vitamin B_9. These occur naturally in food and can also be taken as supplements.

Folate in foods

Leafy vegetables such as spinach and turnip greens, dried beans and peas, fortified cereal products, sunflower seeds and certain other fruits and vegetables are rich sources of folate. Some breakfast cereals (ready-to-eat and others) are fortified with 25% to 100% of the recommended dietary allowance (RDA) for folic acid. A table of selected food sources of folate and folic acid can be found at the USDA National Nutrient Database for Standard Reference.

History

A key observation by researcher Lucy Wills in 1931 led to the identification of folate as the nutrient needed to prevent anemia during pregnancy. Dr. Wills demonstrated that anemia could be reversed with brewer's yeast. Folate was identified as the corrective substance in brewer's yeast in the late 1930s and was extracted from spinach leaves in 1941. It was first synthesised in 1946.

Biological roles

Folate is necessary for the production and maintenance of new cells. This is especially important during periods of rapid cell division and growth such as infancy and pregnancy. Folate is needed to replicate DNA. Thus folate deficiency hinders DNA synthesis and cell division, affecting most clinically the bone marrow, a site of rapid cell turnover. Because RNA and protein synthesis are not hindered, large red blood cells called megaloblasts are produced, resulting in megaloblastic anemia. Both adults and children need folate to make normal red blood cells and prevent anemia.

Biochemistry

In the form of a series of tetrahydrofolate compounds, folate derivatives are substrates in a number of single-carbon-transfer reactions, and also are involved in the synthesis of dTMP (2'-deoxythymidine-5'-phosphate) from dUMP (2'-deoxyuridine-5'-phosphate). It helps convert vitamin B12 to one of its coenzyme forms and helps synthesize the DNA required for all rapidly growing cells.

The pathway leading to the formation of tetrahydrofolate (FH_4) begins when folate (F) is reduced to dihydrofolate (FH_2), which is then reduced to tetrahydrofolate (FH_4). Dihydrofolate reductase catalyses both steps.

Methylene tetrahydrofolate (CH_2FH_4) is formed from tetrahydrofolate by the addition of methylene groups from one of three carbon donors: formaldehyde, serine, or glycine. Methyl tetrahydrofolate ($CH_3–FH_4$) can be made from methylene tetrahydrofolate by reduction of the methylene group; formyl tetrahydrofolate (CHO-FH_4, folinic acid) results from oxidation of methylene tetrahydrofolate.

In other words:

$F \rightarrow FH_2 \rightarrow FH_4 \rightarrow CH_2{=}FH_4 \rightarrow$ 1-carbon chemistry

A number of drugs interfere with the biosynthesis of folic acid and tetrahydrofolate. Among them are the dihydrofolate reductase inhibitors (such as trimethoprim and pyrimethamine), the sulfonamides (competitive inhibitors of para-aminobenzoic acid in the reactions of dihydropteroate synthetase), and the anticancer drug methotrexate (inhibits both folate reductase and dihydrofolate reductase).

1998 RDAs for Folate

Men	Women		
(19+)	(19+)	Pregnancy	Breast feeding
400 μg	400 μg	600 μg	500 μg
1 μg of food folate = 0.6 μg folic acid from supplements and fortified foods			

The National Health and Nutrition Examination Survey (NHANES III 1988-91) and the Continuing Survey of Food Intakes by Individuals (1994-96 CSFII) indicated that most adults did not consume adequate folate. However, the folic acid fortification program in the United States has increased folic acid content of commonly eaten foods such as cereals and grains, and as a result diets of most adults now provide recommended amounts of folate equivalents.

Pregnancy

Folic acid is very important for all women who may become pregnant. Adequate folate intake during the periconceptional period, the time just before and just after a woman becomes pregnant, helps protect against a number of congenital malformations including neural tube defects. Neural tube defects result in malformations of the spine (spina bifida), skull, and brain (anencephaly). The risk of neural tube defects is significantly reduced when supplemental folic acid is consumed in addition to a healthy diet prior to and during the first month following conception. Women who could become pregnant are advised to eat foods fortified with folic acid or take supplements in addition to eating folate-rich foods to reduce the risk of some serious birth defects. Taking 400 micrograms of synthetic folic acid daily from fortified foods and/or supplements has been suggested. The Recommended Dietary Allowance (RDA) for folate equivalents for pregnant women is 600-800 micrograms, twice the normal RDA of 400 micrograms for women who are not pregnant.

Folic acid supplements and masking of B_{12} deficiency

There has been concern about the interaction between vitamin B_{12} and folic acid. Folic acid supplements can correct the anemia associated with vitamin B_{12} deficiency. Unfortunately, folic acid will not correct changes in the nervous system that result from vitamin B_{12} deficiency. Permanent nerve damage could theoretically occur if vitamin B_{12} deficiency is not treated. Therefore, intake of supplemental folic acid should not exceed 1000 micrograms (1000 mcg or 1 mg) per day to prevent folic acid from masking symptoms of vitamin B_{12} deficiency. In fact, to date the evidence that such masking actually occurs is scarce, and there is no evidence that folic acid fortification in Canada or the US has increased the prevalence of vitamin B_{12} deficiency or its consequences.

However one recent study has demonstrated that high folic or folate levels when combined with low B_{12} levels are associated with significant cognitive impairment among the elderly. If the observed relationship for seniors between folic acid intake, B_{12} levels, and cognitive impairment is replicated and confirmed, this is likely to re-open the debate on folic acid fortification in food, event though public health policies tend generally to support the developmental needs of infants and children over slight risks to other population groups.

In any case, it is important for older adults to be aware of the relationship between folic acid and vitamin B_{12} because they are at greater risk of having a vitamin B_{12} deficiency. If you are 50 years of age or older, check your B_{12} status before you take a supplement that contains folic acid.

Health risk of too much folic acid

The risk of toxicity from folic acid is low. The Institute of Medicine has established a tolerable upper intake level (UL) for folate of 1 mg for adult men and women, and a UL of 800 μg for pregnant and lactating (breast-feeding) women less than 18 years of age. Supplemental folic acid should not exceed the UL to prevent folic

acid from masking symptoms of vitamin B_{12} deficiency. A 10000-patient study at Tufts University in 2007 concluded that excess folic acid worsens the effects of B12 deficiency and in fact may affect the absorption of B12.

Research suggests high levels of folic acid can interfere with some antimalarial treatments.

Some current issues and controversies about folate

RESULTS: Patients' with low RBC folate levels had higher mean corpuscular volume (MCV) and red cell distribution width (RDW) values and lower serum folate and B_{12} values than patients with normal RBC folate levels, but there was no difference in degree of anemia, presence of oval macrocytes and/or hypersegmented neutrophils on the peripheral blood smear, LDH, alcohol use, diet, or any other measured clinical parameter. Serum and RBC folate levels were highly correlated and a low RBC folate affected the clinical outcome of three patients (5% of the low RBC folate group). *CONCLUSIONS:* Based upon these retrospective data and a review of the literature, we cannot define significant differences between patients with low RBC folate and randomly selected patients with normal RBC folate that could not have been equally well defined using serum folate values. The sensitivity and specificity of a low RBC folate level in the diagnosis of ill or healthy individuals are undefined, and until prospective studies utilizing some of the newer, more specific biochemical indicators of tissue folate are completed, the interpretation of low RBC folate levels will remain problematic.

Dietary fortification of folic acid

Since the discovery of the link between insufficient folic acid and neural tube defects (NTDs), governments and health organisations worldwide have made recommendations concerning folic acid *supplementation* for women intending to become pregnant. For

example, the United States Public Health Service recommends an extra 0.4 mg/day, which can be taken as a pill. However, many researchers believe that supplementation in this way can never work effectively enough since about half of all pregnancies in the U.S. are unplanned and not all women will comply with the recommendation.

This has led to the introduction in many countries of *fortification*, where folic acid is added to flour with the intention of everyone benefiting from the associated rise in blood folate levels. This is controversial, with issues having been raised concerning individual liberty, and the masking effect of folate fortification on pernicious anaemia (vitamin B_{12} deficiency). However, most North and South American countries now fortify their flour, along with a number of Middle Eastern countries and Indonesia, Mongolia and a number of ex-Soviet republics are amongst those having widespread voluntary fortification; about five more countries (including Morocco, the first African country) have agreed but not yet implemented fortification. In the UK the Food Standards Agency has recommended fortification. To date, no EU country has yet mandated fortification. Australia is considering fortification, but a period for comments ending 2006-07-31 attracted strong opposition from industry as well as academia.

Recent debate has emerged in the United Kingdom and Australia regarding the inclusion of folic acid in products such as bread and flour.

In the USA many grain products are fortified with folic acid.

In 1996, the United States Food and Drug Administration (FDA) published regulations requiring the addition of folic acid to enriched breads, cereals, flours, corn meals, pastas, rice, and other grain products. This ruling took effect 1998-01-01, and was specifically targeted to reduce the risk of neural tube birth defects in newborns. There are concerns that the amount of folate added is insufficient. In October 2006, the Australian press claimed that U.S. regulations

requiring fortification of grain products were being interpreted as disallowing fortification in non-grain products, specifically Vegemite (an Australian yeast extract containing folate). The FDA later said the report was inaccurate, and no ban or other action was being taken against Vegemite.

Since the folic acid fortification program took effect, fortified foods have become a major source of folic acid in the American diet. The Centers for Disease Control and Prevention in Atlanta, Georgia used data from 23 birth defect registries that cover about half of United States births and extrapolated their findings to the rest of the country. This data indicates that since the addition of folic acid in grain-based foods as mandated by the Food and Drug Administration, the rate of neural tube defects dropped by 25% in the United States.

Although folic acid does reduce the risk of birth defects, it is only one part of the picture and should not be considered a cure. Even women taking daily folic acid supplements have been known to have children with neural tube defects.

Heart disease

Adequate concentrations of folate, vitamin B_{12}, or vitamin B_6 may decrease the circulating level of homocysteine, an amino acid normally found in blood. The evidence suggests that high levels of homocysteine may damage coronary arteries or make it easier for blood clotting cells called platelets to clump together and form a clot. However, there is currently no evidence available to suggest that lowering homocysteine with vitamins will reduce your risk of heart disease. Clinical intervention trials are needed to determine whether supplementation with folic acid, vitamin B_{12} or vitamin B_6 can lower your risk of developing coronary heart disease.

The NORVIT trial suggests that folic acid supplementation may do more harm than good. As of 2006, studies have shown that giving

folic acid to reduce levels of homocysteine does not result in clinical benefit. One of these studies suggests that folic acid in combination with B_{12} may even increase some cardiovascular risks.

Stroke

Folic acid appears to reduce the risk of stroke. The reviews indicate only that in some individuals the risk of stroke appears to be reduced, but a definite recommendation regarding supplementation beyond the current recommended daily allowance has not been established for stroke prevention.

Cancer

The association between folate and cancer appears to be complex. It has been suggested that folate may help prevent cancer, as it is involved in the synthesis, repair, and functioning of DNA, our genetic map, and a deficiency of folate may result in damage to DNA that may lead to cancer. Conversely, it has been suggested that excess folate may promote tumor initiation. Although diets high in folate are associated with decreased risk of colorectal cancer, the association is stronger for folate from foods alone than for folate from foods and supplements, and a 2007 randomized clinical trial found that folate supplements did not reduce the risk of colorectal adenomas. A 2006 prospective study of 81,922 Swedish adults found that diets high in folate from foods, but not from supplements, were associated with a reduced risk of pancreatic cancer. Most epidemiologic studies suggest that diets high in folate are associated with decreased risk of breast cancer, but results are not uniformly consistent: one large cancer screening trial reported a potential harmful effect of high folate intake on breast cancer risk, suggesting that routine folate supplementation should not be recommended as a breast cancer preventive, but a 2007 Swedish prospective study found that a high folate intake was associated with a lower incidence of postmenopausal breast cancer.

Antifolates

Folate is important for cells and tissues that rapidly divide. Cancer cells divide rapidly, and drugs that interfere with folate metabolism are used to treat cancer. The antifolate methotrexate is a drug often used to treat cancer because it inhibits the production of the active form, tetrahydrofolate. Unfortunately, methotrexate can be toxic, producing side effects such as inflammation in the digestive tract that make it difficult to eat normally.

Folinic acid is a form of folate that can help "rescue" or reverse the toxic effects of methotrexate. Folinic acid is *not* the same as folic acid. Folic acid supplements have little established role in cancer chemotherapy. There have been cases of severe adverse effects of accidental substitution of folic acid for folinic acid in patients receiving methotrexate cancer chemotherapy. It is important for anyone receiving methotrexate to follow medical advice on the use of folic or folinic acid supplements.

Low dose methotrexate is used to treat a wide variety of non-cancerous diseases such as rheumatoid arthritis, lupus, psoriasis, asthma, sarcoidoisis, primary biliary cirrhosis, and inflammatory bowel disease. Low doses of methotrexate can deplete folate stores and cause side effects that are similar to folate deficiency. Both high folate diets and supplemental folic acid may help reduce the toxic side effects of low dose methotrexate without decreasing its effectiveness. Anyone taking low dose methotrexate for the health problems listed above should consult with a physician about the need for a folic acid supplement.

Depression

Some evidence links low levels of folate with depression. There is some limited evidence from randomised controlled trials that using folic acid in addition to antidepressant medication may have benefits. Researchers at the University of York and Hull York Medical School have confirmed a link between depression and low levels of folate in a research study involving 15,315 . However, the evidence is probably

too limited at present for this to be a routine treatment recommendation.

Memory and mental agility

In a 3-year trial on 818 people over the age of 50, short-term memory, mental agility and verbal fluency were all found to be better among people who took 800 micrograms of folic acid daily—twice the current RDA—than those who took placebo. The study was reported in *The Lancet* on 19 January 2007.

Fertility

Folate is necessary for fertility in both men and women. In men, it contributes to spermatogenesis. In women, on the other hand, it contributes to oocyte maturation, implantation, placentation, in addition to the general effects of folic acid and pregnancy. Therefore, it is necessary to receive sufficient amounts through the diet, in order to avoid subfertility.

Bioavailability of Food Folates and Evaluation of Food Matrix

Folate bioavailability of beef liver, lima beans, peas, spinach, mushrooms, collards, orange juice and wheat germ was estimated with a protocol of folate depletion-repletion using growth and liver, serum and erythrocyte folate of weanling male rats. Diets with 125, 250 and 375 μg folic acid/kg were standards. Individual foods were incorporated into a folate-free amino acid-based diet alone (250 μg folate/kg diet from food) or mixed with folic acid (125 μg folate from food + 125 μg folic acid) to evaluate folate bioavailability and effects of food matrix. Beef liver and orange juice folates were as available as folic acid, whereas those of wheat germ were less bio-available. Folates of peas and spinach were also less available than folic acid using liver and serum folate concentrations and total liver folate as response criteria, but they were not lower when based on growth and

erythrocyte folate concentrations. Lima bean, mushroom and collard folates were as available as folic acid using four of five response criteria. Folate bioavailability of all foods generally exceeded 70%. All response criteria gave approximately equivalent results, indicating that growth and tissue folate levels are appropriate criteria. No food matrix effects were observed for any food except lima beans. Foods rich in polyglutamyl folates were less bio-available than those of foods rich in short-chain folates.

Importance of Folic Acid in human nutrition

Folates accept one-carbon units from donor molecules and pass them on via various biosynthetic reactions. Cellular folates are a mixture of un-substituted polyglutamyl tetrahydrofolates and substituted one-carbon forms of terahydrofolate.

The folates found in food consist of a mixture of reduced folate polyglutamates. The chemical lability of all naturally-occurring folates results in a significant loss of biochemical activity during harvesting, storage, processing, and preparation; up to three quarters of initial folate activity may be lost during these processes. On the contrary, synthetic folate is stable for months to years.

Natural folates found in foods are all conjugated to a polyglutamyl chain. This chain is removed in the brush border of the mucosal cells by folate conjugase, and folate monoglutamate is subsequently absorbed. This process, however, is not complete and reduces the bioavailability of natural folates by 25-50%. (Synthetic folic acid appears to be highly bio-available – 85% or greater). In the intestinal cells, folate is reduced by the enzyme dihydrofolate reductase to the dihydro and tetrahydro forms, and 5-methyltetrahydrofolate is released in to the plasma. Thus, the primary form of folate entering human circulation from intestinal cells is 5-methyltetrahydrofolate monoglutamate.

The poor chemical stability of the natural folate and their low bioavailability determine nutrient recommendations. Fortification of foods as breakfast cereals and flour can add significant amounts of folic acid to diet.

Function of folates: Folates play very important metabolic roles:

1. Formyl group (from 10-formyltetrahydrofolate) is incorporated sequentially into C-2 and C-8 of the purine ring during its biosynthesis.
2. The enzyme, thymidylate synthetase, which catalyses conversion of deoxyuridylate (a precursor to RNA) into thymidylate (a precursor to DNA) requires 5, 10-methylenetetahydrofolate. Thus, folate is essential for the DNA biosynthesis.
3. 5,10-methylenetetrahydrofolate can be channeled into methylation cycle – a process which has multiple roles including methylation lipids, hormones, DNA and proteins. Methylation of myelin, which acts as insulation for nerve cells, may be impaired with deficiency of folate, B 6 and B 12. De-myelination of nerve cells may present with combined degeneration of the spinal cord and peripheral nerves. Clinically it presents with neuropathy (ataxia, paralysis, and if left untreated, ultimately death). This usually occurs when folate deficiency is very severe and prolonged, but not with mild to moderate deficiency, since nerve cells are able to concentrate folate 5 times than in the plasma, thereby protecting themselves from falling short of folate in the face of nutritional deficiency.

High Risk Populations:

a. People who have a restricted diet without leafy vegetables are prone.
b. Malabsorption (including celiac disease and tropical sprue) exacerbate folate deficiency.

c. Since rapid foetal growth in the 2 nd and 3 rd trimester increases demand for folate, pregnant women are at risk of deficiency.
d. During lactation, loss of folate in milk also increases the folate requirement.

Dietary Sources of Folate: Folate in present in many foods but in a relatively low density except in liver. Diets which contain adequate amounts of green leafy vegetables (ie in excess of 3 servings per day) will be good sources of folate. Folate losses during harvesting, storage, distribution, and coking can be considerable. Folate derived from animal products is also subject to loss during cooking. Folic acid content of some staple foods (white rice, corn) can enhance folate intake. This may be especially considered for pregnant and lactating mothers for their increased requirement.

Adequacy of Folate Status:

1. Red cell folate is an important index of folate status; an adequate status is reflected in a red cell folate level of > 150 microgram/l. This is sufficient to prevent anemia but not the neural tube defects; this needs higher levels.
2. Plasma and liver folate levels are considered to be unreliable.
3. N-formino-L-glutamate test has been discredited and abandoned as not having any useful function.
4. Plasma homocysteine has been identified as a very sensitive indicator of folate status.

Important indicators of reduced folate status which are relevant to our situation are:

- **A raised mean corpuscular volume**
- **Hypersegmentation of neutrophils,**
- **Macrocytic anaemia**

Serious Effects of Folate Deficiency:

1) Effect of folate deficiency on the foetus: There is increased risk of neural tube defects, with risk increasing 10-fold as folate status of the mother goes from adequate to poor. Between 21st and 27 th day of conception, the neural plate closes to form what will eventually be the spinal cord and cranium. Their improper closure results, respectively, in spina bifida and anencephaly (collectively called neural tube defects).

There is now conclusive evidence that neural tube defects can be prevented by the ingestion of folic acid near term.

