The Role of Folic Acid in Deficiency States and Prevention of Disease

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Folic acid, a water-soluble vitamin, has been used since the 1940s to treat some cases of macrocytic anemia without neurologic disease. Folate deficiency is best diagnosed with red blood cell folate levels along with macrocytosis and/or megaloblastic anemia. In addition to reversing overt deficiency, the vitamin may reduce the incidence of neural tube defects by 45% in women who receive 400 µg per day. It is recommended that all women of childbearing age take 400 µg of folate per day. Elevations in homocysteine levels, a metabolite intimately associated with folate, are also being found with increasing regularity in those with cardiovascular diseases. Homocysteine levels are reduced by folic acid administration. Therefore, there is some biologic plausibility, but not currently direct proof, for the assumption that folate supplements may prevent heart disease, stroke, and peripheral arterial disease. Controlled trials should take place before widespread food supplementation with folate is carried out on a large scale because of the possibility of outbreaks of permanent B_{12}-related neurologic damage in those with undiagnosed pernicious anemia. However, if a patient has a premature cardiovascular event and has minimal risk factors, ordering a test to determine homocysteine level may be advisable, and if elevated, treating with folic acid supplement as long as B_{12} deficiency does not coexist.

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Folic acid or pteroylglutamic acid was first synthesized and named in the 1940s. Cases of macrocytic anemia that ultimately responded to yeast (in which folate is present in large amounts) were described shortly thereafter. Since that time folate has kept a relatively low profile while other vitamins such as the antioxidants have been touted for disease prevention and treatment. Recently, however, medical findings are thrusting this important vitamin into the "prevention spotlight." In this article, basic information about folic acid and deficiency states are reviewed, and the newer concepts of the use of folate for prevention of neural tube defects and cardiovascular disease are presented.

FOLIC ACID BASICS

The minimum daily requirement or the amount of folate from exogenous sources needed to sustain normality is about 50 µg. The results of a 1961 study[1] concluded that 50 µg per day of folate would meet the minimum daily requirement (MDR) . The study was conducted at a Boston hospital using a synthetic diet devoid of folic acid. On this diet, patients developed overt megaloblastic anemia within a few days. When patients were given 50 µg per day of folate orally, the deficiency resolved along with the anemia.

There is a difference between the MDR and the recommended daily allowance (RDA). The MDR, as mentioned above, is 50 to100 µg per day, while the RDA is set at 200 µg per day for adult men and 180 µg per day for adult women. The RDA for folate is arbitrarily set at a level that is neither the MDR nor necessarily the optimal level of intake, but the level of intake that, on the basis of scientific knowledge, is judged to be adequate to meet the known needs of *almost all* healthy persons.[2]

The World Health Organization has also recommended an intake of around 200 µg per day.[3] This would provide about a 3- to 4-month supply in the event of zero intake. Americans usually take in only 200 to 300 µg per day, according to the US Department of Health and Human Services. This was substantiated in the third National Health and Nutrition Examination Survey (NHANES) study,

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which documented that in a sample size of over 14,000 subjects, men ate a mean of 317 µg per day (median, 266 µg per day) of folate while women ate a mean of 236 µg per day (median, 195 µg per day).[4] Herbert[5] found that the percentage of total folate in liver, yeast, and egg yolk that is absorbed is very high; but the absorbability of the forms of folate in most other foods is probably in the range of 10%. The Food and Nutrition Board reported that the bioavailability of folate in the typical US diet is about one half of crystalline (in vitamins) folic acid, which is efficiently absorbed. The average amount of folate stored in the body is 5 to 10 mg, one half of which is present in the liver. Enterohepatic circulation is thought to play a role in the maintenance of folate levels. Alcohol ingestion, however, interferes with this process and usually causes a negative folate balance.

Folic acid is abundantly present in most food groups. Foods with the highest amounts of folate include yeast, fresh green vegetables, organ meats, and some fresh fruits. Unfortunately, folate is highly susceptible to destruction, up to 50% by cooking or processing.[6,7] Retention of folate is basically the same whether foods are cooked in a microwave or on a conventional range.[7] In general, cooking recommendations for retention of folate are similar to those for any water-soluble vitamin: minimal contact of the vegetable with the cooking water reduces loss of nutrients due to the solution.[7] Using a small amount of cooking water when a vegetable is boiled is recommended for this reason. Vegetables should be cooked only until crisp-tender (barely done). Steaming in a steamer or in a pressure cooker also reduces contact with the water. The vitamin is absorbed from food sources by the proximal third of the small intestine. The manner in which the vitamin is transferred to the circulation is not well understood.

