FOLATE: A KEY TO OPTIMIZING HEALTH AND REDUCING DISEASE RISK IN THE ELDERLY

Inadequate folate status is associated with an increased risk for chronic diseases that may have a negative impact on the health of the aging population. Folate, a water-soluble vitamin, includes naturally occurring food folate and synthetic folic acid in supplements and fortified foods. Inadequate folate status may result in hyperhomocysteinemia, a significant risk factor for atherosclerotic vascular disease, changes in DNA that may result in pro-carcinogenic effects and increased risk for cognitive dysfunction. Folate status may be negatively influenced by inadequate intake, genetic polymorphisms and interactions with various drugs. In the US, folic acid is now added to enriched grain products and continues to be included in the majority of ready-to-eat breakfast cereals. Recent data indicate that the folate status in the US population has improved significantly, presumably due to the effects of fortification. Folic acid (not food folate) intake in excess of the Tolerable Upper Intake Level may mask the diagnosis of a vitamin B12 deficiency, which is more prevalent in the elderly than younger individuals. When folic acid supplements are recommended, a multivitamin that includes vitamin B12 should also be advised. To safely and effectively increase folate intake in the elderly, naturally occurring folate-rich food sources should be promoted. Folate-rich foods include orange juice, dark green leafy vegetables, asparagus, strawberries and legumes. These foods are also excellent sources of other health-promoting nutrients associated with chronic disease risk reduction.

KEY TEACHING POINTS:

- Adequate folate intake and status has been associated with reduced risk for chronic conditions that may particularly affect the elderly, including hyperhomocysteinemia, a risk factor for vascular disease, cancer and cognitive dysfunction.

- Folate status can be impacted negatively by low intake, genetic polymorphisms or use of antifolate medications such as methotrexate, which is commonly used to treat rheumatoid arthritis.

- Folic acid intakes in excess of the Tolerable Upper Intake Level (UL) (1 mg/day) may mask the symptoms associated with a vitamin B12 deficiency and allow for the progression of irreversible neurological damage. The UL is applicable to intakes of synthetic folic acid, but not folate occurring naturally in foods.

- The elderly are at increased risk for vitamin B12 deficiency due to food-bound malabsorption associated with hypochlorhydria or achlorhydria, conditions which are prevalent among the elderly.

- Patients taking antifolate medications or presenting with megaloblastic anemia or hyperhomocysteinemia should be screened for both folate and vitamin B12 deficiency. Chronic therapy with folic acid for the elderly should be coupled with vitamin B12 supplements.

- Practitioners should encourage intake of folate-rich foods such as orange juice, dark green leafy vegetables, asparagus, strawberries and legumes, which can provide a variety of other nutrients beneficial to the health status of the elderly without the danger of exceeding the UL.

INTRODUCTION

By the year 2015, it is estimated that there will be over 45 million individuals 65 years of age and older living in the United States, representing a 31% increase in this age group compared to the 2000 US census [1].

Nutritional status of the elderly is of great significance as it is associated with morbidity and mortality in this age group [2]. Folate nutriture may be especially important since it is associated with several health issues that particularly affect the elderly, such as cardiovascular disease, cancer and cognitive function. This report will review current issues related to folate intake and nutriture in the elderly, including the impact of food fortification with folic acid and the role of folate in reducing risk for chronic disease. Recommendations for practitioners concerning folate intake in the elderly are included.

BACKGROUND

Folate Biochemistry, Food Sources and Bioavailability, Genetic Polymorphisms and Requirements

"Folate" is a generic term used to denote both naturally occurring folate in foods (food folate) and the synthetic form of the vitamin (folic acid). The primary chemical form of folate occurring in nature is a pteroylglutamate compound that contains a side chain of conjugated glutamate molecules. Folate must be cleaved to the monoglutamate form by an intestinal conjugase enzyme before being absorbed in the intestinal tract [3]. Food folates are concentrated in select foods such as orange juice, dark green leafy vegetables, dried beans and peas, asparagus, strawberries and peanuts (Table 1). The synthetic form of the vitamin, folic acid, exists in the monoglutamate form and is more readily absorbed than the natural form because it does not require intestinal enzymatic cleavage prior to absorption. Synthetic folic acid is found in vitamin and other nutritional supplements, as well as fortified foods. Cereal grain products labeled as "enriched" have added folic acid and include flour, corn meal, grits, breads, rice, pasta and hundreds of combination foods containing these

[4]. Grain fortification was mandated by the Food and Drug Administration (FDA) to assist women of childbearing age with meeting folic acid intake recommendations for neural tube defect risk reduction.

Bioavailability estimates for folate range from approximately 50% for naturally occurring food folate [5] to 100 percent for synthetic folic acid consumed on an empty stomach [3]. It is estimated that approximately 85% of synthetic folic acid taken with food or as a component of a fortified food is absorbed [6,7]. The current Dietary Reference Intakes (DRIs) express folate requirements in terms of Dietary Folate Equivalents (DFEs) to take into account this difference in bioavailability between food folate and synthetic folic acid [7,8].

Folate coenzymes participate in biochemical processes involving single carbon group transfers, including the metabolism of amino acids and synthesis of purines and pyrimidines (Fig. 1, reaction #1) that are incorporated into DNA and RNA [9]. Folate is integrally involved in the remethylation of homocysteine to form methionine (Fig. 1, reaction #2), which is subsequently converted to S-adenosylmethionine, the latter being the primary supplier of methyl groups in a considerable number of chemical reactions including methylation of DNA, RNA, proteins, phospholipids and neurotransmitters (Fig. 1, reaction #3) [9]. DNA methylation patterns are thought to play an important role in gene expression [10].