2) Folate and birth-weight: It is possible that maternal folate has some impact on the baby's birth-weight, especially in areas where LBW is widely prevalent (as in India).

3) Folate and Colorectal Cancer: Low folate status has been associated with an increased risk of colorectal cancer. Studies have shown that people who take multivitamin supplements containing folic acid for prolonged periods have a significantly reduced risk of colorectal cancer.

4) CVS and Folate: A raised plasma homocysteine concentration – a very sensitive indicator of folate status - is an independent risk factor for cardiovascular disease and stroke. Even in populations that are apparently normal and consuming diets adequate in folate, there is a range of elevation of plasma homocysteine that could be lowered by an extra 100 or 200 microgram of folic acid.

Table; Recommended Nutrient Intake for Folic acid (expressed as dietary folate equivalents)

Group	Recommended Intake (microg/day)
Infants and Children	80
0-6 months	80
7-12 months	150

1-3 years	200
4-6 years	300
7-9 years	400
Adolescents and Adults	400
10 and above	400
Pregnant Women	600
Lactating women	500

These guidelines are based on the assumption that food is the sole source of folate in the developing world. It must be remembered that bioavailability of dietary folate is only 50%. To be comparable to food folate only half as much folic acid is needed if taken on an empty stomach. However, if synthetic folic acid is taken with food, dose needs to be multiplied by a factor of 1.2.

There is no possibility of folate overdose from dietary sources, but excess intake of folic acid may result in some problems. High levels of folic acid mask the diagnosis of pernicious anemia since they correct anemia allowing the neuropathy to progress to a point where it becomes irreversible. Since the upper limit of safe intake is 1000 mcg, 400 mcg/day of folic acid, over and above the dietary folate, would seem safe.

The intestinal absorption of dietary folates in health and disease

Dietary folates exist as pteroylpolyglutamates (PteGlun) that undergo hydrolysis to pteroylmonoglutamate (PteGlun) forms during the process of intestinal absorption. Using the technique of jejunal perfusion of separately labeled folates, it has been demonstrated that hydrolysis of PteGlun occurs on the surface of the jejunum and is a prerequisite for folate absorption. An intestinal brush border pteroylpolyglutamate hydrolase (BB-PPH) has been identified in

human and pig jejunum with characteristics that are distinct from those of an intracellular hydrolase (IC-PPH). Functional parallels of BB-PPH with in vivo hydrolysis of PteGlun in human and pig intestine and the clinical responsiveness of BB-PPH to different disease states indicate that this enzyme plays the major physiological role in folate absorption. Folate malabsorption is found in diseases which affect the jejunal mucosa and in response to various drugs. In most of these clinical conditions, folate malabsorption results from suppression of both of the processes of hydrolysis of PteGlun and jejunal uptake of PteGlun. Ongoing studies in miniature pigs are aimed at definition of the sequence of development of folate malabsorption in chronic alcoholism.

Sideroblastic Anemia

Sideroblastic anemia is an enzyme disorder in which the body has adequate iron but is unable to incorporate it into hemoglobin. Iron enters the developing red blood cell (erythroblasts); here iron accumulates in the mitochondria giving a ringed appearance to the nucleus (ringed sideroblast). The mitochondria are overloaded with iron and hemoglobin production (heme synthesis) is defective. Sideroblasts are visible with Prussian blue staining and observable under microscopic examination of bone marrow. Because these ringed sideroblasts can develop poorly or not at all into mature red cells, anemia is the consequence.

Sideroblastic anemia (SA) is a complicated disorder and therefore difficult to treat. . Often SA acts like iron deficiency anemia (IDA), but unlike IDA, iron tests are normal or increased with SA.

Three categories of sideroblastic anemia are: hereditary, acquired or idiopathic.

Hereditary Sideroblastic Anemia is due to a genetic defect; the gene is an X-linked recessive (not dominant) gene. It may manifest in both men and women but is seen more commonly in young males, maternal uncles and cousins. Hereditary sideroblastic anemia generally manifests during the first three decades of life especially during adolescence but it has been diagnosed in patients over seventy.

Acquired sideroblastic anemia is due to prolonged exposure to toxins like alcohol, lead, drugs or nutritional imbalances such as deficiency in folic acid, deficiency in copper or excess zinc. Other causes are due to disease such as inflammatory conditions like rheumatoid arthritis, cancerous conditions such as leukemia, lymphoma; kidney disorders causing uremia; endocrine disorders such as hyperthyroidism; metabolic disorders such as porphyria cutanea tarda. Acquired SA is usually seen in patients over 65 year of age but it can be present as early as mid to late fifties.

Idiopathic means the cause is unknown; this category of SA is referred to as myelodysplastic syndrome (MDS). Myelodysplasia is a bone marrow dysfunction disorder which can develop into aplastic anemia requiring bone marrow or stem cell transplantation. Lab findings for acquired/idiopathic sideroblastic anemia: Anemia is usually mild with hemoglobin 10-11.8g/dL Serum iron increased, transferrin iron saturation percentage increased, ferritin increased, transferrin

is decreased. TIBC is normal to decreased; serum transferrin receptor is normal to high Mean Corpuscular Volume (MCV) is normal to slightly increased. Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) are usually normal. Red Cell Distribution Width (RDW) is increased. White blood cells and platelets are decreased.

Symptoms might include enlarged spleen or liver, liver disease, cardiac arrhythmia along with the following specific lab findings:

Treatment depends on the cause; if acquired, remove the offending agent and anemia may disappear. With inflammatory disease such as rheumatoid arthritis, the underlying condition should be treated.

In cases of extreme anemia, whole red blood cell transfusion may be required. Pyridoxine (vitamin B6) 50 to 200mgs taken daily may reverse anemia. Cases of hereditary, acquired and idiopathic anemia have responded to pyridoxine therapy. Pregnant or nursing mothers may wish to limit B6 to 100mg (milligrams) daily, and then resume higher B6 dosage.

Iron overload accompanies sideroblastic anemia. Repeated whole red blood cell transfusion will contribute significantly to this existing iron burden and likely require chelation therapy with desferrioxamine (Desferal).

Desferal (desferrioxamine) is a drug with iron chelating properties. Desferal binds excess body

iron and promotes excretion by the liver and kidneys in urine and bile in feces. It is administered subcutaneously (beneath the skin) by intravenous infusion from a small portable pump.

A patient wears the pump 9-12 hours each day, usually at night while sleeping. The procedure is fairly expensive. Side effects can be varied including pain and swelling at injection site.

Alcohol and certain drugs can be associated with acquired sideroblastic anemia: progesterone like those found in oral contraceptives and in hormone replacement therapy, copper chelating drugs like trientine-used in treatment of Wilson's (excess copper) disease, anti-tuberculosis drugs like isoniazid (type of antibiotic), penicillamine trade name Cuprimine used for extreme arthritic conditions, Wilson's disease and excessive ingestion of zinc.

Leukemia such as acute granulocytic leukemia can develop as a result of acquired sideroblastic anemia. Myelodysplasic syndromes (MDS) are generally observed in the early pre-leukemic stages of disease. More information about MDS or leukemia may be found in the resources section for this topic

The rate of intestinal absorption of natural food folates is not related to the extent of folate conjugation

Background: Evidence is conflicting as to whether the bio-availability of food folates is influenced by the extent of their conjugation.

Objective: The objective was to compare the bioavailability of 3 representative food folate sources with various degrees of glutamylation— egg yolk, spinach, and yeast, whose polyglutamyl folate content measured 0%, 50%, and 100%, respectively.

Design: In a randomized crossover trial, 13 male subjects, after a prestudy folate saturation procedure, received in random order either placebo or 500 μg total folate, which was provided as concentrated freeze-dried extract removed from the normal food matrix of egg yolk, spinach, or yeast. Blood samples ($n = 10$) were collected before and up to 10 h after treatments, which were administered at weekly intervals.

Results: A significant increase from baseline plasma folate concentrations was observed by 0.5 h after treatment with egg yolk folate or spinach folate and by 1 h after treatment with yeast folate, and the concentrations remained significantly elevated for 3–5 h; no plasma folate response was observed after placebo treatment. The overall responses, calculated as plasma folate area under the curve (AUC) for egg yolk, spinach, and yeast folate, were 122.6 ± 23.6, 136.2 ± 21.4, and 102.5 ± 21.1 nmol · h/L, respectively. No significant differences in AUC were seen between monoglutamyl (egg yolk) folate and either of the polyglutamate-containing folates examined.

Conclusion: These results suggest that the ratio of monoglutamate to polyglutamate in natural folates is not a factor that limits the extent of intestinal absorption of food folate.

Key Words: Food folate • polyglutamylation • plasma folate • folate bioavailability

The folate contents of 26 commercial noodle samples were investigated. The impact of ingredients, pH, and cooking on folate content was studied for the 3 predominant styles of noodles: white salted, yellow alkaline, and instant. Some variability was found in the proportion of folate present in the free form and the noodles

generally had low total folate contents. The pH values of the samples covered a wide range, varying from 3.7 to 10.3; however, the results did not provide strong evidence for a relationship between pH and folate content for any of the noodle styles studied. Higher folate levels were typically found in yellow alkaline samples compared to white salted and instant noodles. The storage of noodles in dry or moist forms did not appear to influence total folate contents, and subsequent losses during cooking depended upon the time of exposure to elevated temperatures. The enzymatic treatment of samples was particularly important for cooked noodles, indicating that folates were bound or entrapped during this process.

Folates are currently under intense scrutiny regarding their ability to modulate disease risk, birth defects, CVD/stroke, and possible colon cancer. The objective of this project is to bring together commercial and consumer interests via 7 workpackages which seek to provide folate-rich and enriched foods with specified consumer benefits for optimal bioavailability, function and health. Nutritional scientists, biochemists, clinicians, and food technologists will work together with industry to achieve this objective. Results will include verification of folate efficacy in moderating specific risk factors for chronic disease, quantification of bioavailability of natural folates versus synthetic folic acid added to foods and isolates, and pre-competitive information for development of effective and sustainable dietary strategies to support competitive-edge within the EU food industry, and meet consumer expectations of health benefits.

Objectives

Folic acid significantly reduces the incidence and reoccurrence of neural tube defects (such as Spina bifida) in women. Marginal folate deficiency is also associated with elevated plasma homocysteine, an emerging risk factor for vascular diseases and stroke, and linked to certain cancers, notably colon. Our understanding of the dose-response relationships in these situations is limited and has led to uncertainties over folate requirements for optimal health and

function. Current recommendations suggest that protection from neural tube defects can be achieved through intakes of an extra 400 €g daily of folic acid as supplements, fortified foods or natural food folates. The assumption is that all three routes of administration would have equal effects on folate status.

There is also much debate as to the best means to increase folate intakes in European countries where folic acid fortification is not permitted. Information is required on the relative absorption and utilisation of folates from foods as prepared and delivered to the consumer. The absorption and transport processes of folates from foods are complex and, to large degree, not fully understood. It is not possible to predict bioavailability for a given diet or food, and the influence of food composition and other dietary and physiological variables on folate bioavailability cannot be determined accurately. Understanding factors controlling folate availability is a necessary, pre-competitive step to designing commercial processes, which provide the desired levels of bioavailability and functionality.

There are also concerns as to possible adverse effects, particularly in the elderly, of the high consumption of folic acid from fortified foods, notably masking the diagnosis of vitamin B12 deficiency. Therefore, strategies for increasing the consumption of natural food folates need to be explored, especially, the question as to whether sufficient quantities can be absorbed from these foods to protect against chronic diseases.

Results and achievements

- Development of foods (including improved use of raw materials and optimised food processing techniques) that will enable the diet rich in folates within the range indicated to be protective for human health;
- Verification of the efficacy of folates in moderating specific risk factors for chronic disease;

- Quantification of bioavailability of natural folates versus synthetic folic acid added to foods;
- Pre-competitive information for the development of effective, sustainable, ethically-acceptable dietary strategies for folate-rich foods and folate-enriched products, to support competitive-edge within the European food industry, and meet consumer expectations of health benefits.

Studies on the Uptake of Synthetic Conjugated Folates by Human Marrow Cells

Pteroyltriglutamate shows little ability (between 1% and 5%), compared to pteroylmonoglutamate (folic acid), to enter human marrow cells and to act as a coenzyme in intracellular DNA synthesis. This was shown by comparing the effectiveness of these two forms of the vitamin at stimulating folate-dependent pyrimidine incorporation into DNA in vitro in the bone marrow cells and lymphocytes of patients with megaloblastic anemia. It, therefore, appears that human hemopoietic cells (like *Streptococcus fecalis* rather than *Lactobacillus casei*) are unable to take up efficiently polyglutamyl forms of folate. The suggestion that polyglutamyl analogues of the antifolates might be more effective chemotherapeutic agents than corresponding monoglutamates, while biochemically possible, would appear to be precluded because of failure of transport of these compounds into human hemopoietic cells.

Utilization of yeast polyglutamate folates in man

Ingestion by healthy humans of small amounts of polyglutamate folates from yeast, equivalent to 300 mug of monoglutamate folate and containing 30 mug of "free folate," resulted in an appreciable elevation of the serum folate corresponding to 300 mug of synthetic pteroylmonoglutamate (PGA). Ingestion of higher amounts of polyglutamate folate did not result in higher serum folate elevations than did 300 mug. It is concluded that small amounts of polyglutamate folate from yeast are fully utilized, presumably by

deconjugation in the gut prior to absorption. The relative ineffectiveness of larger doses of polyglutamate folates from yeast may be due to limiting conjugase activity in the gut, unfavorable conditions for its activity (such as unsuitable pH) or to an inhibitor of the enzyme present in impure preparations.

Additional Studies Confirm Folates Role in Preventing Depression

Two new studies provide further evidence that low folate levels may have a role to play in depression. In one study, high folate levels were linked to a greater success rate in treating depressed geriatric patients. In the other study, patients with depression had a more difficult time metabolizing folate.

Researchers undertook the one study because previous findings suggested that lower folate levels are associated with reduced responsiveness to treatment with selective serotonin reuptake inhibitors (SSRI). In addition, depressed geriatric patients have lower levels of folate than controls, and folate supplements have been found to reduce death related to depression.

In the current small, randomized, observational study, researchers with New York University Medical Center gave 12 geriatric patients the SSRI sertraline, while 10 patients received the SSRI nortriptyline for 12 weeks. The researchers measured folate levels at the beginning of the study and after treatment. At the beginning of the study, all patients had folate levels within the normal range. However, patients who had higher folate levels at the beginning of the study achieved greater improvement after treatment.

The reason why folate may help reduce depression is because deficiency of this important B vitamin causes brain levels of mood-boosting serotonin to fall. In addition, low levels of folate triggers a reduction of 5-hydroxyindoleacetic acid, which may contribute to

depression in individuals with a predisposition to depression. Folate also plays a role in the metabolism of S-adenosylmethionine (SAMe), an action which also contributes to proper levels of brain serotonin. The researchers called for additional research to evaluate the role of folate supplements in patients with folate levels in the low normal range.

In the other study, researchers studied folate levels and levels of the amino acid homocysteine in 5,948 subjects aged 46 to 49 years and 70 to 74 years. The study authors found that subjects who had relatively high levels of homocysteine in their blood were almost twice as likely to be depressed, compared to people with the lowest homocysteine blood levels. Depressed subjects also were more likely to have impaired folate metabolism. Homocysteine is an amino acid implicated in heart disease. Folate is known to lower levels of homocysteine. The study authors concluded that folic acid supplements may help prevent depression.

In the middle-aged subjects, low levels of folate also were linked to depression. But even though markers of folate metabolism were altered in depression, actual levels of folate in the blood did not differ between the elderly subjects with and without depression. Researchers suggested that this may be because measuring folate in the blood may not be an accurate indication of how much folate actually resides in cells.

Blood folate levels up in American women: Potential for reduction in birth defects

As the first sign that the nation's effort to fortify foods with folic acid to prevent birth defects is succeeding, the Centers for Disease Control and Prevention announced today new findings showing that folate levels of American women of child-bearing age are on the rise. These increased folate levels, mostly due to food fortification, will potentially reduce women's risk of having a baby born with a birth defect of the spine or brain (spina bifida or anencephaly).

As reported in the October 27 issue of the *Morbidity Mortality Weekly Report*, the CDC's 1999 National Health and Nutrition Examination Survey found that the average level of folic acid in the blood almost tripled from 6.3 to 16.2 nanograms per milliliter and red blood cell folate concentrations, a better measure of long-term folate status, show an average increase from 181 to 315 ng/mL RBC.

Similar increases in folate levels were shown for women who were not pregnant and therefore less likely to use a supplement containing folic acid; for those who were taking a vitamin/mineral supplement; and for those who had not used supplements.

"This is an important step toward reducing the risk of a life-threatening and disabling birth defect in the U.S.," said CDC Director Jeffrey P. Koplan, M.D.. M.P.H.

To prevent neural tube defects, the U.S. Public Health Service recommended in 1992 that women of childbearing age increase daily consumption of the vitamin folic acid. Since then, national efforts have been implemented to increase the use of dietary supplements containing folic acid. In 1996, the U.S. Food and Drug Administration mandated that all enriched cereal grain products be fortified with folic acid by January1998.

Food fortification was determined to be the best strategy for increasing blood folate levels since the critical period for adequate folic acid intake is in the first weeks of pregnancy, before most women know they are pregnant and begin taking prenatal vitamins. Since many pregnancies are unintended or not recognized in the early stages, women are more likely to consume folic acid from food than from supplements.

Dietary folate deficiency with normal red cell folate and circulating blasts

Abstract

This report describes a 26 year old woman, of Pakistani origin, who presented five months postpartum with severe megaloblastic anaemia as a result of nutritional folate deficiency. This case was unusual in that a small number of myeloblasts were present in the peripheral blood at presentation, and this circulating population temporarily increased in size when folate replacement was begun. We also highlight the need to recognise the non-linear relation between haematocrit and red blood cell folate concentration when the haematocrit is very low (< 0.15) and emphasise the importance of the clinical history.

The 20th century saw important and fundamental advances in our understanding of the pathogenesis and treatment of the megaloblastic anaemias. In 1926, Minot and Murphy[1] discovered that pernicious anaemia, a previously fatal disease, could be treated by the ingestion of liver. Vitamin B12, which is the vitamin responsible, was subsequently isolated in 1948.[2,3] Folic acid, originally called Wills's factor, was discovered in 1931 when Dr Lucy Wills,[4] studying nutritional anaemias in India, observed that the macrocytic anaemia of pregnancy was more common in poorer women and was corrected by dietary supplements of yeast or yeast extract (Marmite). Seventy years later, megaloblastic anaemia still holds surprises.

CASE REPORT

Presenting features

A 26 year old woman, originally from Pakistan and living in the UK for two years, presented feeling extremely weak and tired. At the time of presentation she was five months postpartum and continuing to breast feed her healthy son. She was a vegetarian but ate very little. Her main food source was chapattis, which she ate with no accompaniments, and very small amounts of fruit. The patient had been taking ferrous sulfate (200 mg daily) for 10 months because normochromic normocytic anaemia had been noted during pregnancy. No haematinic assays were performed at this time. There was no other relevant past medical history. Physical examination revealed no abnormalities except a tachycardia and extreme pallor.