MACROCYTIC ANEMIA AND FOLATE

Like all nutritional deficiencies, folate deficiency may be caused by a number of factors, including inadequate ingestion, inadequate absorption, inadequate utilization, increased requirement, increased excretion, or increased destruction. This process of deficiency may be divided into four progressive clinical stages.

Stage 1: Early negative folate balance with decreased serum folate levels but normal folate stores as indicated by a normal red blood cell (RBC) folate level.

Stage 2: Folate depletion both in serum values and RBC folate levels.

Stage 3: Folate-deficient red cell production resulting in increasing but not yet grossly abnormal red cell indices and hypersegmentation of neutrophils.

Stage 4: Clinical folate deficiency with macrocytic anemia.

A diagnosis of deficiency is based on findings of macrocytosis with or without anemia or on a suspected deficiency based on high likelihood (Table 1). Unlike B_{12} deficiency, folic acid deficiency does not usually cause neurologic damage. Rare reports have indicated that folate deficiency may lead to a subacute spinal cord syndrome with gait disturbance, weakness, and loss of position sense.[8] Serum folate levels are notoriously unreliable, as they may fluctuate from day to day because of dietary changes. The RBC folate level is a much better indicator of folate status. Deficiency states are best treated by a daily oral dose of 1 mg per day of folic acid. Doses larger than this are excreted in the urine. Toxicity with folic acid administration is exceedingly rare. Although doses of 15 mg intravenously in a nonconvulsive patient have been reported to be without known problems,[9] some sources have noted that intravenous doses over 15 mg may cause epileptiform activity in humans.[10] No such findings have been noted to date with large oral doses of folate. The mechanism of this interaction may be that folate competes with antiepileptic agents for binding positions in both the intestine and the brain.[10]

Folate deficiency will often be found in the elderly, alcoholics, the poor, rheumatoid arthritis patients receiving long-term methotrexate therapy, and AIDS patients using sulfa drugs or pentamidine for pneumocystis prophylaxis. Previous articles have stated that alcohol abuse is the most common cause of folate deficiency in this country.[11] Studies on the cause of macrocytosis with or without anemia have provided conflicting evidence. In two studies the

leading cause was found to be vitamin B_{12} or folate deficiency, followed closely by alcohol abuse.[12,13] In another study, however, an overwhelming preponderance of alcohol abuse was revealed to be the cause, followed by vitamin deficiency.[14] The most common cause most likely differs according to the patient population being seen.

Any patient at risk for vitamin deficiency with significant macrocytosis with or without anemia should have a serum B_{12} and RBC folate determination. If both the RBC folate and the B_{12} levels are abnormal, further metabolic testing with serum or urine determination of homocysteine and methylmalonic acid levels may be necessary. Elevations in homocysteine levels and borderline or low RBC folate levels usually indicate folate deficiency as long as B_{12} levels are above 350 pg/mL. Those being treated with the above-mentioned medications on a long-term basis should be given prophylactic folic acid at a dose of 1 mg per day. Although methotrexate was thought to exert its anti-rheumatic effects by folic acid antagonism, recent studies have refuted this.[15] Patients taking both 5 mg and 27.5 mg per week of folate had significantly less toxicity than their counterparts on placebo. Investigators noticed no deceased efficacy, however.

THE RELATIONSHIP OF B_{12} AND FOLATE

Folic acid and cobalmin (vitamin B_{12}) are closely related, as the latter helps recycle folic acid for reuse in the homocysteine-to-methionine reaction. They both serve as cofactors in this important cycle. Both RBC folate levels[16] and serum folate levels[17] are reduced by cobalmin deficiency. Despite this, these levels are reduced much more by folic acid deficiency. Homocysteine levels and methylmalonic acid levels may be combined with RBC folate and serum B_{12} levels to effectively diagnose both deficiency states (Table 2). It is essential to diagnose the correct deficiency state because the treatment of cobalmin deficiency with folate may result in an inappropriate improvement in

TABLE 1

Causes of Folate Deficiency

Cause of Deficiency	Specific Examples in Humans
Inadequate ingestion (rare in U.S. except in cases of ethanol abuse)	Alcoholism and poor intake of fresh uncooked fruits and vegetables
Inadequate absorption	
Disease	Gluten enteropathy; sprues; congenital enzyme deficiency
Drugs	Anticonvulsants; ethanol; metformin; cholestyramine; azulfidine, oral contraceptives
Inadequate utilization	Metabolic blocks
Folic acid antagonists	Methotrexate; trimethoprim; triamterene; pentamidine; some anticonvulsants
Enzyme deficiency	Congenital or due to liver diseases
Vitamin B_{12} deficiency	Decreased folate uptake
Alcohol	
Vitamin C deficiency	
Increased requirements	
Extra demand	Pregnancy, cancer, lactation
Infancy	
Increased red cell production	
Increased excretion	Kidney dialysis or B_{12} deficiency