Current research interest in folate nutriture includes the effect of genetic variations that influence folate utilization. Several single nucleotide polymorphisms associated with the folate metabolic cycle have been identified, the most intensely studied to date is the methylenetetrahydrofolate reductase (MTHFR) 677C3T variant. The MTHFR enzyme catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (Fig. 1,

TABLE 1 – FOLATE CONTENT OF SELECTED NON-FORTIFIED FOODS		
Food item	**Measure**	**Folate content (µg DFEs)**
Beef liver	1 slice (85 g)	185
Lentils, cooked	½ cup	180
Chickpeas, cooked	½ cup	140
Beans (black, kidney, navy), cooked	½ cup	115–130
Spinach, raw	1 cup	110
Spinach, cooked	½ cup	100
Asparagus, cooked	5 spears	100
Greens (mustard, turnip), cooked	½ cup	85–90
Orange juice, ready to drink	1 cup	80
Strawberries, fresh	8 medium	80
Broccoli, cooked	½ cup	50
Tomato juice	1 cup	50
Peanuts, dry roasted	1 oz	40
Lettuce, romaine	1 cup	40
Cantaloupe, fresh	¼ medium	40
Lettuce, iceberg	1 cup	30
Banana	1 medium	20
Grapefruit	½ medium	15

Abbreviations: DFEs dietary folate equivalents.

reaction #4), which then serves as a carbon donor to homocysteine to form methionine. Individuals who are homozygous for this polymorphism may have MTHFR enzyme activities up to 50% lower than those without the polymorphism [11, 12]. This polymorphism increases the risk for hyperhomocysteinemia when coupled with low folate status [13, 14], which supports the suggestion that individuals who are homozygous for the 677C3T polymorphism have a higher folate requirement [15]. The population frequency of homozygotes for the 677C3T MTHFR polymorphism is approximately 12% for US Caucasians, 21% for US Hispanics and 1% for African Americans [16], suggesting that a substantial portion of the US population may be at increased risk for altered folate metabolism.

Adequate folate status has been defined as a serum or plasma folate concentration above 7 nmol/L (3 ng/mL) and a red blood cell concentration above 305 nmol/L (140 ng/mL) [7]. Plasma homocysteine also has been used as a folate status functional indicator, with 14 μMol/L most often used as a cutoff to define elevated concentrations [7]. The Recommended Dietary Allowance (RDA) for folate for adults aged 51 and greater is 400μg/day of DFEs [7]. The RDA for older adults was largely based on folate requirements in younger individuals because at the time there were no metabolic studies providing data for adults over age 65 [7]. Subsequently, Kauwell et al. [17] have demonstrated that a folate intake of 400 μg/day from diet and supplements was more effective than 200 μg/day in normalizing folate and homocysteine status following moderate folate depletion in healthy postmenopausal women (60–85 years), suggesting that the previous folate RDA of 180 or 200 μg/day [18] may have been insufficient for this age group. As part of the DRIs, a Tolerable Upper Intake Level (UL) was set at 1,000 μg/day of synthetic folic acid. The UL was not based on toxicity criteria since folic acid intakes at or above 15 mg/day have not been found to have adverse effects [7], but rather on the ability of folic acid at high doses to mask the diagnosis of anemia associated with a vitamin B12 deficiency [7,19]. It is important to note that the UL is applicable to intake of synthetic folic acid, and not food folate.

FOLATE'S ROLE IN HEALTH AND DISEASE

Potential Effect of Folic Acid Fortification and Supplementation to Mask the Diagnosis of a Vitamin B12 Deficiency

The issue of masking the diagnosis of the hematological symptoms associated with a vitamin B12 deficiency is of particular concern to the elderly since it is estimated that 10% to 15% of people over the age of 60 are affected by vitamin B12 deficiency [20]. This deficiency primarily occurs because elderly individuals are at higher risk for food-bound vitamin B12 malabsorption related to reduced gastric acid (hypochlorhydria) associated with atrophic gastritis, a condition quite prevalent among the elderly. Hypochlorhydria only affects absorption of protein-bound vitamin B12 in foods and not the crystalline form of the vitamin found in fortified foods and supplements. Pernicious anemia, which affects a smaller percentage of elderly individuals, is associated with a lack of intrinsic factor that may be due to an autoimmune disorder that destroys gastric parietal cells. Intrinsic factor is needed to ensure absorption of vitamin B12; therefore; patients with pernicious anemia are unable to absorb both the protein-bound and crystalline forms of vitamin B12. When a patient with a vitamin B12 deficiency is treated with folic acid, the hematological indices associated with macrocytic anemia revert to normal while the irreversible neurological abnormalities progress. The result is that folic acid treats the symptoms, but not the

disease, and is referred to as "masking" the diagnosis.

There may be many undiagnosed cases of atrophic gastritis and concurrent vitamin B12 deficiency among the elderly and there is concern that folic acid fortification and supplementation may be sufficient to reverse the hematological abnormalities associated with B12 deficiency, thus allowing the deficiency to remain undiagnosed [21,22]. Because a significant number of individuals over age 50 may be affected by atrophic gastritis, the Institute of Medicine recommends that they meet the vitamin B12 RDA of 2.4 μg/day primarily through intake of synthetic vitamin B12 in fortified foods or supplements since absorption of this form of the vitamin is not adversely affected by the condition [7,19].

The FDA estimated that folic acid intake in middle-aged and elderly adults would increase by 70 to 120 μg/day after fortification [23]. A significant increase in blood folate concentrations and decrease in plasma homocysteine concentrations in population subgroups including the elderly has been observed [24, 25] as a result of the folic acid fortification program that became mandatory in January 1998. In addition to folic acid consumed as a component of enriched cereal grain products, ready-to-eat breakfast cereals can provide a significant amount of folic acid in the diets of the elderly. A recent analysis of the folic acid content in ready-to-eat breakfast cereals [26] indicated that one half of the cereals tested contained folic acid amounts exceeding 150% of the label declaration.