Investigations included a full blood count, which revealed: haemoglobin (Hb), 28 g/litre; mean cell volume (MCV), 100 fl; no reticulocytes present; white blood cell count (WBC), 1.4×10^9/litre (neutrophils, 0.3×0^9/litre), and platelets 16×10^9/litre. Blood film examination showed gross anisopoikilocytosis, tear drop poikilocytes, and basophilic stippling of erythrocytes. A small population of myeloblasts was identified in the peripheral blood (fig 1A–C). In addition, circulating megaloblasts were easily found (fig 1D), although there was no neutrophil hypersegmentation. The bone marrow aspirate was hypercellular. Erythropoiesis was greatly expanded, left shifted, and megaloblastic. Granulopoiesis was also hyperplastic and giant metamyelocytes were present. There was no excess of myeloblasts. The bone marrow trephine specimen was hypercellular at 95% with loss of fat spaces. The hyperplastic, left shifted erythroid series was again prominent. Occasional giant metamyelocytes were seen and megakaryocytes were normally represented.

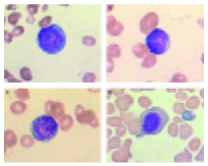


Figure 1
Cells in the peripheral blood (Romanowsky stain). (A–C) Cells with the morphological appearance of myeloblasts and (D) a megaloblast.

Red blood cell folate (RCF) was measured in the normal range at 222 μg/litre (normal range, 160–600). After correction for serum folate the RCF was 200 μg/litre. Serum folate was low at 1.9 μg/litre (normal range, 3.3–13), as was vitamin B12 at 70 ng/litre (normal range, 170–700), although the ferritin concentration was normal. The lactate dehydrogenase (LDH) concentration was very high at 6410 U/litre, with a slightly raised bilirubin at 29 μmol/litre, and reduced haptoglobins. Other liver function tests were normal. Anti-intrinsic factor antibodies and anti-endomysial antibodies were not detected. A Schilling test, undertaken three months after presentation, was normal.

Response to treatment

Given the severity of the pancytopenia, we began vitamin B12 and folate replacement before the haematinic assay results were known, discontinuing the vitamin B12 after three injections, and continuing replacement with folic acid alone for four months. The reticulocyte response was dramatic, reaching a maximum of 608×10^9/litre on the fourth day after treatment. Hypokalaemia has previously been recognised as an important problem in the treatment of megaloblastic anaemia and is an important cause of death in such patients.[5] In our patient, the potassium concentration dipped below normal on day 2 of treatment; potassium supplements were begun and continued for one week. Six weeks after starting treatment, the patient had a normal blood count with: Hb, 124 g/litre; MCV, 87 fl; WBC, 8.9×10^9/litre; and platelets, 371×10^9/litre.

DISCUSSION

The morphological features seen in this case are, in the main, consistent with those of megaloblastic anaemia resulting from

vitamin B12 or folate deficiency. These features have been well described previously.[6] However, we were concerned by the presence of circulating myeloblasts in our patient and the bone marrow aspirate was crucial in demonstrating no excess of blasts in the bone marrow. It is almost 50 years since six similar cases of folate deficiency in the puerperium were described, in which myeloblasts were present in the peripheral blood at diagnosis.[7–9] In three of these patients,[8,9] and in our atient, the leukaemoid reaction became more pronounced in the first few days after starting folate replacement.

In view of the recent pregnancy, ongoing lactation, poor diet, and precipitate presentation, we felt that the probable cause of the megaloblastic anaemia was folate deficiency. Although dietary vitamin B12 deficiency may have played a part, subsequent follow up appeared to support a diagnosis of folate deficiency because the patient continued to follow a strict vegetarian diet and has had no recurrence of vitamin B12 deficiency in the absence of vitamin B12 replacement. Raised LDH and bilirubin, along with reduced haptoglobin values, can be explained by ineffective erythropoiesis and excess intramedullary cell death. These features are well recognised in both severe vitamin B12 and folate deficiency.[6] The low serum folate and serum vitamin B12 values are consistent with folate deficiency. Mollin and colleagues[10] found serum vitamin B12 concentrations of < 100 ng/litre in 15 of 142 patients with folate deficiency. In view of the severe megaloblastosis, we were surprised by the normal RCF results, but believe that these can be explained by limitations of the folate assay system.

"It is clear that life threatening, postpartum nutritional anaemias can occur in a First World country even in the 21st century"

To measure red blood cell and serum folate we use an ion capture assay with fluorimetric quantitation (Axsym; Abbott Laboratories,

Abbot Park, Illinois, USA). Serum folate is assayed along with the total folate in a haemolysate of whole blood. Therefore, the total blood folate so measured equals RCF plus serum folate. The mean red blood cell folate concentration in the cells (RCF) is proportional to total blood folate divided by the haematocrit. The contribution from serum folate is assumed to be insignificant compared with the red blood cell folate contribution.

We believe that the normal red blood cell folate result is spurious and can be explained by limitations in the technique. First, any error or variability in the folate assay will be magnified by the extremely low haematocrit (0.08 in this case). Second, the contribution of serum folate to the total blood folate needs to be considered. Serum folate is assumed to have little impact on red blood cell folate measurements because the folate concentration in red cells is 20–50 times that in serum. In this case, however, the ratio of red blood cells to serum is greatly reduced, so the proportion of serum folate to total folate will be higher, despite the serum folate measurement itself being low. Notably, the red blood cell folate concentration remained in the normal range, in this case, even after correction for serum folate values. Bain and colleagues[11] found a non-linear relation between RCF values and haematocrit, with RCF measurements significantly higher than expected, particularly when the haematocrit was < 0.15, and that this effect persisted even after correction for serum folate values.

CONCLUSIONS

We present a case of severe megaloblastic anaemia resulting from nutritional folate deficiency. We report the unusual feature of circulating blasts in the peripheral blood at presentation and re-emphasise the importance of recognising a pitfall in red cell folate measurement.

Take home messages

- We describe a 26 year old woman who presented five months postpartum with severe megaloblastic anaemia as a result of nutritional folate deficiency
- The case was unusual in that a small number of myeloblasts were present in the peripheral blood at presentation, and this circulating population temporarily increased in size when folate replacement was begun
- This case highlights a pitfall in red cell folate measurement, namely: the non-linear relation between haematocrit and red blood cell folate concentration when the haematocrit is very low (< 0.15)

We also stress the importance of the clinical history rather than over reliance on laboratory tests and the need for close cooperation between clinical teams and laboratory staff.

Our case highlights the importance of the clinical history rather than over reliance on laboratory tests. It also emphasises the ongoing need for close cooperation between clinical teams and laboratory staff. There is no doubt that an understanding of laboratory methods and their limitations is necessary for the useful interpretation of the many investigations that we request daily.

Although our understanding of the anaemias of pregnancy and the puerperium has undoubtedly increased since the descriptions of Sir William Osler in 1919,[12] it is clear that life threatening, postpartum nutritional anaemias can occur in a "First World" country even in the 21st century. It is vital that in these days of ever quickening medical progress, we do not lose sight of the fundamental lessons of the last century.

Abbreviations

- Hb, haemoglobin
- LDH, lactate dehydrogenase
- MCV, mean cell volume

- RCF, red blood cell folate
- WBC, white blood cell count

Folate: What is it?

Folate is a water-soluble B vitamin that occurs naturally in food. Folic acid is the synthetic form of folate that is found in supplements and added to fortified foods [1].

Folate gets its name from the Latin word "folium" for leaf. A key observation of researcher Lucy Wills nearly 70 years ago led to the identification of folate as the nutrient needed to prevent the anemia of pregnancy. Dr. Wills demonstrated that the anemia could be corrected by a yeast extract. Folate was identified as the corrective substance in yeast extract in the late 1930s, and was extracted from spinach leaves in 1941.

Folate helps produce and maintain new cells [2]. This is especially important during periods of rapid cell division and growth such as infancy and pregnancy. Folate is needed to make DNA and RNA, the building blocks of cells. It also helps prevent changes to DNA that may lead to cancer [3]. Both adults and children need folate to make normal red blood cells and prevent anemia [4]. Folate is also essential for the metabolism of homocysteine, and helps maintain normal levels of this amino acid.

What foods provide folate?

Leafy green vegetables (like spinach and turnip greens), fruits (like citrus fruits and juices), and dried beans and peas are all natural sources of folate [5].

In 1996, the Food and Drug Administration (FDA) published regulations requiring the addition of folic acid to enriched breads, cereals, flours, corn meals, pastas, rice, and other grain products [6-9].

Since cereals and grains are widely consumed in the U.S., these products have become a very important contributor of folic acid to the American diet. The following table suggests a variety of dietary sources of folate.

Table 1: Selected Food Sources of Folate and Folic Acid [5]

Food	Micrograms (µg)	% DV^
*Breakfast cereals fortified with 100% of the DV, ¾ cup	400	100
Beef liver, cooked, braised, 3 ounces	185	45
Cowpeas (blackeyes), immature, cooked, boiled, ½ cup	105	25
*Breakfast cereals, fortified with 25% of the DV, ¾ cup	100	25
Spinach, frozen, cooked, boiled, ½ cup	100	25
Great Northern beans, boiled, ½ cup	90	20
Asparagus, boiled, 4 spears	85	20
*Rice, white, long-grain, parboiled, enriched, cooked, ½ cup	65	15
Vegetarian baked beans, canned, 1 cup	60	15
Spinach, raw, 1 cup	60	15
Green peas, frozen, boiled, ½ cup	50	15
Broccoli, chopped, frozen, cooked, ½ cup	50	15
*Egg noodles, cooked, enriched, ½ cup	50	15
Broccoli, raw, 2 spears (each 5 inches long)	45	10
Avocado, raw, all varieties, sliced, ½ cup sliced	45	10
Peanuts, all types, dry roasted, 1 ounce	40	10
Lettuce, Romaine, shredded, ½ cup	40	10

Wheat germ, crude, 2 Tablespoons	40	10
Tomato Juice, canned, 6 ounces	35	10
Orange juice, chilled, includes concentrate, ¾ cup	35	10
Turnip greens, frozen, cooked, boiled, ½ cup	30	8
Orange, all commercial varieties, fresh, 1 small	30	8
*Bread, white, 1 slice	25	6
*Bread, whole wheat, 1 slice	25	6
Egg, whole, raw, fresh, 1 large	25	6
Cantaloupe, raw, ¼ medium	25	6
Papaya, raw, ½ cup cubes	25	6
Banana, raw, 1 medium	20	6

* Items marked with an asterisk (*) are fortified with folic acid as part of the Folate Fortification Program.

^ DV = Daily Value. DVs are reference numbers developed by the Food and Drug Administration (FDA) to help consumers determine if a food contains a lot or a little of a specific nutrient. The DV for folate is 400 micrograms (µg). Most food labels do not list a food's magnesium content. The percent DV (%DV) listed on the table indicates the percentage of the DV provided in one serving. A food providing 5% of the DV or less is a low source while a food that provides 10-19% of the DV is a good source. A food that provides 20% or more of the DV is high in that nutrient. It is important to remember that foods that provide lower percentages of the DV also contribute to a healthful diet. For foods not listed in this table, please refer to the U.S. Department of Agriculture's Nutrient Database Web site: http://www.nal.usda.gov/fnic/cgi-bin/nut_search.pl.

What are the Dietary Reference Intakes for folate?

Recommendations for folate are given in the Dietary Reference Intakes (DRIs) developed by the Institute of Medicine of the National Academy of Sciences [10]. *Dietary Reference Intakes* is the general term for a set of reference values used for planning and assessing nutrient intake for healthy people. Three important types of reference values included in the DRIs are *Recommended Dietary Allowances* (RDA), *Adequate Intakes* (AI), and *Tolerable Upper Intake Levels* (UL). The RDA recommends the average daily intake that is sufficient to meet the nutrient requirements of nearly all (97-98%) healthy individuals in each age and gender group [10]. An AI is set when there is insufficient scientific data available to establish a RDA. AIs meet or exceed the amount needed to maintain a nutritional state of adequacy in nearly all members of a specific age and gender group. The UL, on the other hand, is the maximum daily intake unlikely to result in adverse health effects [10].

The RDAs for folate are expressed in a term called the *Dietary Folate Equivalent*. The Dietary Folate Equivalent (DFE) was developed to help account for the differences in absorption of naturally occurring dietary folate and the more bioavailable synthetic folic acid [10-11]. Table 2 lists the RDAs for folate, expressed in micrograms (µg) of DFE, for children and adults [10].

Table 2: Recommended Dietary Allowances for Folate for Children and Adults [10]

Age (years)	Males and Females (µg/day)	Pregnancy (µg/day)	Lactation (µg/day)
1-3	150	N/A	N/A
4-8	200	N/A	N/A
9-13	300	N/A	N/A
14-18	400	600	500

19+	400	600	500

*1 DFE = 1 µg food folate = 0.6 µg folic acid from supplements and fortified foods

There is insufficient information on folate to establish an RDA for infants. An Adequate Intake (AI) has been established that is based on the amount of folate consumed by healthy infants who are fed breast milk [10]. Table 3 lists the Adequate Intake for folate, in micrograms (µg), for infants.

Table 3: Adequate Intake for folate for infants [10]

Age (months)	Males and Females (µg/day)
0 to 6	65
7 to 12	80

The National Health and Nutrition Examination Survey (NHANES III 1988-94) and the Continuing Survey of Food Intakes by Individuals (1994-96 CSFII) indicated that most individuals surveyed did not consume adequate folate [12-13]. However, the folic acid fortification program, which was initiated in 1998, has increased folic acid content of commonly eaten foods such as cereals and grains, and as a result most diets in the United States (US) now provide recommended amounts of folate equivalents [14].

When can folate deficiency occur?

A deficiency of folate can occur when an increased need for folate is not matched by an increased intake, when dietary folate intake does not meet recommended needs, and when folate excretion increases.

Medications that interfere with the metabolism of folate may also increase the need for this vitamin and risk of deficiency [1,15-19].

Medical conditions that increase the need for folate or result in increased excretion of folate include:

- pregnancy and lactation (breastfeeding)
- alcohol abuse
- malabsorption
- kidney dialysis
- liver disease
- certain anemias

Medications that interfere with folate utilization include:

- anti-convulsant medications (such as dilantin, phenytoin and primidone)
- metformin (sometimes prescribed to control blood sugar in type 2 diabetes)
- sulfasalazine (used to control inflammation associated with Crohn's disease and ulcerative colitis)
- triamterene (a diuretic)
- methotrexate (used for cancer and other diseases such as rheumatoid arthritis)
- barbiturates (used as sedatives)

What are some common signs and symptoms of folate deficiency?

 Folate deficient women who become pregnant are at greater risk of giving birth to low birth weight, premature, and/or infants with neural tube defects.

 In infants and children, folate deficiency can slow overall growth rate.

- In adults, a particular type of anemia can result from long term folate deficiency.

- Other signs of folate deficiency are often subtle. Digestive disorders such as diarrhea, loss of appetite, and weight loss can occur, as can weakness, sore tongue, headaches, heart palpitations, irritability, forgetfulness, and behavioral disorders [1,20]. An elevated level of homocysteine in the blood, a risk factor for cardiovascular disease, also can result from folate deficiency.

Many of these subtle symptoms are general and can also result from a variety of medical conditions other than folate deficiency. It is important to have a physician evaluate these symptoms so that appropriate medical care can be given.

Do women of childbearing age and pregnant women have a special need for folate?

Folic acid is very important for all women who may become pregnant. Adequate folate intake during the periconceptual period, the time just before and just after a woman becomes pregnant, protects against neural tube defects [21]. Neural tube defects result in malformations of the spine (spina bifida), skull, and brain (anencephaly) [10]. The risk of neural tube defects is significantly reduced when supplemental folic acid is consumed in addition to a healthful diet prior to and during the first month following conception [10,22-23]. Since January 1, 1998, when the folate food fortification program took effect, data suggest that there has been a significant reduction in neural tube birth defects [24]. Women who could become pregnant are advised to eat foods fortified with folic acid or take a folic acid supplement in addition to eating folate-rich foods to reduce the risk of some serious birth defects. For this population, researchers recommend a daily intake of 400 µg of synthetic folic acid per day from fortified foods and/or dietary supplements [10].

Who else may need extra folic acid to prevent a deficiency?

People who abuse alcohol, those taking medications that may interfere with the action of folate (including, but not limited to those listed above), individuals diagnosed with anemia from folate deficiency, and those with malabsorption, liver disease, or who are receiving kidney dialysis treatment may benefit from a folic acid supplement.

Folate deficiency has been observed in alcoholics. A 1997 review of the nutritional status of chronic alcoholics found low folate status in more than 50% of those surveyed [25]. Alcohol interferes with the absorption of folate and increases excretion of folate by the kidney. In addition, many people who abuse alcohol have poor quality diets that do not provide the recommended intake of folate [17]. Increasing folate intake through diet, or folic acid intake through fortified foods or supplements, may be beneficial to the health of alcoholics.

Anti-convulsant medications such as dilantin increase the need for folate [26-27]. Anyone taking anti-convulsants and other medications that interfere with the body's ability to use folate should consult with a medical doctor about the need to take a folic acid supplement [28-30].

Anemia is a condition that occurs when there is insufficient hemoglobin in red blood cells to carry enough oxygen to cells and tissues. It can result from a wide variety of medical problems, including folate deficiency. With folate deficiency, your body may make large red blood cells that do not contain adequate hemoglobin, the substance in red blood cells that carries oxygen to your body's cells [4]. Your physician can determine whether an anemia is associated with folate deficiency and whether supplemental folic acid is indicated.

Several medical conditions increase the risk of folic acid deficiency. Liver disease and kidney dialysis increase excretion (loss) of folic acid.

Malabsorption can prevent your body from using folate in food. Medical doctors treating individuals with these disorders will evaluate the need for a folic acid supplement [1].

What are some current issues and controversies about folate?

Folic Acid and Cardiovascular Disease
Cardiovascular disease involves any disorder of the heart and blood vessels that make up the cardiovascular system. Coronary heart disease occurs when blood vessels which supply the heart become clogged or blocked, increasing the risk of a heart attack. Vascular damage can also occur to blood vessels supplying the brain, and can result in a stroke.

Cardiovascular disease is the most common cause of death in industrialized countries such as the US, and is on the rise in developing countries. The National Heart, Lung, and Blood Institute of the National Institutes of Health has identified many risk factors for cardiovascular disease, including an elevated LDL-cholesterol level, high blood pressure, a low HDL-cholesterol level, obesity, and diabetes [31]. In recent years, researchers have identified another risk factor for cardiovascular disease, an elevated homocysteine level. Homocysteine is an amino acid normally found in blood, but elevated levels have been linked with coronary heart disease and stroke [32-44]. Elevated homocysteine levels may impair endothelial vasomotor function, which determines how easily blood flows through blood vessels [45]. High levels of homocysteine also may damage coronary arteries and make it easier for blood clotting cells called platelets to clump together and form a clot, which may lead to a heart attack [38].

A deficiency of folate, vitamin B_{12} or vitamin B_6 may increase blood levels of homocysteine, and folate supplementation has been shown to decrease homocysteine levels and to improve endothelial function [46-48]. At least one study has linked low dietary folate intake with an increased risk of coronary events [49]. The folic acid fortification program in the U. S. has decreased the prevalence of low levels of

folate and high levels of homocysteine in the blood in middle-aged and older adults [50]. Daily consumption of folic-acid fortified breakfast cereal and the use of folic acid supplements has been shown to be an effective strategy for reducing homocysteine concentrations [51].

Evidence supports a role for supplemental folic acid for lowering homocysteine levels, however this does not mean that folic acid supplements will decrease the risk of cardiovascular disease. Clinical intervention trials are underway to determine whether supplementation with folic acid, vitamin B_{12}, and vitamin B_6 can lower risk of coronary heart disease. It is premature to recommend folic acid supplementation for the prevention of heart disease until results of ongoing randomized, controlled clinical trials positively link increased folic acid intake with decreased homocysteine levels AND decreased risk of cardiovascular disease.