TABLE 2

Meaning of Laboratory Results in Folate or B_{12} Deficiency

Laboratory Test	Folic Acid Deficiency	B_{12} Deficiency
Serum folate	Reduced or Normal	Normal or mildly reduced
Serum B_{12}	Normal	Reduced or normal
MCV on CBC	Increased or normal	Increased or normal
Homocysteine	Increased	Increased or normal
RBC folate	Reduced	Normal
Methylmalonic acid	Normal unless renal insufficiency	Increased often rarely normal
Antibody to parietal cell or intrinsic factor	Absent	Present if due to pernicious anemia
Serum gastrin	Normal	Increased most often but can be normal

MCV on CBC denotes mean corpuscular volume on complete blood count.

hematologic levels but may also cause deterioration in the neurologic status of the patient.[18,19] If after testing a determination cannot be made about which deficiency exists, only one specific vitamin should be given, and the homocysteine and methylmalonic acid levels should be followed serially to determine which vitamin is causing the deficiency. Patients in whom cobalmin deficiency should be suspected include the elderly, patients on antiulcer medication, AIDS patients, vegetarians, patients who have undergone bowel surgery, and patients with dementia. A recent review on B_{12} deficiency has been published in this journal if further information is of interest.[20]

FOLATE AND PREVENTION OF NEURAL TUBE DEFECTS

The effectiveness of multivitamins in preventing neural tube defects (NTDs) was reported in 1991 by the Medical Research Council[21] and again in 1992 by Czeizel and Dudas.[22] Other studies have showed that folic acid alone may prevent both first-time occurrence of NTD and recurrent NTDs.[23-27] Investigators and several public health bodies have recommended that all women of childbearing age should receive a 0.4-mg supplement per day of folate. Women who have had a previous child with an NTD should receive 4 to 5 mg per day as near to the time of conception as possible and during early pregnancy. Following these recommendations has been postulated to reduce the chance of NTDs by 48% and higher dosages are estimated not to be any more effective.[27]

Many researchers and health officials have stated that increasing foods high in folate in the diet would also reduce the incidence of NTDs. This does not seem to be true. In a recent British trial, eating an additional 0.4 mg per day of folic acid naturally found in food was compared with foods fortified with similar amounts of folate and with folate supplements.[28] In these three groups, effects on RBC folate were monitored over 3 months. There was no significant change in levels in those assigned to the natural source group, while those in the other two groups had significant elevations in their levels. Therefore, it seems most prudent to advise supplementation for all women of childbearing age.

The reason folate is effective in reducing the incidence of NTDs is not clear. It may be due to a subtle folate deficiency during early pregnancy when the fetus is forming neural tissue. It is known that the fetal and uterine placental units require much larger amounts of the coenzyme folate than the nonpregnant woman.[29] Up to one third of all pregnancies worldwide are complicated by folate deficiencies, while US pregnancies are only affected 1% to 4% of the time.[30] The incidence is increased up to eight times by multiple gestations, teenaged pregnancies, and closely spaced successive pregnancies.[30] These low folate levels have been found with increased frequency in those mothers with NTD babies as compared with controls.[31]

A recent case-control study may point to a sec-

ond possible cause. These authors studied 41 mothers who gave birth to babies with NTDs and compared them with 50 controls.[32] In this study the metabolite homocysteine, which uses folate as a cofactor in its conversion, was studied. Previous studies had shown that homocysteine levels in normal pregnancies in healthy women tend to decrease.[33] The women with NTD births all had higher baseline homocysteine levels or had increased homocysteine levels with a methionine load. This makes the elevated homocysteine levels a possible source of increased risk of NTDs through the mechanism of as yet unknown congenital biochemical defects. Unrelated studies on folic acid and homocysteine have shown that folate administration alone or in combination with vitamin B_6 reduces homocysteine levels. (See discussion below on homocysteine levels and premature cardiovascular disease.) It is not currently recommended by any medical organization that routine folate or homocysteine levels be obtained, since all women who are pregnant should be treated with at least 400 µg of folate supplementation. Most prenatal vitamins contain this amount of folate. RBC folate levels should be obtained in those pregnant women with macrocytic anemia or macrocytosis.