This study also evaluated the amount of ready-to-eat cereal adults would consume and found that median serving sizes were 50% higher for females and 100% higher for males compared to the nutrition label portion. Because cold breakfast cereals are a major contributor of folic acid and a major component of the diet for the elderly [27], they may be consuming substantially more folic acid than stated on the cereal's Nutrition Facts panel.

Daily use of folic acid-containing supplements can contribute significantly to folic acid intake. Depending

FIGURE 1 – PRINCIPAL COMPONENTS OF THE FOLATE BIOCHEMICAL CYCLE
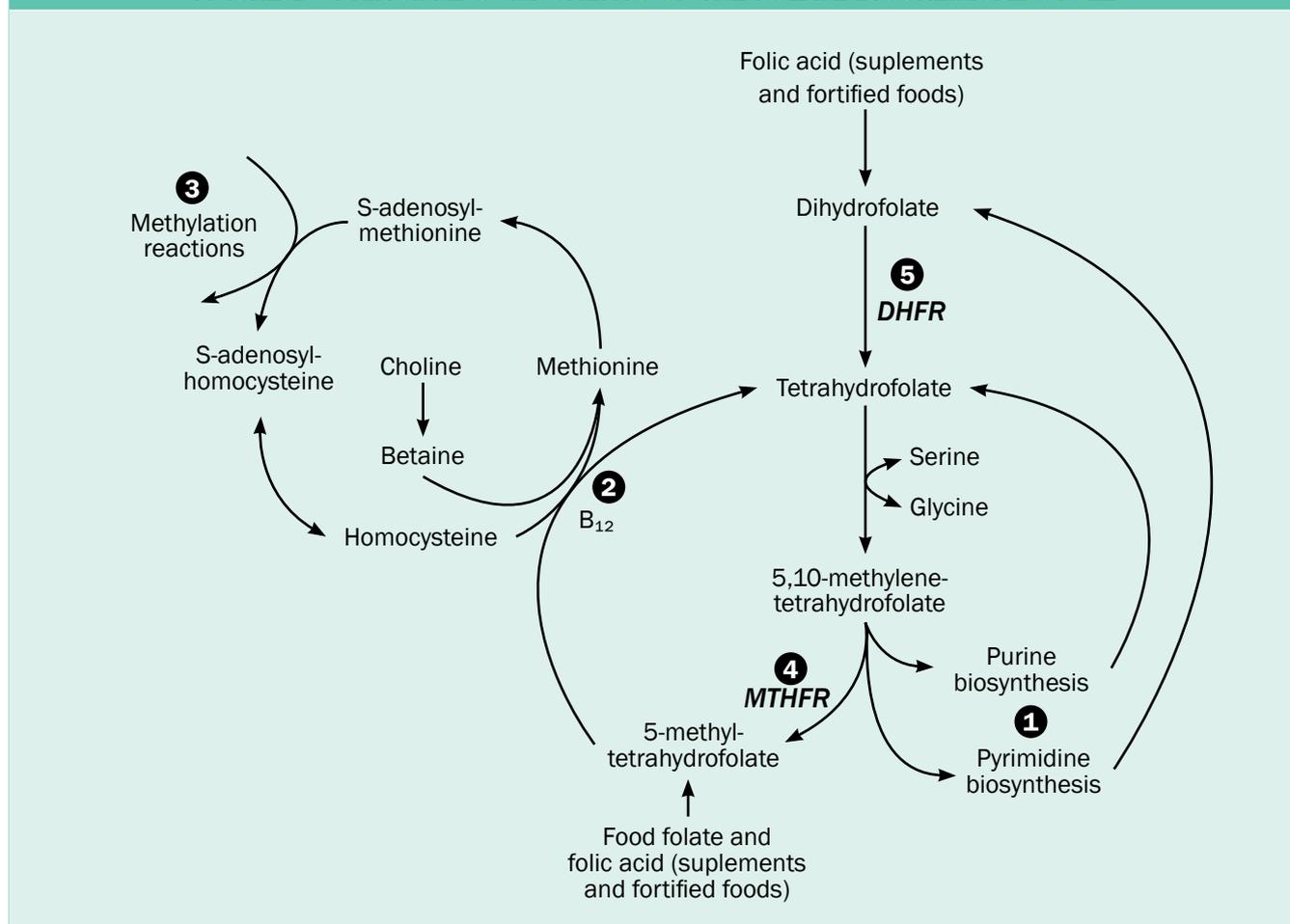
Abbreviations: DHFR _ dihydrofolate reductase; MTHFR _ methylenetetrahydrofolate reductase. Reactions: • Biosynthesis of nucleotides for incorporation into DNA and RNA; • Remethylation of homocysteine to form methionine (vitamin B12 serves as a coenzyme in this reaction); • Methylation of substrates, including DNA, RNA, phospholipids, and proteins; • MTHFR, which catalyzes the formation of 5-methyltetrahydrofolate needed for methylation reactions; • Dihydrofolate reductase enzyme.

on the study, use of any supplements by individuals aged 60 and older ranges from 39 to 55 percent [28–30]. Of concern is the fact that individuals taking a multivitamin containing 400 μg folic acid and consuming one serving of a folic acid containing fortified breakfast cereal (400 μg folic acid/serving) every day may have daily synthetic folic acid intakes of at least 750 μg from these two sources alone. Additional folic acid may be obtained through other fortified foods consumed throughout the day.

Therefore, individuals consuming supplements, fortified cereals and other folic acid fortified foods on a daily basis may exceed the UL of 1,000 μg/day of synthetic folic acid. Future population-based studies evaluating folate intake post-fortification will be useful in determining folic acid intake amounts by the elderly.

Since the health benefits associated with maintaining an optimal folate status are clearly established, it is important that clinicians advise their elderly patients to ensure adequate folate intake through increased consumption of folate-dense food sources. Naturally occurring food folate is not associated with the negative pharmacological effects described above [7] and yet provides the health benefits of this essential nutrient.