Folic Acid and Cancer

Some evidence associates low blood levels of folate with a greater risk of cancer [52]. Folate is involved in the synthesis, repair, and function of DNA, our genetic map, and there is some evidence that a deficiency of folate can cause damage to DNA that may lead to cancer [52]. Several studies have associated diets low in folate with increased risk of breast, pancreatic, and colon cancer [53-54]. Over 88,000 women enrolled in the Nurses' Health Study who were free of cancer in 1980 were followed from 1980 through 1994. Researchers found that women ages 55 to 69 years in this study who took multivitamins containing folic acid for more than 15 years had a markedly lower risk of developing colon cancer [54]. Findings from over 14,000 subjects followed for 20 years suggest that men who do not consume alcohol and whose diets provide the recommended intake of folate are less likely to develop colon cancer [55]. However, associations between diet and disease do not indicate a direct cause. Researchers are continuing to investigate whether enhanced folate intake from foods or folic acid supplements may reduce the risk of cancer. Until results from such clinical trials are available, folic acid

supplements should not be recommended to reduce the risk of cancer.

Folic Acid and Methotrexate for Cancer
Folate is important for cells and tissues that rapidly divide [2]. Cancer cells divide rapidly, and drugs that interfere with folate metabolism are used to treat cancer. Methotrexate is a drug often used to treat cancer because it limits the activity of enzymes that need folate.

Unfortunately, methotrexate can be toxic, producing side effects such as inflammation in the digestive tract that may make it difficult to eat normally [56-58]. Leucovorin is a form of folate that can help "rescue" or reverse the toxic effects of methotrexate [59]. There are many studies underway to determine if folic acid supplements can help control the side effects of methotrexate without decreasing its effectiveness in chemotherapy [60-61]. It is important for anyone receiving methotrexate to follow a medical doctor's advice on the use of folic acid supplements.

Folic Acid and Methotrexate for Non-Cancerous Diseases
Low dose methotrexate is used to treat a wide variety of non-cancerous diseases such as rheumatoid arthritis, lupus, psoriasis, asthma, sarcoidoisis, primary biliary cirrhosis, and inflammatory bowel disease [62]. Low doses of methotrexate can deplete folate stores and cause side effects that are similar to folate deficiency. Both high folate diets and supplemental folic acid may help reduce the toxic side effects of low dose methotrexate without decreasing its effectiveness [63-64]. Anyone taking low dose methotrexate for the health problems listed above should consult with a physician about the need for a folic acid supplement.

Caution About Folic Acid Supplements

Beware of the interaction between vitamin B_{12} and folic acid
Intake of supplemental folic acid should not exceed 1,000 micrograms (µg) per day to prevent folic acid from triggering

symptoms of vitamin B_{12} deficiency [10]. Folic acid supplements can correct the anemia associated with vitamin B_{12} deficiency. Unfortunately, folic acid will not correct changes in the nervous system that result from vitamin B_{12} deficiency. Permanent nerve damage can occur if vitamin B_{12} deficiency is not treated.

It is very important for older adults to be aware of the relationship between folic acid and vitamin B_{12} because they are at greater risk of having a vitamin B_{12} deficiency. If you are 50 years of age or older, ask your physician to check your B_{12} status before you take a supplement that contains folic acid. If you are taking a supplement containing folic acid, read the label to make sure it also contains B_{12} or speak with a physician about the need for a B_{12} supplement.

What is the health risk of too much folic acid?

Folate intake from food is not associated with any health risk. The risk of toxicity from folic acid intake from supplements and/or fortified foods is also low [65]. It is a water soluble vitamin, so any excess intake is usually excreted in urine. There is some evidence that high levels of folic acid can provoke seizures in patients taking anti-convulsant medications [1]. Anyone taking such medications should consult with a medical doctor before taking a folic acid supplement.

The Institute of Medicine has established a tolerable upper intake level (UL) for folate from fortified foods or supplements (i.e. folic acid) for ages one and above. Intakes above this level increase the risk of adverse health effects. In adults, supplemental folic acid should not exceed the UL to prevent folic acid from triggering symptoms of vitamin B_{12} deficiency [10]. It is important to recognize that the UL refers to the amount of synthetic folate (i.e. folic acid) being consumed per day from fortified foods and/or supplements. There is no health risk, and no UL, for natural sources of folate found in food. Table 4 lists the Upper Intake Levels (UL) for folate, in micrograms (µg), for children and adults.

Table 4: Tolerable Upper Intake Levels for Folate for Children and Adults [10]

Age (years)	Males and Females (µg/day)	Pregnancy (µg/day)	Lactation (µg/day)
1-3	300	N/A	N/A
4-8	400	N/A	N/A
9-13	600	N/A	N/A
14-18	800	800	800
19 +	1000	1000	1000

Selecting a healthful diet

As the 2000 *Dietary Guidelines for Americans* states, "Different foods contain different nutrients and other healthful substances. No single food can supply all the nutrients in the amounts you need" [66]. As indicated in Table 1, green leafy vegetables, dried beans and peas, and many other types of vegetables and fruits provide folate. In addition, fortified foods are a major source of folic acid. It is not unusual to find foods such as some ready-to-eat cereals fortified with 100% of the RDA for folate. The variety of fortified foods available has made it easier for women of childbearing age in the US to consume the recommended 400 mcg of folic acid per day from fortified foods and/or supplements [6]. The large numbers of fortified foods on the market, however, also raises the risk of exceeding the UL. This is especially important for anyone at risk of vitamin B_{12} deficiency, which can be triggered by too much folic acid. It is important for anyone who is considering taking a folic acid supplement to first consider whether their diet already includes adequate sources of dietary folate and fortified food sources of folic acid.

Folic acid is a coenzyme necessary for the synthesis of thymidine, which is required for DNA synthesis. The major nutritional source is

vegetables. It is rapidly absorbed in the jejunum and transported from plasma into storage sites (primarily liver). The daily requirement is about 400 ug. There has been considerable interest recently in the importance of folic acid as a nutrient. Much attention has been given to the role of folic acid in the prevention of neural tube defects, as well as to the relationship between low serum folate levels and an increased risk of fatal coronary heart disease. However, the current primary use of folate assays remains the initial investigation of patients with macrocytic anemia (see macrocytosis).

In 1996 the FDA mandated folate fortification of cereals and grains in the USA to reduce the risk of neural tube defects in pregnancy, resulting in a significant decrease in the incidence of folate deficiency. The prevalence of samples with low serum folate levels has decreased significantly over the past two decades as a consequence of food fortification.

Year	**%Folate Deficient**	**% Folate >20 ng/mL**	**Median (ng/mL)**
1986	12.5%	3.6%	5.7
1999	0.7%	9.0%	11.4
2004	0.6%	20%	13.7

Although folate deficiency is now uncommon in the USA, evaluation of folate status is still indicated in a patient with megaloblastic anemia associated with poor nutrition, debilitation, alcoholism or malabsorption.

Two tests have traditionally been used in the evaluation of folate status - serum folate and red cell folate. The rationale for measuring red cell folate has been that it more accurately reflects tissue folate stores than serum folate, since red cells acquire folate when they are produced, and the cellular concentration of folate does not change

during the red cell lifespan. A single folate-rich meal can correct the serum folate in a deficient patient, while a persistently low red cell folate reflects chronically depleted stores. However, a study of folate concentrations in the liver (tissue folate stores) showed that they correlated equally well with serum and red cell folate. There are several significant disadvantages to the red cell folate assay, including a lack of specificity and sensitivity. Red cell folate levels are decreased in up to 63% of patients with vitamin B12 deficiency, and normal levels have been described in a significant number of individuals with documented folate deficiency. Furthermore, the red cell folate assay is more complex and cumbersome to perform than the serum folate assay, resulting in poorer precision and reliability, and increased cost.

Recent studies have evaluated current test ordering practices in the diagnosis of folate deficiency. Several studies have shown good correlation between serum and red cell folate assays. A national pathology benchmarking review in the UK (J Clin Pathol 2003; 56:924-926) performed a 3 year analysis of folate assay ordering practices, and found 3 test-ordering strategies in the 45 laboratories studied - serum folate assay only (42%), red cell folate assay only (45%), and both assays (12.5%). The authors evaluated these approaches by performing a comprehensive literature review. In one study of 1355 samples submitted for folate evaluation, 57 had low red cell folate results, however in only 3 (5%) of these did this finding make a difference to the clinical outcome (that is, serum folate was normal, red cell folate was low, and the patient responded to folate therapy). In other words, red cell folate assay provided no additional clinical information over serum folate in 95% of the patients. The authors concluded that serum folate assay is the most appropriate screening test to detect folic acid deficiency.

For the small proportion of cases (approx. 5%) where serum folate is normal, but there is a high clinical suspicion of folate deficiency, further testing is warranted. Some authorities have suggested that plasma homocysteine is a satisfactory substitute for the problematic red cell folate assay in this situation. An elevation of plasma

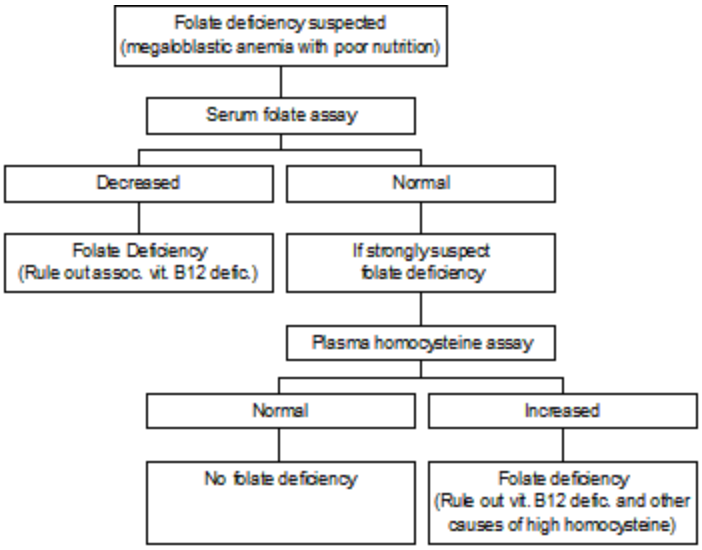
homocysteine is a sensitive and early indicator of functional folic acid deficiency.

Differentiation of Folate & B12 Deficiency

	Serum Folate	**Serum B_{12}**	**HC**	**MMA**
Folate	low	normal-low	high	normal
Vitamin B_{12}	normal-high	low	high	high
Combined	low	low	high	high

HC=homocysteine, MMA=methylmalonic acid

In fact, there is evidence that a rise in plasma homocysteine may precede a decrease below normal in the serum folate level. A normal homocysteine level makes the diagnosis of folate deficiency extremely unlikely. Of course, elevation of homocysteine is not specific for folate deficiency, and is seen in other disorders, including vitamin B12 deficiency (methylmalonic acid is also elevated in this disorder), pyridoxine deficiency, renal failure, and various enzyme defects in the homocysteine metabolic pathway.



In view of the above findings, red cell folate is no longer needs to be performed. In cases of suspected folic acid deficiency, the testing algorithm above is recommended. Specimen requirement for serum folate assay is one SST tube, and for plasma homocysteine is one lavender-top (EDTA) tube. Fasting samples are preferred for both assays.

The low prevalence of folate deficiency calls into question the common practice of simultaneously ordering folate and vitamin B12 assays. Folate deficiency, in the absence of megaloblastic anemia, is now rare in the United States. Even in patients with megaloblastic anemia, there are often clinical indications that would lead to ordering only one of the two tests. For example, a vegetarian is much more likely to have B12 deficiency, while a patient with poor nutrition is more likely to have folate deficiency. Vitamin B12 levels are often ordered during the workup of peripheral neuropathies, but folate levels are not indicated. Routinely ordering both tests not only doubles the laboratory's work, but also increases health care costs.

The most important cause of a falsely elevated red cell folate is a recent blood transfusion, due to the normal folate in the transfused

red cells. A spuriously low red cell folate level may be seen in vitamin B12 deficiency, as described above. Causes of a falsely elevated serum folate level include a recent folate-rich meal, and even slight hemolysis of the sample (since red cell folate is at least 30 times as concentrated as serum folate). A falsely low serum folate level may be seen in the absence of megaloblastosis in patients with recent poor dietary intake, alcoholism, normal pregnancy, and in patients receiving anticonvulsant therapy.

Reference range for serum folate is >3.4 ng/mL.

Specimen requirement for serum folate is one SST tube. Fasting specimens are preferred because recent food intake may increase the serum folate level. The specimen should be protected from light until processing. Hemolyzed specimens cannot be used. The following substances may interfere with folate determination: heparin, ascorbic acid, fluoride, methotrexate, or other folic acid antagonist.

The Food Pyramid

The simplicity of the food guide pyramid is often an oversimplification of necessary nutrient intake. Yes, we often eat too much of the wrong and not enough of the right kinds of foods. Americans are prone to skimping on fruits and vegetables and opting for foods higher in fats and empty calories. On initial observation, it appears that a person need only follow the recommended servings from the five food groups to obtain sufficient nutrient intake. This may be true, but most Americans fall miserably short of those recommended servings and often rearrange the order of the food pyramid (i.e. interchanging meat group with food or vegetable group). Though individuals should strive for a diet consistent with the food pyramid guide, it is not the focus of this article. Of primary importance in this paper is the need for individuals to closely monitor their intake of nutrients and supplement as necessary.

Folic Acid

Most everyone has heard the slogan "Milk, it does the body good" and seen the ads of famous individuals with a milk mustache. The effective advertisement campaign highlights the importance of calcium for strong bone and teeth development and prevention of osteoporosis, brittleness of the bones. A less-well-known, but certainly equally important nutrient is Vitamin B9, also known as folate and folic acid *(click for more information)*. Increased consumption of foods high in folate, carotenes, and Vitamin C combined with physical activity and decreased fat intake, is associated with decreased risk of heart disease, cancer, and stroke *(click for more information)*.

Found in foods such as fruits, green leafy vegetables, beans, orange juice, and rice, one would think these abundant sources would provide adequate folate intake for the recommended daily allowance of 400 micrograms per day for individuals 11 years of age and older. According to data gathered from the NHAMES III study, the mean intake of folate across the lifespan was 275 micrograms. Males had consistently higher intakes across the lifespan with peak intake for males in the 30-39 age group (359 mcg) and the 60-69 age group for females (279 mcg). Different groups of people need varying amounts of folic acid across their lifespan. The remainder of the article will focus on three specific populations at risk when either not enough or too much folic acid is present.

At-Risk population #1: Women of Childbearing Age

Table 16 of the NHANES III study *(click for more information)* revealed women's substantial deficit of recommended folic acid intake. The deficiency is exponentially compounded when a woman becomes pregnant. If she does not begin to immediately supplement her diet with vitamins, she is not only not meeting her own daily intake, she is also not meeting an additional requirement recommended for the developing fetus, possibly leading to devastating consequences.

Birth Defects:

The most infamous effect of folate deficiency is neural tube defects where the infant's brain and spinal cord do not close properly early in fetal development. Neural tube defects are caused by both genetic and environmental contributions. An adequate amount of folate does not preclude having a child born with a neural tube defect, just as a folate deficiency does not ensure the development of one, but studies have proven that a woman drastically increases her chances of having a child born with neural tube defects if folate levels are low (Oakley, Erickson, Levy, Mulinare & Cordero, 1994).

A Wise Warning: Because over half of the pregnancies occurring in the United States are unplanned, many of the expectant mothers are not taking supplemental vitamins and often do not confirm their pregnancy until the 6th or 8th week (Greenwood Genetic Center Factsheet—Issue # 92-1). The tragedy of this situation is that additional folate is needed (again no guarantees, regarding deficient or sufficient pre-pregnancy nutritional level) *very* early to aid in the proper closure of the brain and spine which occurs in the 4th week, when most women are, at most, a few days late for their period.

The consequences are ***Spina Bifida*** (opening along spine—child could have weak or total paralysis of legs and later poor bladder and bowel control); ***Anencephaly*** (partial or no formation of skull—brain damage and imminent premature death); or ***Encephalocele*** (defect or weakening of the skull where baby's brain pushes through defect—most have mental retardation). The mechanism by which folic acid decreases neural tube defects was examined by Lewis, Van Dyke, Stumbo, and Berg (1998) through an effect on methionine-homocysteine metabolism. Concluding that chance of toxicity is minimal, the authors proposed higher levels of cereal grain fortification (200 mcg/100 g of grain) from the proposed 140 mcg/100 g of grain).

Genetic Contribution to Low Folate Levels:

Although the FDA's fortification has already been approved, the National Institute of Health (NIH) recently issued a news alert agreeing with the need for even higher doses of fortification. In addition to taking oral contraceptives, smoking, or drinking decreasing the metabolism of ingested folate, a study of Irish women indicate the presence of a gene mutation that inhibits folate metabolism even more.

The presence of two abnormal recessive genes for 5,10 methylenetetrahydrofolate reductase was associated with lower blood folate levels and higher homocysteine levels (the topic for the third population at risk). These women need even higher intakes of folate for minimal intake levels. The prevalence of individuals with both defective genes ranged from an estimated 5 to 15%, a percentage not to be taken lightly. Although men were obviously not evaluated in this study, the maintenance of low homocysteine levels is important across gender, race and age.

At Risk Population #2: Everyone who may be at risk for Cardiovascular Disease:

Many meta-analyses of many clinical trials have been performed (Boushey, Beresford, Omenn, & Motulsky, 1995; Brattstrom, Isreelsson, Jeppsson, & Hultberg, 1998; Jacobsen, 1998; Robinson, Arheart, Refsum, et. al, 1998; Schorah, Devitt, Lucock, & Dowell, 1998; Swain & St.Clair, 1997;) all coming to similar conclusions—***to lower the homocysteine level of an individual, increase the amount of folate***. Even without longitudinal follow-up outcome studies available, Abby, Harris, and Harris (1998) endorse a daily multivitamin high enough in folic acid to ensure 400 mcg/day for everyone. A word of caution regarding the unpredictableness of serum homocysteine levels intra-individually raises the need for appropriate multiple baseline measures to ensure that an accurate difference score can be obtained for evaluating the effectiveness of

folate supplementation (Santhosh-Kumar, Deutsch, Ryder, Kolhouse, 1997).

Effects in Healthy and Impaired Populations:

In healthy, young women with normal homocysteine levels, Dierkes, Kroesen, and Pietrzik (1998), found that folic acid supplementation decreased homocysteine levels by 11.5%. Similar effects were seen for young patients with arteriosclerosis and hyperhomocysteinemia (Van den Berg, Granken, Boers, Blom, et. al, 1994), for chronic renal failure patients (Chauveau, Chadefauz, Coude, Aupetit, Kamoun, & Jungers, 1996), and for patients who have recently suffered an acute myocardial infarction (Landgre, Israelsson, Lindgren, Hultberg, Anderson, and Brattstrom, 1995). The high degree of similar outcomes across stratified populations coupled with folic acid's inexpensive status demand a closer look at the suggested Recommended Daily Allowance.

At Risk Population #3: The Elderly

As seen in number two, multiple clinical trials have proven a strong inverse relationship between folate and homocysteine concentrations in various-aged samples. The same inverse relationship was also demonstrated for individuals in the original Framingham study aged 67 to 96 years (Selhum, Wilson, Rush, Rosenberg, 1993).