HOMOCYSTEINE LEVELS AND PREMATURE CARDIOVASCULAR DISEASE

Homocystinuria, which is a rare genetic disorder associated with very high homocysteine levels, has been known to cause premature atherosclerosis.[34] Other conditions that are much more common, however, including folate deficiency, may elevate homocysteine levels to a lesser extent.[35] Early retrospective case-control studies indicated a possible relationship of these milder homocysteine elevations and atherosclerotic vascular disease.[35, 36] A prospective case-control study was later published in 1992 that also suggested this association.[37] This study revealed a mild increase in serum levels of homocysteine in men with myocardial infarctions. These increased levels could be responsible for a threefold increased incidence of myocardial infarction after other factors were controlled for. A retrospective cohort study recently published by Morrison et al[38] revealed that those with the lowest ranges of serum folic acid levels were found to have the highest levels of fatal myocardial infarction. This group had an overall risk 1.69 times that of those with the highest folate levels.

Homocystinemia does not appear to be a risk factor solely for ischemic heart disease either. A recent cross-sectional study involving over 1000 elderly patients from the Framingham cohort was performed to analyze the risk of strokes.[39] Forty-three percent of the men and 34% of the women had more than a 25% stenosis of the carotid arteries. Of these subjects, those who had high homocysteine levels were twice as likely to have this degree of stenosis as their counterparts with lower homocysteine levels. Plasma folate levels and intake of dietary folate were both inversely associated with the degree of carotid artery stenosis. Another study on stroke risk was performed with data from the First National Health and Nutrition Examination Survey by Giles et al.[40] This study showed that those patients with low folic acid levels were 1.4 times more likely to have a stroke than their counterparts with higher folic acid levels. Two other studies have had conflicting outcomes. One study using data from the Physician's Health Study reported that men with higher homocysteine levels had 1.2 times the risk of a stroke compared with those with lower homocysteine levels.[41] Conversely, another study showed no such increased risk with elevations in the homocysteine levels.[42]

Elevations in the homocysteine levels have also been postulated to be a risk factor for deep venous thrombosis (DVT). Two early studies showed that there was a possible association between DVT and homocysteine levels both in patients younger than age 40[43] and in the older population.[44] Finally, in a case-control format, 269 patients with DVT and 269 controls had their plasma homocysteine levels determined.[45] Twenty-eight of 269 patients with DVT had high levels of homocysteine as compared with only 13 of 269 controls.

What can we conclude from all these data? Homocysteine levels may be just a marker or even a major risk factor for many forms of vascular disease. A meta-analysis of 21 studies[46] recently was completed to analyze homocysteine. It estimated a risk 1.6 times greater for heart disease in men and 1.8 for women for each increase of 5 µmol/L in the homocysteine level. The authors estimated that 10% of the population's risk for coronary artery disease (CAD)

appears related to elevations in this metabolite. It also seemed to be an independent risk factor for cerebrovascular disease, as those with high homocysteine levels were 1.5 times more likely to experience stroke than those with lower levels. A similar effect was seen in peripheral vascular disease. Folate supplementation, according to statistical analysis, was theorized to be able to prevent 13,500 to 50,000 CAD deaths per year in the United States.

To date, it is unclear whether homocysteine is merely a marker for impending cardiovascular disease or more of a directly toxic metabolite intricately involved in the atherosclerotic process. One recent study[47] analyzed the effect of other cardiovascular risk factors on homocysteine levels. Investigators found that homocysteine levels were higher in men than in women and increased with age. In addition, levels also increased markedly with smoking and were directly related to cholesterol levels, blood pressure, and heart rate. They were inversely related to physical activity. On the other hand, some studies have shown that homocysteine is directly toxic to the vascular endothelium[48-50] and also that it facilitates oxidation of the LDL molecule thereby encouraging atherosclerotic plaques.[51-53] Randomized, blinded, placebo-controlled trials should be carried out before the benefits of folate supplementation can be proved. However, if an individual patient has an adverse cardiovascular event and has minimal other risk factors, a test for homocysteine level should be ordered. If the level is elevated and B_{12} deficiency does not coexist, supplementation with 1 mg of folic acid per day may be indicated.

SUMMARY

Though folic acid deficiency with macrocytic anemia is uncommon, studies are revealing new reasons to consider folate supplementation other than overt deficiency. Supplementation of 400 µg per day to women of childbearing age may reduce the chance of neural tube defects by 45%. Also, the role of homocysteine and folate administration may be intricately involved in the atherosclerotic process. Folate administration, although proven to reduce homocystine levels, has not currently been shown to reduce levels of any cardiovascular disease. The family physician should monitor developments in this important area. Some governmental agencies are recommending folate enrichment for some foods. Unfortunately, widespread supplementation of food with folate alone may cause outbreaks of neurologic damage in those with undiagnosed pernicious anemia.

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