INTERACTIONS WITH DRUGS, FOODS, AND NUTRIENTS

Several drugs have been known to have a significant interaction with folate either through their effects on absorption or metabolism. A common drug used by the elderly is methotrexate, which is used to treat neoplastic disease and works as a folate antagonist by targeting a key enzyme in folate metabolism (dihydrofolate reductase, Fig. 1, reaction #5) and thereby reducing production of nucleotides needed for DNA synthesis. However, low-dose methotrexate also has been found to be efficacious in treating non-neoplastic

conditions including asthma, inflammatory bowel disease, psoriasis and especially rheumatoid arthritis [9]. For rheumatoid arthritis patients, the primary reason for discontinuation of methotrexate treatment is side effects associated with toxicity. Many of the side effects mimic folate deficiency and include gastrointestinal problems (nausea, diarrhea), stomatitis, headache, vertigo, pneumonitis, leucopenia, thrombopenia, hair loss and infections [31]. Individuals treated for rheumatoid arthritis using methotrexate have reduced blood folate concentrations [32–34] and elevated plasma homocysteine concentrations [34–36]. Supplementation with folic acid in conjunction with methotrexate treatment has been found to either reduce the incidence of side effects or improve folate and homocysteine status without significantly decreasing treatment efficacy [31, 34, 37, 38]. It is recommended that individuals treated with methotrexate for rheumatoid arthritis be concurrently supplemented with folic acid [34, 36], while treatment efficacy should be closely monitored.

Chronic use of the anticonvulsants diphenylhydantoin (e.g., phenytoin, Dilantin®) and phenobarbital has been associated with impaired folate metabolism [7]. Patients with inflammatory bowel disorders who are treated with salicylazosulfapyridine (e.g., sulfasalazine, Azulfidine®) are also at risk of developing a folate deficiency since this drug has been shown to inhibit folate absorption and metabolism in humans [7]. The folate status of epileptic patients being treated with anticonvulsant drugs or patients with inflammatory bowel disease treated with sulfasalazine should be carefully monitored.

Chronic use of alcohol also has been associated with folate deficiency. Alcohol intake may impair absorption and hepatobiliary metabolism of folate and may exacerbate the effects of low folate intake often observed in chronic alcohol users [39]. When mod-

erate alcohol consumption is coupled with low folate intake, the risk of certain types of cancer significantly increases (see next section).

Potential interactions between grapefruit juice and certain prescription drugs such as antihistamines, antihypertensives and cholesterol-lowering statins have been reported and recently reviewed [40]. Although not considered to be a good dietary source of folate, grapefruit juice has been shown to positively contribute to folate intake in the elderly [41], and they may additionally benefit from other nutrients (e.g., vitamin C, potassium) found in grapefruit juice. At this time, only a limited number of prescription drugs are known to be affected [42]. However, reports of potential drug interactions may dissuade individuals from continuing to include grapefruit juice in their diet. Patients should consult with their physicians or pharmacists to determine if a drug they use is one of a small number that might be affected.

CHRONIC DISEASE

Folate serves as a coenzyme in the remethylation of homocysteine to form methionine (Fig. 1, reaction #2). It is now widely accepted that elevated blood homocysteine concentrations are an independent risk factor for vascular disease involving the coronary, cerebral and peripheral vessels as well as thromboembolism [43–45]. Independent of other factors that may influence homocysteine concentrations, blood homocysteine increases with age and is generally found to be higher in men than women [46,47]. Folic acid supplementation (+/- 500 μg/day) has been found to effectively reduce blood homocysteine concentrations to a plateau beyond which additional folic acid has no further homocysteine lowering effect [43,48,49].

Individuals with elevated homocysteine or who have low blood folate concentrations benefit the most from folic acid supplementation.

Individuals who are homozygous for the MTHFR polymorphism have higher homocysteine concentrations compared to those without the polymorphism [50, 51]. However, there appears to be little evidence that these individuals are at increased risk for vascular disease [12, 50]. The question remains as to whether supplemental folic acid can reduce the risk for vascular disease, and a number of intervention trials are currently in progress [52].

Adequate folate intake or status has also been associated with reduced risk for certain cancers, most notably colorectal and cervical cancer [53], and more recently breast cancer.

Potential mechanisms include disruption of DNA integrity or repair systems or altered methylation [54]. Hypo- or undermethylation of DNA has been associated with increased risk for carcinogenesis [54], and hypomethylation of lymphocyte DNA has been observed in postmenopausal women fed moderately low folate diets [55,56]. The most convincing epidemiological evidence associating folate intake with cancer risk exists for colorectal cancer, with the results of several prospective cohort studies supporting a statistically significant inverse association with the risk of pre-cancer adenomas [57] and colorectal cancer [58,59]. In the Physician's Health Study, the MTHFR polymorphism was associated with a significantly decreased risk of colon cancer [60], although this protective effect was negated with either low folate status or increased alcohol intake. Presence of the MTHFR polymorphism also has been associated with decreased risk of acute lymphocytic leukemia [61]. The exact nature of this protective effect is not yet understood. The association between folate and cervical cancer risk is less supported, although there is evidence to suggest that low folate status coupled with the presence of other risk factors for cervical cancer, such as human papil-lomavirus, may increase risk [62, 63].

There is mounting evidence that folate is associated with the risk for breast cancer, particularly in combination with alcohol intake. Alcohol has been known to adversely affect folate metabolism [39]. Recent data from cohort groups including the Nurses' Health Study [64], the Iowa Women's Health Study [65] and the Canadian National Breast Screening Study [66], support an inverse association between risk of breast cancer and folate intake with increased alcohol intake. The nature of the folate/alcohol interaction is such that increased folate intake appears to impart protection in individuals within a defined alcohol intake level (greater than 14 g/day) compared to those within the same alcohol intake level, but with lower folate intakes [64,66]. Intervention trials will be needed to further elucidate folate's potential role in cancer prevention.