Postmenopausal Women:

In a study by Jacob et. al (1998),postmenopausal women were housed in a metabolic unit for 12 weeks and fed diets of 56 mcg/day of folate for five weeks, 111 mcg/day for four weeks, and 286-516 mcg/day for the final three weeks of the study. During the course of

the fist nine weeks of the study, the women's baseline homocysteine levels were significantly elevated. However, during the final three weeks higher concentrations of homocysteine (516 vs. 286 mcg/day diet) decreased the elevated levels of homocysteine, making an argument for higher folate RDA's than current standards to maintain a low plasma homocysteine level.

Arterial Disease:

Aronow and Ahn (1998) used stepwise multiple regression to determine both high plasma homocysteine values and low plasma folate levels to be significant risk factors for peripheral arterial disease. After adjusting for age, sex, smoking status, HDL concentration, systolic blood pressure, and other risk factors, Selhub et. al (1996) found the same inverse relationship of folate and homocysteine concentrations for associations with extracranial carotid stenosis. These are two very important reasons to monitor folate intake and supplement as necessary with the evident benefit of decreasing risks for heart disease in the older population.

Can Elderly Persons get too much Folate and, if so, what are the Consequences?

Is there a level where too much of a good thing becomes bad? The answer is "Yes", but in unusual circumstances. Tucker, Mahnken, Wilson, Jacques, and Selhub (1996) raise an important caution when prescribing increased intake levels of folate. The risk of Vitamin B12 deficiency from increased exposure to folate can mask or precipitate clinical symptoms similar to dementia. Going undiagnosed, the results can be devastating to the person. As always, nutritional intake should be suspect and properly evaluated before psychiatric intervention is prescribed. Even with this risk, the authors endorsed cereal grain fortification due to the greater benefits reaped from lowering homocysteine levels in the elderly population.

Brattstrom (1996) recommended supplementation for the elderly to include folic acid and cyanocobalamin to maintain low levels of homocysteine while preventing Vitamin B12 deficiency.

Hyperhomocystinaemia is common in the psychogeriatric population (Milsson, et. al, 1996). It was shown that significantly higher homocysteine concentrations were found in patients of dementia of a vascular cause or a history of occlusive arterial disease. Even after controlling for history of vascular disease, Riggs, Spiro, Tucker and Rush (1996) used a battery of cognitive tests for an older male sample, and found low concentrations of Vitamin B12 and folate along with high concentrations of homocysteine to be associated with poorer performance of spatial copying skills making a stronger argument for folate fortification.

Conclusions:

So, Are we getting enough Folate ? For most individuals the answer is a resounding "Of Course Not!" For those select individuals who may be at increased risk for Vitamin B12 deficiency, the primary care physician should always monitor vitamin and mineral levels to rule out a deficiency.

The Take Home Messages from these Populations at Risk are consistent and clear:

- If you are a woman of childbearing age, TAKE YOUR VITAMINS!!

- Higher Folate Levels are strongly inversely associated with Lower Homocysteine Levels.

- High Homocysteine levels are positively associated with vascular disease which could lead to development of CHD.

 Elderly Individuals should maintain similar folate intakes, but special care should be taken to monitor vitamin levels for possible B12 deficiency.

Folic acid

From Wikipedia, the free encyclopedia

Jump to: navigation, search

Folic acid	
IUPAC name	N-[4(2-Amino-4-hydroxy pteridin-6-ylmethylamino) benzoyl]-L(+)-glutamic acid.
Other names	pteroyl-L-glutamic acid; Vitamin B_9, Vitamin M; Folacin
Identifiers	
CAS number	59-30-3
RTECS number	LP5425000
SMILES	C1=CC(=CC=C1C(=O)NC (CCC(=O)O)C(=O)O) NCC2=CN=C3C(=N2) C(=O)N=C(N3)N
Properties	
Molecular formula	$C_{19}H_{19}N_7O_6$
Molar mass	441.403 g/mol
Appearance	yellow-orange crystalline powder
Melting point	250 °C (523 K), decomp.

Solubility in water	8.5 g/100 ml (20 °C)
Acidity (pK_a)	1^{st}: 2.3, 2^{nd}: 8.3
Hazards	
Main hazards	non-toxic, non-flammable
Except where noted otherwise, data are given for materials in their standard state (at 25 °C, 100 kPa) Infobox disclaimer and references	

Folic acid and **folate** (the anion form) are forms of the water-soluble Vitamin B_9. These occur naturally in food and can also be taken as supplements. Folate gets its name from the Latin word *folium* ("leaf").

Contents

[hide]

[edit] Folate in foods

Leafy vegetables such as spinach and turnip greens, dried beans and peas, fortified cereal products, sunflower seeds and certain other fruits and vegetables are rich sources of folate. Some breakfast cereals (ready-to-eat and others) are fortified with 25% to 100% of the recommended dietary allowance (RDA) for folic acid. A table of selected food sources of folate and folic acid can be found at the USDA National Nutrient Database for Standard Reference.

[edit] History

A key observation by researcher Lucy Wills in 1931 led to the identification of folate as the nutrient needed to prevent anemia during pregnancy. Dr. Wills demonstrated that anemia could be reversed with brewer's yeast. Folate was identified as the corrective substance in brewer's yeast in the late 1930s and was extracted from spinach leaves in 1941. It was first synthesised in 1946 by Yellapragada Subbarao.

[edit] Biological roles

Folate is necessary for the production and maintenance of new cells.[1] This is especially important during periods of rapid cell division and growth such as infancy and pregnancy. Folate is needed to replicate DNA. Thus folate deficiency hinders DNA synthesis and

cell division, affecting most clinically the bone marrow, a site of rapid cell turnover. Because RNA and protein synthesis are not hindered, large red blood cells called megaloblasts are produced, resulting in megaloblastic anemia.[2] Both adults and children need folate to make normal red blood cells and prevent anemia.[3]

[edit] Biochemistry

In the form of a series of tetrahydrofolate compounds, folate derivatives are substrates in a number of single-carbon-transfer reactions, and also are involved in the synthesis of dTMP (2'-deoxythymidine-5'-phosphate) from dUMP (2'-deoxyuridine-5'-phosphate). It helps convert vitamin B12 to one of its coenzyme forms and helps synthesize the DNA required for all rapidly growing cells.

The pathway leading to the formation of tetrahydrofolate (FH_4) begins when folate (F) is reduced to dihydrofolate (FH_2), which is then reduced to tetrahydrofolate (FH_4). Dihydrofolate reductase catalyses both steps.[4]

Methylene tetrahydrofolate (CH_2FH_4) is formed from tetrahydrofolate by the addition of methylene groups from one of three carbon donors: formaldehyde, serine, or glycine. Methyl tetrahydrofolate (CH_3–FH_4) can be made from methylene tetrahydrofolate by reduction of the methylene group; formyl tetrahydrofolate (CHO-FH_4, folinic acid) results from oxidation of methylene tetrahydrofolate.

In other words:

$F \rightarrow FH_2 \rightarrow FH_4 \rightarrow CH_2{=}FH_4 \rightarrow$ 1-carbon chemistry

A number of drugs interfere with the biosynthesis of folic acid and tetrahydrofolate. Among them are the dihydrofolate reductase inhibitors (such as trimethoprim and pyrimethamine), the sulfonamides (competitive inhibitors of para-aminobenzoic acid in

the reactions of dihydropteroate synthetase), and the anticancer drug methotrexate (inhibits both folate reductase and dihydrofolate reductase).

1998 RDAs for Folate			
Men	**Women**		
(19+)	**(19+)**	**Pregnancy**	**Breast feeding**
400 μg	400 μg	600 μg	500 μg
1 μg of food folate = 0.6 μg folic acid from supplements and fortified foods			

The National Health and Nutrition Examination Survey (NHANES III 1988-91) and the Continuing Survey of Food Intakes by Individuals (1994-96 CSFII) indicated that most adults did not consume adequate folate.[5][6] However, the folic acid fortification program in the United States has increased folic acid content of commonly eaten foods such as cereals and grains, and as a result diets of most adults now provide recommended amounts of folate equivalents.[7]

[edit] Folate deficiency

See Folate deficiency

[edit] Pregnancy

Folic acid is very important for all women who may become pregnant. Adequate folate intake during the periconceptional period, the time just before and just after a woman becomes pregnant, helps protect against a number of congenital malformations including neural tube defects.[8] Neural tube defects result in malformations of the spine (spina bifida), skull, and brain (anencephaly). The risk of neural tube defects is significantly reduced when supplemental folic acid is consumed in addition to a healthy diet prior to and during the first month following conception.[9][10] Women who could become pregnant are advised to eat foods fortified with folic acid or take

supplements in addition to eating folate-rich foods to reduce the risk of some serious birth defects. Taking 400 micrograms of synthetic folic acid daily from fortified foods and/or supplements has been suggested. The Recommended Dietary Allowance (RDA) for folate equivalents for pregnant women is 600-800 micrograms, twice the normal RDA of 400 micrograms for women who are not pregnant.[11]

[edit] Folic acid supplements and masking of B_{12} deficiency

There has been concern about the interaction between vitamin B_{12} and folic acid.[12]Folic acid supplements can correct the anemia associated with vitamin B_{12} deficiency. Unfortunately, folic acid will not correct changes in the nervous system that result from vitamin B_{12} deficiency. Permanent nerve damage could theoretically occur if vitamin B_{12} deficiency is not treated. Therefore, intake of supplemental folic acid should not exceed 1000 micrograms (1000 mcg or 1 mg) per day to prevent folic acid from masking symptoms of vitamin B_{12} deficiency. In fact, to date the evidence that such masking actually occurs is scarce, and there is no evidence that folic acid fortification in Canada or the US has increased the prevalence of vitamin B_{12} deficiency or its consequences.[13]

However one recent study has demonstrated that high folic or folate levels when combined with low B_{12} levels are associated with significant cognitive impairment among the elderly.[14] If the observed relationship for seniors between folic acid intake, B_{12} levels, and cognitive impairment is replicated and confirmed, this is likely to re-open the debate on folic acid fortification in food, even though public health policies tend generally to support the developmental needs of infants and children over slight risks to other population groups.

In any case, it is important for older adults to be aware of the relationship between folic acid and vitamin B_{12} because they are at greater risk of having a vitamin B_{12} deficiency. If you are 50 years of

age or older, ask your physician to check your B_{12} status before you take a supplement that contains folic acid.

[edit] Health risk of too much folic acid

The risk of toxicity from folic acid is low.[15] The Institute of Medicine has established a tolerable upper intake level (UL) for folate of 1 mg for adult men and women, and a UL of 800 μg for pregnant and lactating (breast-feeding) women less than 18 years of age. Supplemental folic acid should not exceed the UL to prevent folic acid from masking symptoms of vitamin B_{12} deficiency.[16]

Research suggests high levels of folic acid can interfere with some antimalarial treatments.[17]

A 10000-patient study at Tufts University in 2007 concluded that excess folic acid worsens the effects of B12 deficiency and in fact may affect the absorption of B12. [18]

[edit] Some current issues and controversies about folate

[edit] Dietary fortification of folic acid

Since the discovery of the link between insufficient folic acid and neural tube defects (NTDs), governments and health organisations worldwide have made recommendations concerning folic acid *supplementation* for women intending to become pregnant. For example, the United States Public Health Service (see External links) recommends an extra 0.4 mg/day, which can be taken as a pill. However, many researchers believe that supplementation in this way can never work effectively enough since about half of all pregnancies in the U.S. are unplanned and not all women will comply with the recommendation.

This has led to the introduction in many countries of *fortification*, where folic acid is added to flour with the intention of everyone benefiting from the associated rise in blood folate levels. This is

controversial, with issues having been raised concerning individual liberty, and the masking effect of folate fortification on pernicious anaemia (vitamin B_{12} deficiency). However, most North and South American countries now fortify their flour, along with a number of Middle Eastern countries and Indonesia. Mongolia and a number of ex-Soviet republics are amongst those having widespread voluntary fortification; about five more countries (including Morocco, the first African country) have agreed but not yet implemented fortification. In the UK the Food Standards Agency has recommended fortification.[19][20][21] To date, no EU country has yet mandated fortification.[22] Australia is considering fortification, but a period for comments ending 2006-07-31 attracted strong opposition from industry as well as academia.[23]

Recent debate has emerged in the United Kingdom[24] and Australia[25] regarding the inclusion of folic acid in products such as bread and flour.

In the USA many grain products are fortified with folic acid.

In 1996, the United States Food and Drug Administration (FDA) published regulations requiring the addition of folic acid to enriched

breads, cereals, flours, corn meals, pastas, rice, and other grain products.[26][27] This ruling took effect 1998-01-01, and was specifically targeted to reduce the risk of neural tube birth defects in newborns.[28] There are concerns that the amount of folate added is insufficient[3]. In October 2006, the Australian press claimed that U.S. regulations requiring fortification of grain products were being interpreted as disallowing fortification in non-grain products, specifically Vegemite (an Australian yeast extract containing folate). The FDA later said the report was inaccurate, and no ban or other action was being taken against Vegemite.[4]

Since the folic acid fortification program took effect, fortified foods have become a major source of folic acid in the American diet. The Centers for Disease Control and Prevention in Atlanta, Georgia used data from 23 birth defect registries that cover about half of United States births and extrapolated their findings to the rest of the country. This data indicates that since the addition of folic acid in grain-based foods as mandated by the Food and Drug Administration, the rate of neural tube defects dropped by 25% in the United States.[29]

Although folic acid does reduce the risk of birth defects, it is only one part of the picture and should not be considered a cure. Even women taking daily folic acid supplements have been known to have children with neural tube defects.

Heart disease

Adequate concentrations of folate, vitamin B_{12}, or vitamin B_6 may decrease the circulating level of homocysteine, an amino acid normally found in blood. There is evidence that an elevated homocysteine level is an independent risk factor for heart disease and stroke.[30] The evidence suggests that high levels of homocysteine may damage coronary arteries or make it easier for blood clotting cells called platelets to clump together and form a clot.[31] However, there is currently no evidence available to suggest that lowering homocysteine with vitamins will reduce your risk of heart disease.

Clinical intervention trials are needed to determine whether supplementation with folic acid, vitamin B_{12} or vitamin B_6 can lower your risk of developing coronary heart disease. The NORVIT trial suggests that folic acid supplementation may do more harm than good.[32]

As of 2006, studies have shown that giving folic acid to reduce levels of homocysteine does not result in clinical benefit. One of these studies suggests that folic acid in combination with B_{12} may even increase some cardiovascular risks.[33][34][35]

[edit] Stroke

Folic acid appears to reduce the risk of stroke. The reviews indicate only that in some individuals the risk of stroke appears to be reduced, but a definite recommendation regarding supplementation beyond the current recommended daily allowance has not been established for stroke prevention.[36]

[edit] Cancer

The association between folate and cancer appears to be complex.[37] It has been suggested that folate may help prevent cancer, as it is involved in the synthesis, repair, and functioning of DNA, our genetic map, and a deficiency of folate may result in damage to DNA that may lead to cancer.[38] Conversely, it has been suggested that excess folate may promote tumor initiation.[39] Although diets high in folate are associated with decreased risk of colorectal cancer, the association is stronger for folate from foods alone than for folate from foods and supplements,[40] and a 2007 randomized clinical trial found that folate supplements did not reduce the risk of colorectal adenomas.[41] A 2006 prospective study of 81,922 Swedish adults found that diets high in folate from foods, but not from supplements, were associated with a reduced risk of pancreatic cancer.[42] Most epidemiologic studies suggest that diets high in folate are associated with decreased risk of breast cancer, but results are not uniformly consistent: one large cancer screening trial reported a potential

harmful effect of high folate intake on breast cancer risk, suggesting that routine folate supplementation should not be recommended as a breast cancer preventive,[43] but a 2007 Swedish prospective study found that a high folate intake was associated with a lower incidence of postmenopausal breast cancer.[44]

[edit] Antifolates

Folate is important for cells and tissues that rapidly divide.[1] Cancer cells divide rapidly, and drugs that interfere with folate metabolism are used to treat cancer. The antifolate methotrexate is a drug often used to treat cancer because it inhibits the production of the active form, tetrahydrofolate. Unfortunately, methotrexate can be toxic,[45][46][47] producing side effects such as inflammation in the digestive tract that make it difficult to eat normally.

Folinic acid is a form of folate that can help "rescue" or reverse the toxic effects of methotrexate.[48] Folinic acid is *not* the same as folic acid. Folic acid supplements have little established role in cancer chemotherapy.[49][50] There have been cases of severe adverse effects of accidental substitution of folic acid for folinic acid in patients receiving methotrexate cancer chemotherapy. It is important for anyone receiving methotrexate to follow medical advice on the use of folic or folinic acid supplements.

Low dose methotrexate is used to treat a wide variety of non-cancerous diseases such as rheumatoid arthritis, lupus, psoriasis, asthma, sarcoidoisis, primary biliary cirrhosis, and inflammatory bowel disease.[51] Low doses of methotrexate can deplete folate stores and cause side effects that are similar to folate deficiency. Both high folate diets and supplemental folic acid may help reduce the toxic side effects of low dose methotrexate without decreasing its effectiveness.[52][53] Anyone taking low dose methotrexate for the health problems listed above should consult with a physician about the need for a folic acid supplement.

[edit] Depression

Some evidence links low levels of folate with depression.[54] There is some limited evidence from randomised controlled trials that using folic acid in addition to antidepressant medication may have benefits.[55] Researchers at the University of York and Hull York Medical School have confirmed a link between depression and low levels of folate in a research study involving 15,315 .[56] However, the evidence is probably too limited at present for this to be a routine treatment recommendation.

[edit] Memory and mental agility

In a 3-year trial on 818 people over the age of 50, short-term memory, mental agility and verbal fluency were all found to be better among people who took 800 micrograms of folic acid daily—twice the current RDA—than those who took placebo. The study was reported in *The Lancet* on 19 January 2007.[57]

[edit] Fertility

Folate is necessary for fertility in both men and women. In men, it contributes to spermatogenesis. In women, on the other hand, it contributes to oocyte maturation, implantation, placentation, in addition to the general effects of folic acid and pregnancy. Therefore, it is necessary to receive sufficient amounts through the diet, in order to avoid subfertility.[58]

[edit] Induction of Acute Renal Failure

Folic acid is used in extremely high doses to induce Acute renal failure in murine models. It should be noted that the dose reported below represents about 120 years of the recommended daily intake [0.4 mg for adults] in one application, an experiment irrelevant to human nutrition. The exact method through which folic acid induces kidney injury in such massive dose is unknown, however it is characterized by the appearance of folic acid crystals in renal tubules and acute tubular necrosis. This method of renal injury is also linked to increased expression of Tumor necrosis factor-alpha. The dose of

folic acid used to induce renal injury is usually around 250mg of folic acid per kg of body weight. The folic acid is usually administered in a vehicle of 0.3mmol/L of sodium bicarbonate.[59]

High folate, vitamin B-6 levels may improve woman's chances of preventing breast cancer:

Women with highest recorded folate levels 27 percent less likely to develop breast cancer

Building on preliminary data, researchers at Harvard-affiliated Brigham and Women's Hospital (BWH) have documented that high folate (vitamin B-9) and vitamin B-6 levels may improve a woman's chances of preventing breast cancer. Additionally, researchers observed that adequate folate levels may be particularly important for women who are at higher risk of breast cancer due to higher alcohol consumption. The new findings are the latest results from the landmark BWH-based Nurses' Health Study, and appear in the March 5 issue of The Journal of the National Cancer Institute.

"The benefits of folic acid in reducing birth defects and cardiovascular disease have been well established; however, its protective impact on breast cancer has been less clear," said Shumin Zhang of BWH and the School of Public Health. "The findings from this large study suggest more precisely that by ensuring adequate levels of folate and vitamin B-6 - by consuming foods rich in these nutrients and taking vitamin supplements - a woman's risk of breast cancer may be reduced."

The researchers found that women with the highest recorded folate levels in their blood appeared to be 27 percent less likely to develop breast cancer, compared to women with the lowest folate levels. This association between folate and breast cancer risk was strongest among women who consumed moderate amounts of alcohol - a group already at greater risk for the disease. When plasma folate and alcohol intake were examined in combination, higher alcohol intake only appeared to increase risk of breast cancer in women with low

folate levels. The research team observed that for women consuming less than one glass of alcohol a day, high levels of folate eliminated their increased risk of breast cancer by 28 percent. An inverse association between vitamin B-6 levels and breast cancer was also observed; however, it appeared most significant in postmenopausal women.

Folate and vitamin B-6 are found naturally in foods such as oranges and leafy green vegetables. Breakfast cereals and wheat flour have been fortified with folic acid (the synthetic form of folate) since 1998, following a mandate by the Food and Drug Administration to increase vitamin B levels in the average diet. Though still not fully understood, scientists hypothesize that folates role in DNA construction may explain why it helps stave off disease and is instrumental in reducing some birth defects by up to 70 percent.

"The prospect that folate and vitamin B-6 may have the potential to be protective against breast cancer is encouraging as we look toward more ways to proactively fight disease onset," said Zhang, also of Harvard Medical School. "Women may want to monitor their intake of these nutrients more closely, especially those who consume moderate amounts of alcohol, where it appears high folate levels play a role in offsetting their increased risk of breast cancer."

These findings were based on analysis of 712 breast cancer patients and 712 control subjects selected from a pool of 32,826 women enrolled in the Nurses' Health Study who provided blood samples at the onset of the study. The subjects were followed for six years, during which individual health and diet information was collected.

Higher folate levels linked to faster mental decline in elderly

People who consume a lot of folate, or take large doses of folic acid in supplement form, may have a faster rate of mental decline when older, say researchers on a large population study.

The findings are unexpected as folate, a B vitamin, helps break down the amino acid homocysteine, high levels of which are linked to Alzheimer's disease.

High levels of homocysteine have also been linked to an increased risk of stroke.

The study measured cognitive decline in more than 3,700 elderly people living in Chicago, aged at least 65 years-old at baseline. They were followed up after three years, and again after six years, using the average score of four different cognition tests. Folate intake was assessed using a food frequency questionnaire.

Those with the highest folate intake, an average 742mcg per day, had more than twice the rate of cognitive decline as those in the lowest fifth of intake (186mcg per day), said the researchers.

"A faster rate of cognitive decline was also associated with high folate intake from food and with folate vitamin supplementation of more than 400mcg daily compared with nonusers," write the researchers in this month's issue of the *Archives of Neurology* (vol 62, pp641-645).

The findings could add fuel to the European debate over fortifying food with the vitamin to reduce incidence of neural tube defects. In the US, where the research was carried out, flour has been fortified with folic acid since 1998, increasing intake of the vitamin across all segments of the population.

The UK opted not to introduce such a policy, for fear of masking B12 deficiency in the elderly. The new study could offer some signs of this effect.

However Paul Finglas, senior research scientist at the Institute for Food Research, told NutraIngredients.com that the study data was *"prone to large error"*.

"They've assessed the folate intake using a food frequency questionnaire, which could underestimate or overestimate folate intake by as much as 50 per cent," he said.

A blood sample would have helped to validate the information gathered by the questionnaire and could also assess folate status over the long-term, he added, giving the hypothesis better support.

"The study does not show that high folate intake leads to faster cognitive decline and I don't think evidence for the association is particularly strong," added Finglas, also co-ordinator for the European Union-funded research project on folate, FolateFuncHealth.

The researchers also tested for the impact of vitamin B12 and found that high intake of the vitamin was only associated with slower mental decline among the oldest study participants.

Predictors of red cell folate level in women attempting pregnancy

To identify predictors of red cell folate level in women attempting to become pregnant. DESIGN: Cohort study. SETTING: A health maintenance organization serving the Minneapolis-St Paul, Minn, area. PARTICIPANTS: A total of 189 healthy, primarily white women aged 22 to 35 years enrolled in the Diana Project, a population-based prospective study of preconceptional and prenatal risks to reproductive outcomes. The sample represents 189 of 219 enrolled women who were sequentially selected from the total Diana Project sample to receive additional laboratory analyses. MAIN OUTCOME MEASURE: Red cell folate level. RESULTS: Folic acid supplements, folic acid intake from fortified cereals, vitamin C supplements, and serum zinc level (inverse) were found to predict red cell folate levels. Previous research has shown that red cell folate levels higher than 906 nmol/L (400 ng/mL) may be optimal for the prevention of folate-responsive neural tube defects. For folic acid supplement users, folate intakes of 450 microg per day and higher corresponded to these protective levels of red cell folate. In nonusers

of supplements, intakes of more than 500 microg of folate per day from foods and folic acid-fortified cereals may be needed to attain red cell folate levels higher than 906 nmol/L (400 ng/mL). Red cell folate levels higher than 906 nmol/L (400 ng/mL) were primarily found in women who took folic acid supplements. Only 1 in 4 women had red cell folate levels higher than 906 nmol/L (400 ng/mL), while 1 in 8 had red cell folate levels indicative of a negative folate balance. Addition of a daily, 400-microg folic acid supplement to the usual diet would result in red cell folate levels over 906 nmol/L (400 ng/mL) in a majority of women in this study. CONCLUSIONS: Supplementation of diets of women of childbearing potential with 400 microg of folic acid per day would effectively raise red cell folate to levels associated with a low risk of folate-responsive neural tube defects. Protective levels of red cell folate may also be obtained by ample consumption of vegetables, fruits, and folic acid-fortified breakfast cereals. Efforts to increase folic acid supplement use and folate consumption among women of childbearing potential must go beyond fortification of refined cereal and grain products and reach women within all educational and income groups.

Low Serum and Red Blood Cell Folate Are Moderately, but Nonsignificantly Associated with Increased Risk of Invasive Cervical Cancer in U.S. Women

Stephanie J. Weinstein, Regina G. Ziegler[1], Edward A. Frongillo, Jr.[*], Neville Colman[†], Howerde E. Sauberlich[**],

Louise A. Brinton, Richard F. Hamman , Robert S. Levine[††],

Katherine Mallin , Paul D. Stolley[#] and Carole A. Bisogni[*]

Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD 20892;[*] Division of Nutritional Sciences, Cornell University, Ithaca, NY 14853;[†]Department of Pathology and Laboratory Medicine, St.

Luke's-Roosevelt Hospital Center and Columbia University, New York, NY 10025;[**] Department of Nutrition Sciences, University of Alabama, Birmingham, AL 35294; Department of Preventive Medicine and Biometrics, University of Colorado School of Medicine, Denver, CO 80262;[##] Meharry Medical College, School of Medicine, Occupational and Preventive Medicine, Nashville, TN 37208; Division of Epidemiology and Biostatistics, School of Public Health, University of Illinois at Chicago, Chicago, IL 60612; and[#] Department of Epidemiology and Preventive Medicine, University of Maryland School of Medicine, Baltimore, MD 21201

[1]To whom correspondence should be addressed. E-mail: zieglerr@mail.nih.gov .

ABSTRACT

Previous observational epidemiologic studies of folate and cervical cancer, as well as folate supplementation trials for cervical dysplasia, have produced mixed results. We examined the relationship between serum and RBC folate and incident invasive cervical cancer in a large, multicenter, community-based case-control study. Detailed in-person interviews were conducted, and blood was drawn at least 6 mo after completion of cancer treatment from 51% of cases and 68% of

controls who were interviewed. Blood folate was measured with both microbiologic and radiobinding assays. Included in the final analyses were 183 cases and 540 controls. Logistic regression was used to control for all accepted risk factors, including age, sexual behavior, smoking, oral contraceptive use, Papanicolaou smear history and human papillomavirus (HPV)-16 serology. For all four folate measures, the geometric mean in cases was lower than in controls (e.g., 11.6 vs. 13.0 nmol/L, $P < 0.01$ for the serum radiobinding assay). Folate measures using microbiologic and radiobinding assays were correlated (serum: $r = 0.90$; RBC: $r = 0.77$). For serum folate, multivariate-adjusted odds ratios (OR) in the lowest vs. highest quartile were 1.3 [95% confidence interval (CI) = 0.8–2.9] and 1.6 (0.9–2.9), using the microbiologic and radiobinding assays, respectively. For RBC folate, comparable OR were 1.2 (0.6–2.2) and 1.5 (0.8–2.7). Similar risks were obtained when restricting analyses to subjects with a history of HPV infection. Thus, low serum and RBC folate were each moderately, but nonsignificantly, associated with increased invasive cervical cancer risk. These findings support a role for one-carbon metabolism in the etiology of cervical cancer.

KEY WORDS: • *cervix neoplasms* • *serum folate* • *red blood cell folate* • *microbiologic folate assay* • *radiobinding folate assay* • *humans*

INTRODUCTION

For the past 25 years, there has been credible speculation that folate inadequacy might be a risk factor for cervical neoplasia (1,2). The hypothesis that folate is involved in human carcinogenesis in general, and cervical carcinogenesis in particular, is biologically plausible. Low folate status may be important in cancer etiology because folate is required for DNA synthesis, repair and methylation (3,4,5). In cervical carcinogenesis, low folate may facilitate the incorporation of human papillomavirus (HPV),[2] a factor believed to be responsible for >90% of all invasive cervical cancers (6,7) into the host genome. HPV integrates into the host genome of several cervical cancer cell lines at fragile sites made susceptible to breakage by inadequate folate (8,9,10).

The role of folate in the etiology of cervical cancer has been evaluated in many studies, but with mixed results (2,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27). Of three clinical intervention trials with folate supplementation, one found improvement of cervical dysplasia (2), whereas two others did not (17,22). Case-control studies using dietary measures generally showed no association or only weak associations between folate intake and risk of cervical dysplasia or cancer (11,12,13,14,15,19,20,21,24,25). In many of these studies, crude associations were substantially attenuated when adjusted for accepted cervical cancer risk factors. Additionally, few early studies incorporated any measure of HPV infection. Finally, assessment of folate intake in these studies may have been imprecise because the usual adult diet is difficult to quantify, and nutrient databases for folate are limited by the multiple forms, instability and variable bioavailability of folate in foods (28,29,30,31,32,33).

Serologic measures of folate allow better measurement of folate status than dietary intake measures (29✦ ,34)✦ , and RBC folate is a more reliable measure of folate status than serum folate because it integrates folate intake over several months, whereas serum folate fluctuates with daily intake (29✦ ,35✦ 36✦ 37)✦ . Case-control studies of cervical dysplasia and cancer that measured blood folate have generated mixed results (16✦ ,18✦ ,20✦ ,23✦ ,25✦ 26✦ 27)✦ . Of the five reports with invasive cervical cancer cases (12✦ ,13✦ ,15✦ ,16✦ ,26)✦ , only two (16✦ ,26)✦ examined serum folate and none have yet examined RBC folate; therefore, additional studies of invasive cervical cancer that use serologic measures of folate status, especially RBC folate, and that also consider HPV status, may be informative.

In the 1980s, the National Cancer Institute conducted a large case-control study of incident invasive cervical cancer in five U.S. communities (38✦ 39✦ 40)✦ . Analyses of dietary data from this study found no clear association between folate intake and risk of invasive (13)✦ or in situ (14)✦ cervical cancer. The current paper examines the relationship between invasive cervical cancer risk and serologic measures of folate status. Because of the complexities of measuring folate, serum and RBC folate were measured with both radiobinding and microbiologic assays, which is rare in a large epidemiologic study. To facilitate adequate control for confounding, history of HPV infection was assessed with a serologic HPV-16 antibody assay, and all other known cervical cancer risk factors were assessed by a detailed in-person interview.

SUBJECTS AND METHODS

TOP
ABSTRACT
INTRODUCTION
SUBJECTS AND METHODS
RESULTS
DISCUSSION
REFERENCES

Study design.

Eligible subjects were all women, aged 20–74 y, with histologically confirmed, primary incident invasive cervical cancer diagnosed from April 1982 through December 1983 in five areas reporting to the Comprehensive Cancer Patient Data System. Twenty-four hospitals in areas centered around Birmingham, AL; Chicago, IL; Denver, CO; Miami, FL; and Philadelphia, PA, participated. Up to two potential controls, matched by age (± 5 y), ethnicity (Caucasian, African American, Hispanic) and neighborhood (first six digits of a 10-digit telephone exchange), were identified by random digit dialing for each case. Approximately 25% of potential controls who had a previous hysterectomy were replaced.

Trained staff conducted interviews in the subjects' homes with structured questionnaires to obtain detailed information on demographic characteristics, sexual behavior, reproductive and menstrual history, exogenous hormone use, personal and familial medical history, smoking and diet. Diet was assessed using a 75-item food-frequency questionnaire asking "usual adult frequency of consumption, ignoring any recent changes" (13). All study participants provided informed written consent. The study was approved by the Institutional Review Boards of the National Cancer Institute and of the five participating study centers. Additional details of the study design have been published (38, 39, 40).

For the biochemical component of the study, blood samples were drawn at least 6 mo after completion of treatment for cervical disease to minimize any effects of treatment or disease on blood nutrient status. Treatment included surgery (44%), localized radiation (18%) or both (28%). A small percentage of subjects (4%) received chemotherapy in addition to other treatments, and 6% of subjects

were missing treatment information. Between March 1983 and October 1985, nonfasting blood samples were obtained; aliquots were stabilized with ascorbic acid (0.5% for serum and ~0.9% for whole blood) and frozen at -70°C until assayed (October 1988–January 1991). Hematocrits were determined in duplicate at the time of the blood collection.

Participation.

A total of 480 eligible cases (73%) and 801 eligible controls (72%) were interviewed. Blood was obtained from 245 cases and 545 controls (51 and 68% of those interviewed, respectively). Reasons for nonparticipation in the blood draw included death (17% of cases, 0.4% of controls), contact and scheduling difficulties (15 and 17%), subject refusal (9 and 13%), hospital refusal (6 and 0%), cases who were not yet 6 mo post-treatment at the completion of the study (2 and 0%), and unsuccessful blood draws (2 and 1%), respectively.

Excluded from the epidemiologic analyses were all cases who received chemotherapy treatment (n = 11) and/or who had advanced (stage III or IV) disease (n = 17), cases with nonsquamous cell cervical cancer (n = 28), one control who reported possible cervical cancer and subjects whose ethnicity was other than Caucasian, African American or Hispanic (n = 7 cases, 2 controls). Other reasons for exclusion included insufficient blood for the folate assays, use of an antibiotic by the subject, which could have interfered with the microbiologic folate assay, and missing hematocrit data. Data from two serum microbiologic assay batches (8 cases, 22 controls) and 3 serum radiobinding assay batches (14 cases, 31 controls) were excluded due to quality control problems with these specific batches (see below). Because serum folate values are used to calculate RBC folate values, these samples were excluded for the RBC analyses as well. These exclusions were not mutually exclusive. The number of cases and controls included in each epidemiologic analysis were as follows: serum microbiologic assay, 170, 505; serum radiobinding

assay, 169, 506; RBC microbiologic assay, 169, 504; and RBC radiobinding assay, 162, 496, respectively.

Laboratory methods.

Serum and whole blood folate were measured in duplicate with a microbiologic assay (41) using *Lactobacillus rhamnosus* ATCC #7469 (formerly called *L. casei*) and with a radiobinding assay (42 ,43) using SimulTrac Slurry Kits (Becton Dickinson, Franklin Lakes, NJ). RBC folate was calculated from serum and whole blood folate measurements, corrected for hematocrit (42) . Matched cases and controls were assayed consecutively within the same batch. Laboratory personnel were unaware of the case/control status of the samples.

In addition to each laboratory's own internal quality control procedures, laboratory reproducibility for the serum assays was monitored using blinded serum samples at low and normal folate concentrations. These samples were randomly inserted into each batch to comprise ~10% of the total number of samples. National Cancer Institute staff requested that four microbiologic and one radiobinding batches that failed to meet the Westgard multirule criteria (44) be repeated. At the time the assays were conducted, it was not possible to prepare whole-blood samples with predetermined folate concentrations to be used as blinded quality control samples. However, each laboratory's internal quality control samples included with the whole-blood study samples were evaluated using the Westgard rules, and eight microbiologic (but no radiobinding batches) had to be repeated. Additional laboratory problems suggested by review of the quality control samples after study completion resulted in the exclusion of two microbiologic serum assay and three radiobinding serum assay batches from the epidemiologic analysis. The CV for the remaining batches, based on the quality control material, was calculated using the variance component estimation procedure in SAS (45) and incorporated both within- and between-batch variability. Using blinded quality control material, the CV for

the serum microbiologic assay was 11.6% and for the serum radiobinding assay was 5.2%. Using the laboratory's own quality control material, the CV for the whole-blood microbiologic assay was 11.4% and for the whole-blood radiobinding assay was 10.3%.

A test for HPV type-16 antibodies in serum has been developed only recently. In November–December 1998, we tested for HPV-16 seropositivity using a well-characterized virus-like particle ELISA (46). Samples were tested in duplicate; before they were averaged, the optical density (OD) readings of each duplicate were adjusted according to results of three control samples run in triplicate in each batch, to control for between-day and between-batch variability. An OD < 0.904 was classified as seronegative; an OD > 1.017 was classified as seropositive; and an OD between these values (3.6% of subjects tested) was considered indeterminate (47).

Statistical analyses.

Statistical analyses were conducted using SAS version 6.12 for Windows (45). Correlations were measured with Spearman's rank order correlation coefficient. χ^2 tests were used to determine whether significant differences for selected demographic and behavioral factors existed between cases and controls. Geometric means were calculated by transforming folate values with the natural logarithm, calculating the mean and then transforming back to standard units.

The odds ratio (OR) was the measure of association used to estimate the relative risk of cervical cancer. Folate quartiles were based on the frequency distribution among the controls. The highest quartile was used as the referent, or comparison, group. Logistic regression was used to obtain maximum likelihood estimates of the OR and 95% confidence intervals (CI), while adjusting for potential confounders (48). Comparable OR were found using unconditional regression models, adjusting for the study-matching factors, and conditional regression models. Therefore, unconditional regression models, which retained all of the cases and controls whose matched subjects

did not participate in the blood draw portion of this study, were chosen for the detailed analyses and are presented throughout the results. Unless otherwise specified, all OR are adjusted for study matching factors (age, ethnicity, study site) and the following exposures related to risk in this study: HPV-16 seropositivity, number of sexual partners, age at first intercourse, years since last Papanicolaou (Pap) smear, number of pregnancies, smoking status and intensity, oral contraceptive use, education and income. Potential confounding variables were entered into the models as categorical variables with missing data retained in a separate category. Control for confounding was considered adequate when the addition of a potential confounder or an increase in the number of strata of a confounder did not change the adjusted OR by ≥0.1. Analyses with the RBC radiobinding assay data were adjusted for kit lot because two different kit lots were used for the whole-blood determinations. Tests for trend were obtained by assigning to each quartile the median folate concentration of the controls in that quartile and treating this as a continuous variable. Effect modification was assessed by examining stratum-specific OR and by using the likelihood ratio test to compare models with and without the interaction terms (49). All statistical tests were two-tailed. Differences with $P < 0.05$ or a CI that excluded 1.0 were considered significant.