COGNITIVE FUNCTION, DEMENTIA, AND ALZHEIMER'S 'S DISEASE

A growing body of evidence supports a role for folate or homocysteine in cognitive function, dementia and Alzheimer's disease. Several studies, as summarized by Selhub et al. [67], identify associations between low folate status [68–76] or elevated homocysteine concentrations [71,73,75–78] and cognitive dysfunction. In the Nun Study, serum folate concentrations were strongly negatively correlated with brain atrophy in Catholic sisters with a significant number of Alzheimer's disease lesions [79]. A strength of this study is that participants ate from the same kitchen and had a very similar lifestyle and environment throughout their lives. Recently, an evaluation of the Framingham Study cohort found 40% to 80% higher adjusted relative risks for dementia and Alzheimer's disease, respectively, for each one standard deviation increase in plasma homocysteine as compared to baseline measurements [80].

Relative risks were doubled when plasma homocysteine concentrations exceeded 14 μMol/L, a value commonly used as a cutoff to define elevated concentrations. Other recent reports, including a UK cohort study [81], a Swedish population-based longitudinal study [82] and an NHANES study [83], support an association between folate or homocysteine status and cognitive function or Alzheimer's disease. The mechanism by which folate is associated with dementia is not clear, but may involve folate's role in the synthesis of S-adenosylmethionine used for adequate methylation of neurotransmitters, phospholipids or myelin in brain tissue, or the potential ability of elevated homocysteine to cause vascular disease that may result in brain ischemia [67]. Randomized trials are needed to establish whether there is a causal relationship between folate status and dementia or if low folate status simply exacerbates an existing condition.

CONCLUSION

Studies have shown an association between folate and vascular disease, certain cancers, and dementia and Alzheimer's disease, conditions that can significantly impact on the elderly population. These associations identify folate as an important nutrient that may contribute to reduced morbidity and mortality in the elderly. However, practitioners must also consider important issues for the elderly, including the presence of undiagnosed vitamin B12 deficiency and chronic use of drugs (including alcohol) that may interfere with folate metabolism and impair folate status.

Recent studies evaluating folate status in the general population suggest that folate intakes have increased substantially following food fortification and that many older individuals may now be consuming folic acid in excess of the UL. This may be of particular concern in individuals who take a vitamin supplement containing folic acid and consume ready-to-eat fortified

breakfast cereals every day. Individuals presenting with a macrocytic megaloblastic anemia should be screened for both folate and vitamin B12 deficiency, and folic acid and vitamin B12 supplementation in the elderly should be coupled to reduce the chance of neurological damage from an undiagnosed B12 deficiency. Serum vitamin B12 concentrations have limited sensitivity and specificity for diagnosing a vitamin B12 deficiency in the elderly [84], and serum methylmalonic acid as well as plasma homocysteine concentrations in conjunction with serum B12 may be useful in this diagnosis. Individuals taking medications known to interact with folate should be screened for low folate status.

Practitioners should educate patients about the benefits of foods rich in folate. Because naturally occurring folate is not widespread in the food supply, it is important for practitioners to educate patients about which foods can supply ample amounts of folate (Table 1). Consumption of folate-rich foods provides the added benefit of increasing intake of other nutrients and food components associated with good health, including the antioxidant vitamins A and C, potassium, fiber and a variety of phytochemicals. Many folate-rich foods are fruits and vegetables, adequate intakes of which have been strongly associated with decreased risk for chronic disease [85, 86].

Consumption of foods containing folate can help individuals meet public health recommendations for fruit and vegetable intake [87, 88]. Folate-rich foods also tend to be lower in calories, fat and sodium and higher in fiber, so they may be especially suitable for inclusion in diets to assist with high blood pressure, cardiovascular health or diabetes management. Fruits and vegetables also serve as a source of water that can support hydration, an important health issue for the elderly. Practitioner awareness of issues surrounding folate nutriture along with promotion of folate-rich foods can assist this population group with optimizing their health status.

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References

1. United States Census Bureau: "Table NP-T3-D. Projections of the Total Resident Population by 5-Year Age Groups, and Sex with Special Age Categories: Middle Series, 2011 to 2015." US Census Bureau Website. http://www.census.gov/population/www/projections/popproj.html Accessed December 3, 2002.

2. Wahlqvist ML, Savige GS: Interventions aimed at dietary and lifestyle changes to promote healthy aging. Eur J Clin Nutr 54: S148–S156, 2000.

3. Gregory 3rd JF: Bioavailability of folate. Eur J Clin Nutr 51(Suppl 1):S54–S59, 1997.

4. Food Standards: "Amendment of the Standards of Identity for Enriched Grain Products to Require Addition of Folic Acid. Final Rule." (Codified at 21 CFR Part 136, 137, and 139) 61 Federal Register 8781–8807, 1996.

5. Sauberlich HE, Kretsch MJ, Skala JH, Johnson HL, Taylor PC: Folate requirement and metabolism in nonpregnant women. Am J Clin Nutr 46:1016–1028, 1987.

6. Pfeiffer CM, Rogers LM, Bailey LB, Gregory 3rd JF: Absorption of folate from fortified cereal-grain products and of supplemental folate consumed with or without food determined by using a dual-label stable-isotope protocol. Am J Clin Nutr 66:1388–1397, 1997.

7. Institute of Medicine, Food and Nutrition Board: "Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline." Washington, DC: National Academy Press, 1998.

8. Suitor CW, Bailey LB: Dietary folate equivalents: Interpretation and application. J Am Diet Assoc 100:88–94, 2000.

9. Bailey LB, Moyers S, Gregory 3rd JF: Folate. In Bowman BA, Russell RM (eds): "Present Knowledge in Nutrition," 8th ed. Washington, DC: ILSI Press, pp 214–229, 2001.