RESULTS

TOP
ABSTRACT
INTRODUCTION
SUBJECTS AND METHODS
RESULTS
DISCUSSION
REFERENCES

Demographic and behavioral characteristics of study participants.

In the original study design, potential controls had been individually matched to eligible cases on the bases of age, ethnicity and neighborhood. Among the subjects from whom blood was successfully drawn, the distribution of cases remained comparable to that of the controls on age, ethnicity and study site (Table 1). However, cases who donated blood appeared to be of a lower socioeconomic status than controls, based on their report of less education ($P = 0.001$) and lower income ($P = 0.001$).

View this table: [in this window] [in a new window]

Table 1. Distribution of selected demographic variables for women from five U.S. communities who provided a blood sample, by case/control status

Serum and RBC folate.

For each of the four folate measures (serum and RBC folate measured by both the microbiologic and radiobinding assays), cases had lower geometric mean folate than controls (Table 2). The Third National Health and Nutrition Examination Survey reports somewhat higher blood folate using a radiobinding assay than we report here. For Caucasian women, mean age 43.2 y, unadjusted mean

serum folate was 16.4 ± 0.5 nmol/L and RBC folate was 483.4 ± 9.7 nmol/L (50)✦ .

View this table: [in this window] [in a new window]

Table 2. Geometric mean serum and RBC folate levels for women from five U.S. communities, using microbiologic and radiobinding assays[1]

On the basis of a serum folate cut-off point of <6.8 nmol/L (<3 ng/mL) (51)✦ , 5–8% of subjects were classified as folate deficient by the two assays. On the basis of an RBC folate cut-off point of <317 nmol/L (<140 ng/mL) (51)✦ , only 1.5% of subjects were deficient using the microbiologic assay, whereas 27% were deficient using the radiobinding assay. However, RBC folate measured by the microbiologic assay used in this study tended to run high and probably underestimated the percentage of deficient subjects. In addition, it is important to recognize that elevated disease risks may occur at folate concentrations above the cut-off point for clinical deficiency (52)✦ .

Folate values using the microbiologic and radiobinding assays were correlated for both the serum ($r = 0.90$) and the RBC ($r = 0.77$) measures. This was reassuring because it implies that although absolute values might differ, both assays ranked individuals similarly, and thus reliably. The correlation between serum and RBC folate within each measure was less than between the two methodologies ($r = 0.72$ for serum and RBC folate using the microbiologic assay, and $r = 0.63$ for serum and RBC folate using the radiobinding assay).

Blood folate and invasive cervical cancer risk.

The risk of invasive cervical cancer was moderately elevated (OR = 1.2–1.6 in the multivariate-adjusted models) in the lowest folate quartile compared with the highest folate quartile for all four blood folate measures (Table 3). Little confounding by HPV-16 status or other cervical cancer risk factors was observed.

View this table: [in this window] [in a new window]

Table 3. Invasive cervical cancer risk by serum and RBC folate levels, using microbiologic and radiobinding assays, for women in five U.S. communities

The OR adjusted for age, race, site and HPV-16 seropositivity were recalculated by octile of blood folate to explore the risk gradient over a wider range of exposure. For both the serum and RBC models using the microbiologic assay, the OR between extreme octiles were similar to those between extreme quartiles. However, using the radiobinding assay, the OR between extreme octiles were greater than between extreme quartiles (for serum < 7.9 nmol/L, OR = 2.58, 95% CI = 1.3–5.3; for RBC < 268 nmol/L, OR = 1.78, 95% CI = 0.9–3.7).

Inclusion of education and income in the models slightly attenuated the serum OR, but not the RBC OR. Adjustment for these should help control for inadequately measured lifestyle factors and provide a conservative estimate of risk. Inclusion of intake of provitamin A carotenoids or vitamin C, other micronutrients postulated to reduce

the risk of cervical cancer, modestly increased, rather than decreased the folate OR.

To integrate both serum folate measures and both RBC folate measures, risks were examined among subjects concurrently in the lowest quartile by both assay types compared with those concurrently in the highest quartile by both assay types. Similarly, elevated OR were noted in the low folate groups for both serum and RBC measures (OR = 1.6 for serum and 1.5 for RBC, Table 4). Risks were not noticeably strengthened by combining the microbiologic and radiobinding assays, probably because of the high correlation between the two. To simplify presentation of further epidemiologic analyses, these combined exposure models are presented.

View this table: [in this window] [in a new window]

Table 4. Invasive cervical cancer risk integrating two serum assays and two RBC assays for women from five U.S. communities

HPV is believed to be responsible for >90% of all invasive cervical cancers (6 ,7) . Therefore, we examined the association between folate and cervical cancer risk using only the controls seropositive for HPV-16. All cases were used in this analysis because we assumed that all cases had been exposed to oncogenic HPV at one time. We used the combined exposure model to examine subjects in the lowest quartile by both assay types compared with those in the highest quartile by both assay types. Low serum and RBC folate were nonsignificantly associated with increased cervical cancer risk, after controlling for exposure to oncogenic HPV in this manner (Table 5

). Comparable results were observed when only HPV-16 seropositive cases were included; adjusted OR for low folate in the combined exposure models were 2.0 (0.5–8.9) for serum and 1.2 (0.2–5.9) for RBC.

View this table: [in this window] [in a new window]

Table 5. Invasive cervical cancer risk among women from five U.S. communities with a likely history of human papillomavirus (HPV) infection

The number of HPV-16 seropositive controls was small, generating potentially unstable results, and it is possible that many of the other control subjects had been previously exposed to HPV-16 or other oncogenic HPV types. We therefore considered number of sexual partners and age at first intercourse, which are accepted proxy variables for HPV exposure. We examined risks among all of the cases and only the controls with ≥2 sexual partners, and among all of the cases and only the controls with age at first intercourse ≤20 y. With the combined exposure model, risks were nonsignificantly but consistently elevated in the lowest folate quartiles for both serum and RBC measures (OR = 1.5–2.2) (Table 5).

We also assessed the relationship between folate and infection with oncogenic HPV. Among the controls, folate status was not predictive of detection of HPV-16 antibodies. OR with the combined exposure model, adjusted for age, ethnicity and study site, were 0.7 (0.3–1.9) for low serum folate and 1.2 (0.4–3.7) for low RBC folate.

Because of previous hypotheses linking oral contraceptive use to low folate status and thus increased risk of cervical abnormalities (1,2), we closely investigated these associations. Geometric mean serum and RBC folate, using either assay and adjusted for age, ethnicity and study site, was not significantly different between women who had used oral contraceptives and women who had not. We stratified women by never/ever oral contraceptive use and examined the association between blood folate and invasive cervical cancer risk within each stratum. We observed no elevation in risk by folate status among users of oral contraceptives, although it had been hypothesized that oral contraceptive use would have depleted cervical folate stores. Unexpectedly, however, we did find elevated risks for low folate among women who never used oral contraceptives. Among never-users, OR for low compared with high folate quartiles, using the combined exposure models adjusted for age, ethnicity, study site, years since last Pap smear and HPV serology, were 5.9 (1.9–21.4) for serum folate and 3.4 (0.9–12.5) for RBC folate. The test for effect modification was not significant for either combined exposure model ($P = 0.08$ for serum and 0.21 for RBC).

We further examined risks by duration of oral contraceptive use. Women with high folate had a pattern of increasing risks with increased years of oral contraceptive use, whereas women with low folate, hypothesized to be more susceptible to folate depletion by oral contraceptive use, had a pattern of constant risks with increased years of oral contraceptive use (Table 6). The test for effect modification was not significant for either combined exposure model ($P = 0.31$ for serum and 0.40 for RBC).

View this table: [in this window]

Table 6. Invasive cervical cancer risk by duration of oral contraceptive use for women from five U.S. communities[12]

[in a new window]

Subjects who participated in the blood draw component of the study, relative to all those who participated in the interview, were more often Caucasian, came preferentially from certain study sites and were of higher socioeconomic status, as measured by education and income. Thus, we explored whether there were differences in participation between the cases and controls that might lead to bias. The cases and controls who donated blood were comparable to each other in age, ethnicity and study site (Table 1)✦ ; controls had been individually matched to cases on these factors in the original study design. To examine whether cases and controls differentially participated in the blood draw component by socioeconomic status, number of sexual partners, age at first intercourse, time since last Pap smear, vitamin supplement use or other cervical cancer risk factors, we compared OR among all of the interviewed subjects with OR among only those participating in the blood draw. For each of these exposures, similar patterns of risk were seen, suggesting that participation bias was minimal.

We found some evidence of differential participation by folate intake. However, the correlation between folate intake and blood folate status was low (Spearman $r = 0.08–0.16$), indicating that the differential participation would have little influence on blood folate status. In addition, the OR between folate intake and cervical cancer risk were similar among blood donors and nondonors, again suggesting that participation bias was minimal.

To examine the possibility of low blood folate being the result of systemic effects of disease or treatment, we compared mean blood folate concentrations of the cases by stage of cancer and treatment received (surgery or radiation). None of the women included in our analyses had received chemotherapy. We found no evidence that

either disease or treatment had reduced blood folate concentrations (Table 7) by the time blood was drawn, at least 6 mo after completion of treatment.

View this table: [in this window] [in a new window]

Table 7. Serum and RBC folate levels by stage of cervical cancer and treatment for women from five U.S. communities[12]

At the time of blood draw, subjects were questioned concerning whether they had changed their diet in the past 3 y (this time period encompassed diagnosis and treatment, if any, for the cases). For the subjects in this analysis, 33.9% of cases and 43.1% of controls reported they had made changes to their diet. Only 7.1% of cases and 8.5% of controls reported they had increased their fruit, vegetable and/or grain consumption; these percentages were not significantly different ($P = 0.55$). Of these, only two cases (1.1% of total) reported that they made these healthy improvements to their diet as a result of their cancer. Other subjects reported decreasing their food intake for reasons including weight loss, health (such as reducing cholesterol levels) and, for five cases (2.7%), a loss of appetite due to illness and/or treatment. The percentage of cases (16.4%) and controls (18.9%) who reported decreasing their food intake was similar ($P = 0.45$). Only two cases (1.1%) and two controls (0.4%) specifically reported a decreased intake of fruits, vegetables, or grains.

DISCUSSION

Low blood folate was moderately and consistently associated with an increased risk of invasive cervical cancer (OR = 1.2–1.6), although the risks were not significant (Table 3)[+] . Risk was elevated for each of the four measures of folate status, i.e., serum folate measured with microbiologic and radiobinding assays, and RBC folate measured with both assays. The effect remained after adjustment for history of HPV infection and all other accepted cervical cancer risk factors. A threshold effect was evident, with risk clearly elevated among women in the lowest folate quartile. When subjects were concurrently classified by both microbiologic and radiobinding assays, OR were 1.6 for low relative to high serum folate and 1.5 for RBC folate.

Ziegler et al. (13[+] ,14)[+] , using the same study population as the current analysis, did not detect an association between folate intake and risk of invasive or in situ cervical cancer. In the current analysis, improved measurement of folate status using serologic measures could explain this discrepancy. Serologic measures assess folate status more accurately than dietary intake measures due to difficulty quantifying usual adult diet and limitations in databases for folate in foods (28[+] 29[+] 30[+] 31[+] 32[+] 33[+] 34)[+] . Some case-control studies relying on serum and RBC folate have provided evidence for a protective effect of folate (18[+] ,20[+] ,23)[+] , whereas others have not (16[+] ,25[+] ,27)[+] , and all studies but one (16)[+] examined precancerous conditions, not invasive cancer. Like our retrospective study, the single prospective study reported nonsignificantly reduced risks with elevated serum folate (OR = 0.60, 95% CI 0.19–1.88) but was based on only 13 invasive and 26 in situ cervical cancer cases (26)[+] .

Among the women in our study, we found a strong and significant positive association between serum homocysteine and invasive cervical cancer risk (OR = 2.4–3.2, all 95% CI excluded 1.0, in the

three highest homocysteine quartiles relative to the lowest quartile) (53). These results provide evidence that our moderate folate association is real. Elevated serum homocysteine is a sensitive indicator of folate inadequacy and an emerging biomarker of problems in one-carbon metabolism (54, 55, 56, 57, 58). Serum homocysteine was moderately and inversely correlated with blood measures of folate status in this population (Spearman r = -0.3 to -0.4). Homocysteine may be more predictive of cervical cancer risk than low folate because of problems in assessing dietary (29, 30) and blood folate status (59) or, more likely, because it identifies additional abnormalities in one-carbon metabolism beyond low folate. Homocysteine can be elevated in response to low folate or low vitamin B-12 because both micronutrients are necessary for the conversion of homocysteine to methionine, or in response to low vitamin B-6, which is required for homocysteine degradation (58). Genetic polymorphisms that alter enzyme activity in the one-carbon metabolism pathway, such as C677T methylenetetrahydrofolate reductase (MTHFR), can also result in elevated homocysteine (57).

In most large epidemiologic studies, cost, feasibility and subject refusal limit the number of times blood can be drawn from subjects. A criticism of these studies is the relevance of data from a single blood draw to "usual" nutrient levels. A strength of the current study is that, although blood was drawn only once, blood folate status was measured with both long-term (RBC) and recent (serum) blood folate markers. However, RBC folate was not more predictive of reduced risk in this study; the OR for cervical cancer risk were similar with the serum and the RBC data. It is possible that folate intake was relatively stable for the women in our study. If similar results are found in other studies using both measures, this will simplify study design for epidemiologists because sample collection and assay is much simpler for serum folate.

We were also able to compare results using a microbiologic and a radiobinding assay. The assays were surprisingly well correlated (r = 0.9 for serum and 0.8 for RBC). The radiobinding assay was more

reproducible (CV for serum folate = 5.2% for radiobinding and 11.6% for microbiologic), which may explain the modestly stronger associations seen with the radiobinding assay. Ultimately, however, it is not clear which assay is better for epidemiologic studies because the assays may not be measuring the same folate forms, and it is not known what folate forms are especially relevant to cancer. To complicate the picture further, the radiobinding and microbiologic assays give different results for RBC folate for subjects whose one-carbon metabolism is altered by the C677T MTHFR polymorphism (60).

A stronger association may exist at folate concentrations lower than those found in our study. The percentage of folate-deficient subjects in our study was relatively low (<10% for three of the assays) compared with another study that reported strong associations (with 14–24% of subjects deficient for serum folate and 41–52% for RBC folate) (20). However, folate values can vary greatly among laboratories (59) and complicate these comparisons. Cervical cancer is the third most common cancer in women worldwide (61); thus, associations at low folate concentrations may be magnified in developing countries.

Whitehead et al. (1) found megaloblastic cervical abnormalities in 19% of women using oral contraceptives, in the absence of low blood folate or vitamin B-12. No similar abnormalities were found in women not using oral contraceptives. Folic acid therapy was given to eight women using oral contraceptives and their abnormalities were reversed. The authors hypothesized that a localized folate deficiency existed in the cervical tissue of these women. Butterworth et al. (2) further postulated that this localized deficiency could provide an environment that could lead to cervical dysplasia; in a blind, randomized trial, women using oral contraceptives, with mild or moderate cervical dysplasia, showed significant improvement with folate supplementation of 10 mg daily for 3 mo ($P < 0.05$). In an additional sample of 40 healthy hospital workers, RBC folate was 30% lower in oral contraceptive users compared with nonusers ($P <$

0.01), and among oral contraceptive users, RBC folate was 15% lower in women with dysplasia compared with healthy volunteers (*P* reported as not significant) (2). However, in a follow-up study among women with mild or moderate cervical dysplasia (80% of whom were oral contraceptive users), no significant improvement was found with folate supplementation of 10 mg/d for 6 mo (17). In the current study, there was no difference in geometric mean serum or RBC folate between women who had used oral contraceptives and those who had not. We did not find an increased risk with low serum or RBC folate among women who used oral contraceptives, even when we focused on women who used oral contraceptives the longest. In fact, we found the strongest inverse association with folate among women who never used oral contraceptives, although the interaction was not significant ($P = 0.08$ for serum and 0.21 for RBC). We did not have a measure of localized folate status in the cervix.

HPV infection is believed to be etiologically associated with most cases of cervical cancer, although only a small minority of women who are HPV-positive progress to cervical cancer (62). If folate helps prevent the incorporation of the HPV virus into the genome, this may explain why only some women infected with HPV progress to cervical cancer. When we restricted our analyses to women believed to have a history of HPV infection, using only controls seropositive to HPV-16, with multiple sexual partners or first intercourse at an early age, the association between folate and cervical cancer risk remained. Thus, low folate could be involved in the progression of cervical cancer after HPV infection. However, among the controls in our study, folate was not predictive of detection of HPV-16 antibodies in serum, suggesting that low folate is unrelated to risk of being infected with HPV.

Our serologic characterization of a history of HPV infection had several important limitations. The HPV-16 virus-like particle ELISA test, which uses serum, may be insensitive relative to DNA hybridization assays, which require cervical tissue scrapings (62),

and HPV antibody titers may decrease after surgical treatment for cervical cancer (63). Furthermore, we tested only for antibodies to HPV-16, the most prevalent oncogenic HPV type, which accounts for >50% of invasive cervical cancer in the United States (6), but other oncogenic HPV types exist. Given these limitations, although only 36% of the cases tested seropositive for HPV-16, for the purpose of the HPV stratified analysis, we assumed that all cases, irrespective of their current status by this assay, had once been infected with an oncogenic HPV. Among controls, 15% tested seropositive for HPV-16, similar to a 12% prevalence recently reported among U.S. blood donors, using the same ELISA serologic HPV-16 assay that we used (46). We therefore did not assume false negatives among the controls.

The elevated risk noted in this study is unlikely to be the result of confounding by inadequately measured exposures. Adjustment for potential confounding by accepted cervical cancer risk factors had little effect on the OR. Addition of HPV-16 serologic status to the models actually increased the OR; thus, it is unlikely that better measurement of history of HPV infection would substantially attenuate the effect. Inclusion in the multivariate models of education and income, indicators of poor diet and/or unhealthy lifestyle, only slightly attenuated the OR, suggesting that other lifestyle factors would have little influence on risks. Folate sources such as orange juice and green leafy vegetables are also sources of vitamin C and carotenoids. However, adjustment for intake of these two micronutrients did not attenuate the folate associations.

Participation bias is also unlikely to explain our findings. Cases and controls who participated in the blood phase of the study did not differ from each other in the study matching factors of age, ethnicity and study site. In addition, the same patterns of risk were seen for education, income and other cervical cancer risk factors in all subjects interviewed and in the subgroup who participated in the blood draw. Furthermore, although 17% of cases had died before the blood draw,

any bias would have attenuated the OR if low folate was associated with more advanced disease.

To minimize the possibility that advanced disease or deteriorating health influenced the results, we excluded all stage III and IV cases (n = 17) from our analyses. To minimize any treatment effect, blood was collected at least 6 mo after completion of treatment, and those who received chemotherapy (n = 11) were excluded from the analyses. In addition, for the women in the analyses, we found no evidence that either disease stage or the treatment received reduced blood folate. Finally, in a small prospective cohort study, serum folate measured at baseline was inversely related to subsequent cervical cancer incidence (26), suggesting that low folate preceded disease.

It is unlikely that the folate would have degraded during storage. Folate was found to be stable in plasma samples frozen for 4 y at -20°C (64), and our samples were stored at -70°C. In addition, both our serum and RBC samples were stabilized with ascorbic acid to keep the folate in a reduced state (65). If any degradation did occur, the resulting misclassification of folate status would have attenuated the OR.

It is unlikely that deliberate improvements in diet after diagnosis of cervical cancer could have biased our results. Because diet-disease relationships with cervical cancer were not well established or publicized at the time our study was conducted, it is not likely that subjects made long-term, healthy diet changes as a result of their disease. Furthermore, at the time of blood draw, subjects were questioned concerning whether they had changed their diet in the past 3 y. An analysis of these data indicated that only two cases reported that, as a result of their cancer, they increased fruit, vegetable and/or grain intake, which are potential sources of folate. A much larger number of cases and controls reported these improvements to their diet for a variety of health reasons. Similarly, only five cases reported a loss of appetite due to illness or treatment,

whereas many more cases and controls decreased their intake for reasons such as weight loss.

The increase in invasive cervical cancer risk that we observed with low serum and RBC folate was moderately strong and consistently seen across our four measures of folate status. Our aim was to include new measures of folate status, as well as consider HPV status and other cervical cancer risk factors, to test a hypothesis that has not been consistently supported or refuted in the epidemiologic literature. Because of its size and design, our study provides a robust test of whether folate may be critical at any stage of cervical carcinogenesis. The relationship is biologically plausible, due to folate's role in DNA synthesis, repair and methylation, and is also supported by our strong homocysteine results. The U.S. Food and Drug Administration now requires that enriched grain products be fortified with folic acid at 140 μg/100 g of grain product (66)♣ . This requirement was established to help women consume at least 400 μg of folic acid daily to reduce the incidence of neural tube defects. Future studies should explore the effects of fortification on the folate status of women at high risk for cervical cancer and monitor its effect on cervical cancer incidence.

Red blood cell folate concentrations increase more after supplementation with [6S]-5-methyltetrahydrofolate than with folic acid in women of childbearing age.

The Effect of High Folate Levels

What are the Findings

Studies about the potential adverse effects associated with high intakes of folic acid is still underway.Read about the different findings linked with high folate intake.

The Food and Drug Administration (FDA) chose levels of 140 mg of folic acid per 100g of enriched cereal-grain products. Included in this category were breads, buns, rolls, flour, and corn-meals, rice, macaroni, spaghetti and noodles. It was later reviewed that many of

these fortified folic acid products contained significantly more total folate than what was required by federal regulation. These findings raise a concern about the effects of excessive folic acid intakes.

A combination of the use of folic acid fortification, supplementation and other fortified foods may pose a risk of adverse health effects to particular target groups such as children and women in their childbearing years.

On-going surveillance about the effectiveness and safety of folic acid fortification is still underway and many questions still remain about the potential adverse effects associated with such high intakes of folic acid.

Inconsistent results have been reported concerning the effects of high folate levels on human epileptics. There have been indications of high levels of negative excitatory effects causing increases in the frequency and severity of seizures and reducing the effectiveness of anti-convulsants, while others have shown no such effects. Up to date there is no available evidence-based information about the effectiveness or dosing of folic acid supplements in women with epilepsy . Studies have shown no adverse effects of high oral doses of folates in animals.

However, doses exceeding 250mg/day have shown to produce epileptic responses and renal hypertrophy in rats.Current research suggests that folate may form a non-absorbable complex with Zn, thus antagonizing the utilization of that essential trace element at levels of high intake of thevitamin. Studies with animal models have not consistently shown such antagonism and most results indicate that even at high levels of intake, folate does not affect Zn status.

The most commonly cited risk of folic acid, especially in pregnancy, is the condition megaloblastic anemia, which is caused by a low folate status. In normal women on a folate-deficient diet, megaloblastic anemia develops after 15 to 18 weeks. In pregnant women this condition develops more rapidly and presents abnormalities such as a

fall in erythrocyte count and an increased mean corpuscular erythrocyte volume. High doses of folate (5 mg/day oral) have been shown to correct megaloblastic anemia. However, because folate treatment does not affect the neurological lesions of the vitamin B12 deficiency, pernicious anemia, concern has been raised that random use of large folate supplements may mask and potentially aggravate the neurological consequences of a vitamin B12 deficiency in their early and more easily treated stages.

The Important Role of Folic Acid in Human Health

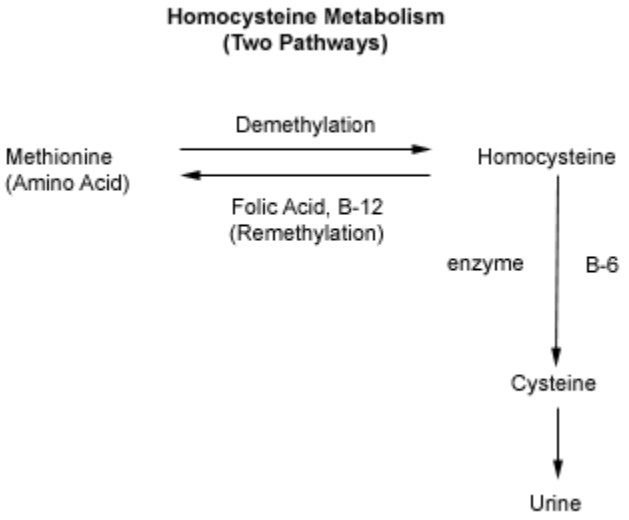
New research and clinical studies have shown that the role of folic acid in human health is far more important than its use as a vitamin and dietary supplement; in fact, folic acid is an important compound that is highly effective in preventing birth-defects, cardiovascular and cerebrovascular diseases, and certain types of cancer. In recognition of its importance, the federal government has mandated the fortification of cereal grains with 0.14 mg (140 micrograms) of folic acid per 100 grams of grain. The goal of this decision is to reduce the risk of heart diseases and the risk of women giving birth to babies with neural tube defects (spina bifida) and orofacial clefts. Women of child-bearing age are encouraged to consume 400 mcg of folic acid a day. The protective effects of folic acid are even more pronounced when it is combined with a high dietary intake of vitamin B6 and vitamin B12. It is now proven that vitamins B6 and B12 markedly increase the homocysteine-lowering effect of folic acid in cardiovascular diseases.[1]

Folic Acid and Cardiovascular Disease

Nine well-known risk factors can help predict the likelihood of heart attacks and strokes: advancing age, heredity, male gender, cigarette smoking, high blood pressure, diabetes, obesity, lack of physical activity, and abnormal blood cholesterol levels. The more risk factors a person has, the greater the likelihood of becoming ill. Age, heredity, and gender cannot be changed, but the other factors can be influenced by individual behavior. Modifying these factors can lower

the risk of having a heart attack, cancer and certain other diseases. The role of folic acid in these diseases is discussed in this article.

As stated, folic acid has been found effective in lowering high blood levels of homocysteine. Homocysteine is an amino acid, but not an essential amino acid and therefore not a building block of proteins. Homocysteine (**FIGURE 1**) is formed in human tissues during the metabolism of methionine, a sulfur containing essential amino acid in proteins. Folic acid blocks the formation of homocysteine from methionine.[2]



It is generally accepted that a high blood level of homocysteine is a significant risk factor for cardiovascular and cerebrovascular diseases. Several studies have concluded that high homocysteine levels are associated with coronary artery disease (CAD). It is also believed that homocysteine promotes atherosclerosis by encouraging dysfunction of the lining of the arteries (endothelial dysfunction) and interference with blood clots.[3] Epidemiological studies have shown that a prolonged lowering of homocysteine levels by 1 micromol/L could theoretically result in a 10% reduction in risk of cardiovascular diseases. It has been estimated that 40% of Americans get too little folate and over 20% have homocysteine levels high enough to result in vascular disease.[4] It has been found that men have higher levels of homocysteine than women and that these levels increase significantly with age.

A normal desirable blood level of homocysteine is less than 10 micro-mol/L. A level of 12 micromol/L is considered borderline, and levels of 15 micromol/L or above are considered to be indicative

of increased risk factor for cardiovascular diseases. The high levels can be lowered effectively by consuming 400-800 micrograms/day of folic acid.[5]

A recently completed study, the Physicians' Health Study, showed that men with a high level of homocysteine (15-20 micromol/L) had a three times higher risk of myocardial infarction (MI) than men with lower levels. The study showed that the level of homocysteine in the blood is inversely proportional with level and dietary intake of folic acid. It also concluded that a minimum daily intake of 400 micrograms per day of folate is required to maintain a stable low level of homocysteine (6 micromol/L). A recent study at the Johns Hopkins University found that a 5 micromol/L increase in homocysteine level is associated with a 50% increase in the risk of cerebrovascular diseases (stroke) and other thrombotic events.[6]

Researchers at the Centers for Disease Control and Prevention now report that low blood levels of folic acid are associated with substantially increased risk of dying from cardiovascular disease.[7] Other research studies report evidence that supplementation with folic acid, vitamin B6, and vitamin B12 is associated with a 60% decrease in abnormal exercise electrocardiographs?important markers for atherosclerosis.

The above reports indicate that folic acid is an essential factor in the daily diet to promote human health. Eating folate-rich foods does not result in improved folate status and supplementation of folic acid up to 800 micrograms/day is necessary. This is because bioavailability (absorption) of folate from food is significantly less than that from a folic acid supplement. Folic acid is present in foliage or dark leafy vegetable (broccoli and spinach) and citrus fruits, but even a well balanced diet may not provide a minimum of 400 micrograms per day.

It is estimated that the treatment of preventable illnesses absorbs as much as 70% of total health costs in the U.S. It has been clearly

demonstrated that $20 billion in hospital charges alone could be saved every year if women of childbearing age were to supplement with zinc and folic acid.[8]

Folic Acid and Cancer
Worldwide research reveals that sufficient folic acid supplementation in daily diets shows an important protective role in certain types of cancer.

Breast Cancer: Folic acid has been shown to be highly effective in preventing breast cancer in both pre- and postmenopausal women. A recent study shows a clear correlation between dietary intake of folic acid and the risk of breast cancer. Women with a dietary intake of 400 micrograms/day or higher had a 40% lower risk than did women with intake of less than 200 micrograms/day. Women who took vitamins B6 and B12 and methionine had a 53% lower risk. It is now believed that both of these vitamins in addition to folic acid are vital co-factors required to lower homocysteine levels. Researchers believe folic acid helps to regenerate methionine, a vital component in DNA synthesis.[9]

Pancreatic Cancer: A review of dietary records of patients with pancreatic cancer has revealed that adequate folic acid intake could materially reduce the risk of developing cancer. In this study of 27,000 male smokers aged 50-69 years, men with a dietary supplement of folic acid of more than 373 micrograms/day had half the risk of pancreatic cancer that did men with an intake of less than 200 micrograms/day. A baseline serum folate level above 4.45 ng/mL was associated with 55% risk reduction when compared to levels below 3.33 ng/mL.[10]

Colon Cancer: Researchers at the Harvard Medical School reported in a study involving 88,750 female nurses that women with high folate intake decreased their risk of developing colon cancer by as much as 75%. Statistical analysis showed that women who had supplemented with multivitamins containing folic acid (>400

micrograms/day) for 15 years or more had a four times lower risk of colon cancer that did women whose daily intake had been 200 micrograms/day or less. The risk reduction with folate supplementation was particularly evident among women with low methionine levels. Folate is essential in the regeneration of methionine, and deficiency may lead to abnormalities in DNA synthesis and repair-mechanism that may influence the development of colon cancer. Certain birth control pills and drugs such as methotrexate can markedly lower folate levels in the body.[11]

Colorectal Cancer: Cancer of the colon and rectum is now the second most common cause of cancer death in the U.S. Studies show that a high consumption of meat and a low intake of fruits and vegetables substantially increase the risk of developing colorectal cancer. A Canadian research study shows that colorectal cancer is preceded by the occurrence of malignant polyps. Non-malignant polyps may also be present in the colon and rectal area. The study indicates that the folic acid level in the lining (mucosa) of the colon is significantly lower among patients with malignant polyps than among patients with benign polyps. Blood levels of homocysteine were also found to correlate well with mucosal folate levels, and elevated homocysteine levels were also found to correlate with the presence of malignant polyps. The study concludes that people with malignant polyps may have an impairment in their folate metabolism, which would account for their higher homocysteine levels.[12] A recent study at the New York University School of Medicine shows women with low levels of folate in their blood serum have twice the risk of colorectal cancer than do women with higher levels. The analysis showed women with low levels of folate had higher levels of homocysteine (more than 12 micromol/L) and had a 70% increase risk compared with women who had higher levels of folate and lower levels of homocysteine (8 micromol/L). The study concluded that sufficient folic acid supplementation may protect against colorectal cancer.[13]

In clinical trials, Dr. Young-In Kim of the University of Toronto concluded that a moderate increase in folate intake can materially help reduce the risk of certain cancers, but cautions that people who already have cancer should not increase their intake as there is evidence that high folate levels may accelerate the growth of existing tumors.[14]

Folate Deficiency in Other Diseases

In addition to cardiovascular diseases and certain types of cancer, folic acid deficiency has been linked to Crohn's disease, Alzheimer's disease, depression, Parkinson's disease and chronic fatigue syndrome. Patients with end-stage renal disease and elderly persons with hearing loss have also been reported to suffer from vitamin B12 and folic acid deficiency.

Crohn's Disease: People with inflammatory bowel disease (Crohn's disease and ulcerative colitis) tend to be at greater risk for thromboembolic events (blood clots and stroke). Researchers believe they may have found the reason for this health problem. In a study including 105 men and women with Crohn's disease, the researchers compared blood levels of homocysteine (a known risk factor for blood clots), folic acid and vitamin B12 to the levels found in healthy subjects. They found a significantly higher level of homocysteine (above 20 micromol/L) and lower levels of folic acid and vitamin B12 in patients with mild to moderately active Crohn's disease. The average level of folic acid was 5.9 pg/mL (normal range 5-17 pg/mL). They noted that patients with Crohn's disease may benefit from supplementing with folic acid and vitamin B12.[15]

Alzheimer's Disease: Recent studies have shown that a low concentration of folic acid (folate) in blood is associated with increased risk of dementia and Alzheimer's disease (AD). Researchers at the University of Kentucky report that low folate levels are directly associated with a high degree of atrophy of the cerebral cortex. The neocortex atrophy was especially associated with patients who had been diagnosed with AD. The average levels of folate in patients with

significant AD was 45 nmol/L as compared to 61 nmol/L in patients without significant Alzheimer's disease.[16]

Other research teams from the Universities of Oxford (England) and Bergen (Norway) also report that low folate and vitamin B12 levels are associated with an increased risk of developing AD. Their patients had significantly higher levels of the amino acid homocysteine. It was noted through the study that disease progression was more rapid among AD patients with high homocysteine levels. They conclude that this risk factor for AD can be significantly reduced by folate and vitamin B12 supplementation in the average Western population.[17]

Depression: A low level of folate has been detected in 15% to 38% of adults suffering from depression. There is significant evidence that supplementation with therapeutic amounts of folate can significantly improve the conditions of depressed patients. Folate supplementation (15 mg/day of methylfolate) has been found to markedly improve the effect of standard antidepressants. Scientists at the Harvard Medical School point out that many drugs, some chronic diseases (rheumatoid arthritis), certain cancer treatments, alcoholism and a poor diet can all lead to a folate deficiency and potential for depression. They caution that daily folate dosage has to be carefully determined in depressed patients as too much of a dose may cause sleeping disorders, irritability and hyperactivity.[18]

Parkinson's Disease: People suffering from Parkinson's disease (PD) have an increased risk of heart attack and stroke. Some German and Swiss medical scientists believe they have found a solution to these patients' problem. They studied a group of 48 to 73 year-old patients with PD. One group was treated with levodopa plus Sinemet, one group was not treated with any drug, and the last group were healthy subjects. All participants had their homocysteine blood levels measured after a 12-hour fast. The drug-treated group had an average of 17 micromol/L homocysteine and the other two groups had a blood level of close to 9 micromol/L. The researchers found

that prolonged treatment with levodopa and Sinemet increased the blood levels of homocysteine. The conclusion of their study is that patients with PD who are treated with levodopa should have their homocysteine levels monitored on a regular basis and should supplement their diet with folic acid as required. Folic acid is nontoxic and no cases of overdosing have ever been reported.[19]

Chronic Fatigue Syndrome: A researcher at UCLA Medical School has reported a thorough review of nutritional deficiencies involved in Chronic Fatigue Syndrome (CFS). He indicated that several vitamin deficiencies such as various B vitamins, vitamin C, folic acid and minerals like magnesium, zinc and sodium are at lower levels of normal in these patients. Although he believes there is some evidence the deficiencies are caused by the disease itself rather than inadequate diet, he recommends the following three-month supplementation trial to improve the CFS healing process: Folic acid (1?0 mg/day); Vitamin B12 (6?0 mg, IM per week); Vitamin C (10?5 g/day); and Zinc (135 mg/day) for 15 days only. Certain amounts of carnitine, 5-hydroxytryptophan, co-enzyme Q10 and essential fatty acids are also included in his regimen. This supplementation formula should be administered under medical supervision.[20]

Hearing Loss: One of the common problems in the elderly with heart diseases, hypertension, and arthritis is hearing loss. Scientists from the University of Georgia Medical Center believe that age-related hearing loss may be partially caused by vitamin deficiency. They just released a report that supports this hypothesis. In their study, 55 women aged 60?1 years were given a standard hearing test and had a fasting blood sample taken. Samples of their red blood cells were analyzed for vitamin B12, serum folate, and folate. After the study they discovered women with impaired hearing had a 38% lower serum level of vitamin B12 and 31% lower folate in their red blood cells as well as 25% lower folate serum levels. They believe poor folate and vitamin B12 status may cause deterioration of the nerves and blood vessels supplying the auditory system, perhaps through a mechanism involving homocysteine.[21]

Patients with Kidney Disease: Patients with end-stage renal disease have been found to have higher levels of homocysteine in their blood. In a study of 176 dialysis patients, blood levels of homocysteine were found to be 21 micromol/L (normal: 10 micromol/L). These patients were found to have a three times higher risk of cardiovascular diseases throughout the study period. The researchers concluded that supplementation with folic acid (15 mg/day) and adequate doses of B6 and B12 may be effective in preventing the development of cardiac disease.

What does a high Folate level mean?

I have seen a couple neurologists, one who specializes in Multiple Sclerosis. I had a brain MRI a couple months ago and they found multiple lesions in the white matter. The first neurologist tested me for Lupus, Lymes and a few other things and the tests came back negative. I decided to go to someone who specialized in MS just to make sure. He said it could be MS, but it wasn't a textbook case. He did some more blood work and my Folate levels came back high. The normal range is up to 21 and mine was 38. The B12 levels came back normal. Does this mean anything?

Answer

I tend to get low Folate levels, and I eat Beef Liver, spinach, Great Northern Beans, (dried beans, which you soak overnight and cook with meat).
Did they tell you this was too high, normal B12 levels, mean you do not have to have iron supplements.
Now I do know that folate levels can temporarily rise in patients who have just eaten foods that contain folic acid.
It used to be easy to diagnose people who had pernicious anemia, but now because of the food in most grocery stores contain folic acid it is very hard to diagnose.
the next time you have blood work, watch the tech, does he/she

slowly tilt the vial with the red rubber top back and forth or does he/she shake it? It can make a difference in your test results.

Source(s):

I give myself Dexrerrum injections for anemia. If you find out what caused the high reading, please get back to me, Interesting.