10. Momparler RL, Bovenzi V: DNA methylation and cancer. J Cell Physiol 183:145–154, 2000.

11. Kang S-S, Zou J, Wong PWK, Kowalisyn J, Strokosch G: Intermediate homocysteinemia: A thermolabile variant of methylenetetrahydrofolate reductase. Am J Hum Genet 43:414–421, 1988.

12. Bailey LB, Gregory 3rd JF: Polymorphisms of methylenetetrahydrofolate reductase and other enzymes: Metabolic significance, risks and impact on folate requirement. J Nutr 129:919–922, 1999.

13. Jacques PF, Bostom AG, Williams RR, Ellison RC, Eckfeldt JH, Rosenberg IH, Selhub J, Rozen R: Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. Circulation 93:7–9, 1996.

14. Kauwell GPA, Wilsky CE, Cerda JJ, Herrlinger-Garcia K, Hutson AD, Theriaque DW, Boddie A, Rampersaud GC, Bailey LB: Methylenetetrahydrofolate reductase mutation (677C3T) negatively influences plasma homocysteine response to marginal folate intake in elderly women. Metabolism 49:1440–1443, 2000.

15. Rosenberg IH, Rosenberg LE: The implications of genetic diversity for nutrient requirements: The case of folate. Nutr Rev 56: S47–S53, 1998.

16. Botto LD, Yang Q: 5,10-methylenetetrahydrofolate reductase gene variants and congenital anomalies: A HuGE review. Am J Epidemiol 151:862–877, 2000.

17. Kauwell GPA, Lippert BL, Wilsky CE, Herrlinger-Garcia K, Hutson AD, Theriaque DW, Rampersaud GC, Cerda JJ, Bailey LB: Folate status of elderly women following moderate folate depletion responds only to a higher folate intake. J Nutr 130:1584–1590, 2000.

18. Food and Nutrition Board: "Recommended Dietary Allowances," 10th ed. Washington, DC: National Academy of Science, 1989.

19. Ho C, Kauwell GPA, Bailey LB: Practitioners' guide to meeting the vitamin B-12 Recommended Dietary Allowance for people aged 51 years and older. J Am Diet Assoc 99:725–727, 1999.

20. Baik HW, Russell RM: Vitamin B12 deficiency in the elderly. Annu Rev Nutr 19:357–377, 1999.

21. Rothenberg SP: Increasing the dietary intake of folate: Pros and cons. Semin Hematol 36:65–74, 1999.

22. Mills JL: Fortification of foods with folic acid—how much is enough? N Eng J Med 342:1442–1445, 2000.

23. Food Standards: amendment of the standards of identity for enriched grain products to require addition of folic acid. Fed Regist 58(197):53305–53312, 1993.

24. Jacques PF, Selhub J, Bostom AG, Wilson PWF, Rosenberg IH: The effect of folic acid fortification on plasma folate and total homocysteine concentrations. N Engl J Med 340:1449–1454, 1999.

25. Choumenkovitch SF, Jacques PF, Nadeau MR, Wilson PWF, Rosenberg IH, Selhub J: Folic acid fortification increases red blood cell folate concentrations in the Framingham Study. J Nutr 131: 3277–3280, 2001.

26. Whittaker P, Tufaro PR, Rader JI: Iron and folate in fortified cereals. J Am Coll Nutr 20:247–254, 2001.

27. Tucker KL, Selhub J, Wilson PWF, Rosenberg IH: Dietary intake pattern relates to plasma folate and homocysteine concentrations in the Framingham Heart Study. J Nutr 126:3025–3031, 1996.

28. U.S. Department of Health and Human Services: "Use of Dietary Supplements in the United States, 1988–94." Series 11: Data from the National Health Examination Survey, the National Health and Nutrition Examination Surveys and the Hispanic Health and Nutrition Examination Survey No. 244. DHHS Publication No. 99–1694. Hyattsville, MD: National Center for Health Statistics, 1999.

29. Vitolins MZ, Quandt SA, Case LD, Bell RA, Arcury TA, Mc-Donald J: Vitamin and mineral supplement use by older rural adults. J Gerontol 55A:M613–M617, 2000.

30. Koehler KM, Baumgartner RN, Garry PJ, Allen RH, Stabler SP, Rimm EB: Association of folate intake and serum homocysteine in elderly persons according to vitamin supplementation and alcohol use. Am J Clin Nutr 73:628–637, 2001.

31. van Ede AE, Laan RFJM, Blom HJ, De Abreu RA, van de Putte LBA: Methotrexate in rheumatoid arthritis: An update with focus on mechanisms involved in toxicity. Semin Arthritis Rheum 27: 277–292, 1998.

32. Leeb BF, Witzmann G, Ogris E, Studnicka-Benke A, Andel I, Schweitzer H, Smolen JS: Folic acid and cyanocobalamin levels in serum and erythrocytes during low-dose methotrexate therapy of rheumatoid arthritis and psoriatic arthritis patient. Clin Exp Rheumatol 13:459–463, 1995.

33. Andersen LS, Hansen EL, Knudsen JB, Wester JU, Hansen GVO, Hansen TM: Prospectively measured red cell folate levels in methotrexate treated patients with rheumatoid arthritis: Relation to withdrawal and side effects. J Rheumatol 24:830–837, 1997.

34. Morgan SL, Baggott JE, Lee JY, Alarcon GS: Folic acid supplementation prevents deficient blood folate levels and hyperhomocysteinemia during longterm, low dose methotrexate therapy for rheumatoid arthritis: Implications for cardiovascular disease prevention. J Rheumatol 25:441–446, 1998.

35. Haagsma CJ, Blom HJ, van Riel PLCM, van't Hof MA, Giesendorf BAJ, van Oppenraaij-Emmerzaal D, van de Putte LBA: Influence of suphasalazine, methotrexate, and the combination of both on plasma homocysteine concentrations in patients with rheumatoid arthritis. Ann Rheum Dis 58:79–84, 1999.

36. Slot O: Changes in plasma homocysteine in arthritis patients starting treatment with low-dose methotrexate subsequently supplemented with folic acid. Scan J Rheumatol 30:305–307, 2001.

37. Ortiz Z, Shea B, Suarez-Almazor ME, Moher D, Wells GA, Tugwell P: The efficacy of folic acid and folinic acid in reducing methotrexate gastrointestinal toxicity in rheumatoid arthritis. A metaanalysis of randomized controlled trials. J Rheumatol 25:36–43, 1998.

38. Van Ede AE, Laan RFJM, Rood MJ, Huizinga TWJ, van de Laar MAFJ, van Denderen CJ, Westgeest TAA, Romme TC, de Rooij DRAM, Jacobs MJM, de Boo TM, van der Wilt G-J, Severens JL, Hartman M, Krabbe PFM, Dijkmans BAC, Breedveld FC, van de Putte LBA: Effect of folic or folinic acid supplementation on the toxicity and efficacy of methotrexate in rheumatoid arthritis. Arthritis Rheum 44:1515–1524, 2001.

39. Halsted CH: Alcohol and folate interactions: Clinical implications. In Bailey LB (ed): "Folate in Health and Disease." New York: Marcel Dekker, pp 313–327, 1995.

40. Kane GC, Lipsky JJ: Drug-grapefruit juice interactions. Mayo Clin Proc 75:933–942, 2000.

41. Koehler KM, Pareo-Tubbeh SL, Romero LJ, Baumgartner RN, Garry PJ: Folate nutrition and older adults: Challenges and opportunities. J Am Diet Assoc 97:167–173, 1997.

42. Greenblatt DJ, Patki KC, von Moltke LL, Shader RI: Drug interactions with grapefruit juice: An update. J Clin Psychopharmacol 21:357–359, 2001.

43. Boushey CJ, Beresford SAA, Omenn GS, Motulsky AG: A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. JAMA 274:1049–1057, 1995.

44. Danesh J, Lewington S: Plasma homocysteine and coronary heart disease: Systematic review of published epidemiological studies. J Cardiovasc Risk 5:229–232, 1998.

45. Refsum H, Ueland PM, Nygard O, Vollset SE: Homocysteine and cardiovascular disease. Annu Rev Medicine 49:31–62, 1998.

46. Selhub J, Jacques PF, Wilson PWF, Rush D, Rosenberg IH: Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. JAMA 270:2693–2698, 1993.

47. Durand P, Prost M, Loreau N, Lussier-Cacan S, Blache D: Impaired homocysteine metabolism and atherothrombotic disease. Lab Invest 81:645–672, 2001.

48. Homocysteine Lowering Trialists' Collaboration: Lowering blood homocysteine with folic acid based supplements: Meta-analysis of randomised trials. BMJ 316:894–898, 1998.

49. Brouwer IA, van Dusseldorp M, Thomas CMG, Duran M, Hautvast JGAJ, Eskes TKAB, Steegers-Theunissen RPM: Low-dose folic acid supplementation decreases plasma homocysteine concentrations: A randomized trial. Am J Clin Nutr 69:99–104, 1999.

50. Brattstrom L, Wilcken DEL, Ohrvik J, Brudin L: Common methylenetetrahydrofolate reductase gene mutation leads to hyperhomocysteinemia but not to vascular disease. The result of a metaanalysis. Circulation 98:2520–2526, 1998.

51. Gudnason V, Stansbie D, Scott J, Bowron A, Nicaud V, Humphries S: C677T (thermolabile alanine/valine) polymorphism in methylenetetrahydrofolate reductase (MTHFR): Its frequency and impact on plasma homocysteine concentration in different European populations. Atherosclerosis 136:347–354, 1998.

52. Eikelboom JW, Lonn E, Genest J, Hankey G, Yusuf S: Homocyst(e)ine and cardiovascular disease: A critical review of the epidemiologic evidence. Ann Intern Med 131:363–375, 1999.

53. Rampersaud GC, Bailey LB, Kauwell GPA: Folate: Relationship to colorectal and cervical cancer. Review and recommendations for practitioners. J Am Diet Assoc 102:1273–1282, 2002.

54. Choi S-W, Mason JB: Folate and carcinogenesis: An integrated scheme. J Nutr 130:129–132, 2000.

55. Jacob RA, Gretz DM, Taylor PC, James SJ, Pogribny IP, Miller BJ, Henning SM, Swendseid ME: Moderate folate depletion increases plasma homocysteine and decreases lymphocyte DNA methylation in postmenopausal women. J Nutr 128:1204–1212, 1998.

56. Rampersaud GC, Kauwell GPA, Hutson AD, Cerda JJ, Bailey LB: Genomic DNA methylation decreases in response to moderate folate depletion in elderly women. Am J Clin Nutr 72:998–1003, 2000.

57. Giovannucci E, Stampfer MJ, Colditz GA, Rimm EB, Trichopoulos D, Rosner BA, Speizer FE, Willett WC: Folate, methionine, and alcohol intake and risk of colorectal adenoma. J Natl Cancer Inst 85:875–884, 1993.

58. Giovannucci E, Stampfer MJ, Colditz GA, Hunter DJ, Fuchs C, Rosner BA, Speizer FE, Willett WC: Multivitamin use, folate, and colon cancer in women in the Nurses' Health Study. Ann Intern Med 129:517–524, 1998.

59. Su LJ, Arab L: Nutritional status of folate and colon cancer risk: Evidence from NHANES I Epidemiologic Follow-up Study. Ann Epidemiol 11:65–72, 2001.

60. Ma J, Stampfer MJ, Giovannucci E, Artigas C, Hunter DJ, Fuchs C, Willett WC, Selhub J, Hennekens CH, Rozen R: Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. Cancer Res 57:1098–1102, 1997.

61. Skibola CF, Smith MT, Kane E, Roman E, Rollinson S, Cartwright RA, Morgan G: Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. PNAS 96:12810–12815, 1999.

62. Butterworth CE, Hatch KD, Macaluso M, Cole P, Sauberlich HE, Soong S-J, Borst M, Baker VV: Folate deficiency and cervical dysplasia. JAMA 267:528–533, 1992.

63. Kwasniewska A, Tukendorf A, Semczuk M: Folate deficiency and cervical intraepithelial neoplasia. Eur J Gynaec Oncol 18:526–530, 1997.

64. Zhang S, Hunter DJ, Hankinson SE, Giovannucci EL, Rosner BA, Colditz GA, Speizer FE, Willett WC: A prospective study of folate intake and the risk of breast cancer. JAMA 281:1632–1637, 1999.

65. Sellers TA, Kushi LH, Cerhan JR, Vierkant RA, Gapstur SM, Vachon CM, Olson JE, Therneau TM, Folsom AR: Dietary folate intake, alcohol, and risk of breast cancer in a prospective study of postmenopausal women. Epidemiology 12:420–428, 2001.

66. Rohan TE, Jain MG, Howe GR, Miller AB: Dietary folate consumption and breast cancer risk. J Natl Cancer Inst 92:266–269, 2000.

67. Selhub J, Bagley LC, Miller J, Rosenberg IH: B vitamins, homocysteine, and neurocognitive function in the elderly. Am J Clin Nutr 71:614S–620S, 2000.

68. Goodwin JS, Goodwin JM, Garry PJ: Association between nutritional status and cognitive functioning in a healthy elderly population. JAMA 249:2917–2921, 1983.

69. Renvall MJ, Spindler AA, Ramsdell JW, Paskvan M: Nutritional status of free-living Alzheimer's patients. Am J Med Sci 298:20–27, 1989.

70. Kristensen MO, Gulmann NC, Christensen JEJ, Ostergaard K, Rasmussen K: Serum cobalamin and methylmalonic acid in Alzheimer dementia. Acta Neurol Scand 87:475–481, 1993.

71. Nilsson K, Gustafson L, Faldt R, Andersson A, Brattstrom L, Lindgren A, Israelsson B, Hultberg B: Hyperhomocysteinaemia-a common finding in a psychogeriatric population. Eur J Clin Invest 26:853–859, 1996.

72. Ortega RM, Manas LR, Andres P, Gaspar MJ, Agudo FR, Jimenez A, Pascual T: Functional and psychic deterioration in elderly people may be aggravated by folate deficiency. J Nutr 126:1992–1999, 1996.

73. Riggs KM, Spiro 3rd A, Tucker K, Rush D: Relations of vitamin B-12, vitamin B-6, folate, and homocysteine to cognitive performance in the Normative Aging Study. Am J Clin Nutr 63:306–314, 1996.

74. La Rue A, Koehler KM, Wayne SJ, Chiulli SJ, Haaland KY, Garry PJ: Nutritional status and cognitive functioning in a normally aging sample: A 6-y reassessment. Am J Clin Nutr 65:20–29, 1997.

75. Joosten E, Lesaffre E, Riezler R, Ghekiere V, Dereymaeker L, Pelemans W, Dejaeger E: Is metabolic evidence for vitamin B-12 and folate deficiency more frequent in elderly patients with Alzheimer's disease? J Gerontol A Biol Sci Med Sci 52A:M76–M79, 1997.

76. Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L, Ueland PM: Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease. Arch Neurol 55:1449–1455, 1998.

77. Lindenbaum J, Healton EB, Savage DG, Brust JCM, Garrett TJ, Podell ER, Marcell PD, Stabler SP, Allen RH: Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. N Engl J Med 318:1720–1728, 1988.

78. Bell IR, Edman JS, Selhub J, Morrow FD, Marby DW, Kayne HL, Cole JO: Plasma homocysteine in vascular disease and in nonvascular dementia of depressed elderly people. Acta Psychiatr Scand 86:386–390, 1992.

79. Snowdon DA, Tully CL, Smith CD, Riley KP, Markesbery WR: Serum folate and the severity of atrophy of the neocortex in Alzheimer disease: Findings from the Nun Study. Am J Clin Nutr 71:993–998, 2000.

80. Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, Wilson PWF, Wolf PA: Plasma homocysteine as a risk factor for dementia and alzheimer's disease. N Engl J Med 346:476–483, 2002.

81. Duthie SJ, Whalley LJ, Collins AR, Leaper S, Berger K, Deary IJ: Homocysteine, B vitamin status, and cognitive function in the elderly. Am J Clin Nutr 75:908–913, 2002.

82. Wang H-X, Wahlin A, Basun H, Fastbom J, Winblad B, Fratiglioni L: Vitamin B12 and folate in relation to the development of Alzheimer's disease. Neurology 56:1188–1194, 2001.

83. Morris MS, Jacques PF, Rosenberg IH, Selhub J: Hyperhomocysteinemia associated with poor recall in the third National Health and Nutrition Examination Survey. Am J Clin Nutr 73:927–933, 2001.

84. Stabler SP, Lindenbaum J, Allen RH: Vitamin B-12 deficiency in the elderly: Current dilemmas. Am J Clin Nutr 66:741–749, 1997.

85. Steinmetz KA, Potter JD: Vegetables, fruit, and cancer prevention: A review. J Am Diet Assoc 96:1027–1039, 1996.

86. World Cancer Research Fund: "Food, Nutrition and the Prevention of Cancer: A global perspective." Washington, DC: American Institute for Cancer Research, 1997.

87. 5-A-Day for Better Health Program. National Cancer Institute, National Institutes of Health, Bethesda, MD. Available online at http://www.5aday.gov Accessed December 3, 2002.

88. US Department of Health and Human Services: "Dietary Guidelines for Americans," 5th ed. Home and Garden Bulletin No. 232. Washington DC: US Department of Health and Human Services, 2000.