Folate Deficiency, Homocysteine and Dementia

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Folate deficiency is relatively common in older adults. There is now growing interest in the roles played by folate and B vitamins in the metabolism of homocysteine. Recent studies have suggested a link between elevated levels of homocysteine and the risk of Alzheimer disease. In this article, we will focus on the physiology and pathophysiology related to folate and homocysteine metabolism. We have also included a discussion of the laboratory evaluation of these compounds. Finally, we review the evidence supporting the relationships between folate deficiency, hyperhomocysteinemia and the development of dementia.

Key words: folic acid, vitamin B_{12}, deficiency diseases, homocysteine, dementia.

Introduction

Folate deficiency is relatively common in older adults, and may result from nutritional deficits, malabsorption, medication effects or other causes. Interest in folate and B vitamins has increased in recent years due to their role in homocysteine metabolism. Elevated levels of homocysteine have been implicated in cardiovascular disease,[1] and there also have been several recent observational studies linking elevated levels of homocysteine to Alzheimer disease and other dementias.[2-7] In this article, we will focus on the physiology and pathophysiology of folate and homocysteine, and the evidence supporting an association with dementia. An accompanying article discusses the evaluation and management of vitamin B_{12} deficiency in the elderly (see article, page 16).

Physiology and Pathophysiology of Folate Metabolism

The sequence of folate metabolism helps to classify the various causes of folate deficiency (Table 1). The term folic acid refers, in the strictest sense, to a specific compound, pteroylglutamic acid (PGA), but it is more often used generally to describe the polyglutamate folates, a class of compounds with similar nutritional activity.[8] Folates are found in a wide variety of foods, including fruits, vegetables, dairy products and cereals. Folate may be degraded or leach into the water in large quantities when such foods are cooked.[8]

The daily requirement for folate is 200–300mg / day in healthy individuals. The total body stores are normally 5–10mg. As the ratio of daily intake to body stores is much higher for folate than vitamin B_{12}, folate deficiency can develop much more quickly than vitamin B_{12} deficiency.[8] This is especially true when requirements increase, as in pregnancy. In order to help reduce the incidence of neural tube defects in newborns, cereals have been fortified with folate in North America since the late 1990s. Women also are advised to increase their folate intake during pregnancy.[9]

Figure 1 outlines the steps involved in folate absorption and transport. Although synthetic folate is a monoglutamate (PGA_1), the naturally occurring folates in the diet are polyglutamates (PGA_n, where n=2–9).[10] As a result, dietary folates must be deconjugated to the monoglutamate form by enzymes on the jejunal brush-border surface to allow absorption into the circulation. Some drugs (e.g., sulfasalazine) may interfere with this deconjugation and thereby inhibit absorption.[8] Diseases affecting the jejunum (e.g., celiac disease, inflammatory bowel disease) may also interfere with folate absorption, but malabsorption alone is an uncommon cause of folate deficiency. In the circulation, one-third of folate is free whereas the rest binds non-specifically to albumin.[10] Intracellular uptake is mediated by folate-binding receptors. The principal storage site is the liver, and there is an enterohepatic recirculation via the biliary system. Alcohol ingestion can interfere with this system, leading to a fall in serum folate levels.[8] Folate and cobalamin participate in pathways leading to DNA synthesis.[10] After folate enters a target cell—such as a red blood cell precursor—its levels remain relatively stable throughout the cell's lifespan. As a

Table 1
Selected Causes of Folate Deficiency

1. Inadequate intake
– elderly "tea and toast" diet
– alcoholism
2. Increased demands
– pregnancy
– increased cell turnover (e.g., chronic hemolysis, leukemia, exfoliative dermatitis)
3. Malabsorption
– intestinal diseases (e.g., celiac disease, inflammatory bowel disease)
4. Drug-induced
– anticonvulsants (e.g., phenytoin)
– oral contraceptives
– sulfasalazine
– methotrexate
– alcohol

result, red blood cell folate levels represent the folate status at the time the cell was formed, and are more reflective of body stores and are less prone to fluctuate with changes in diet than are serum folate levels.[8]

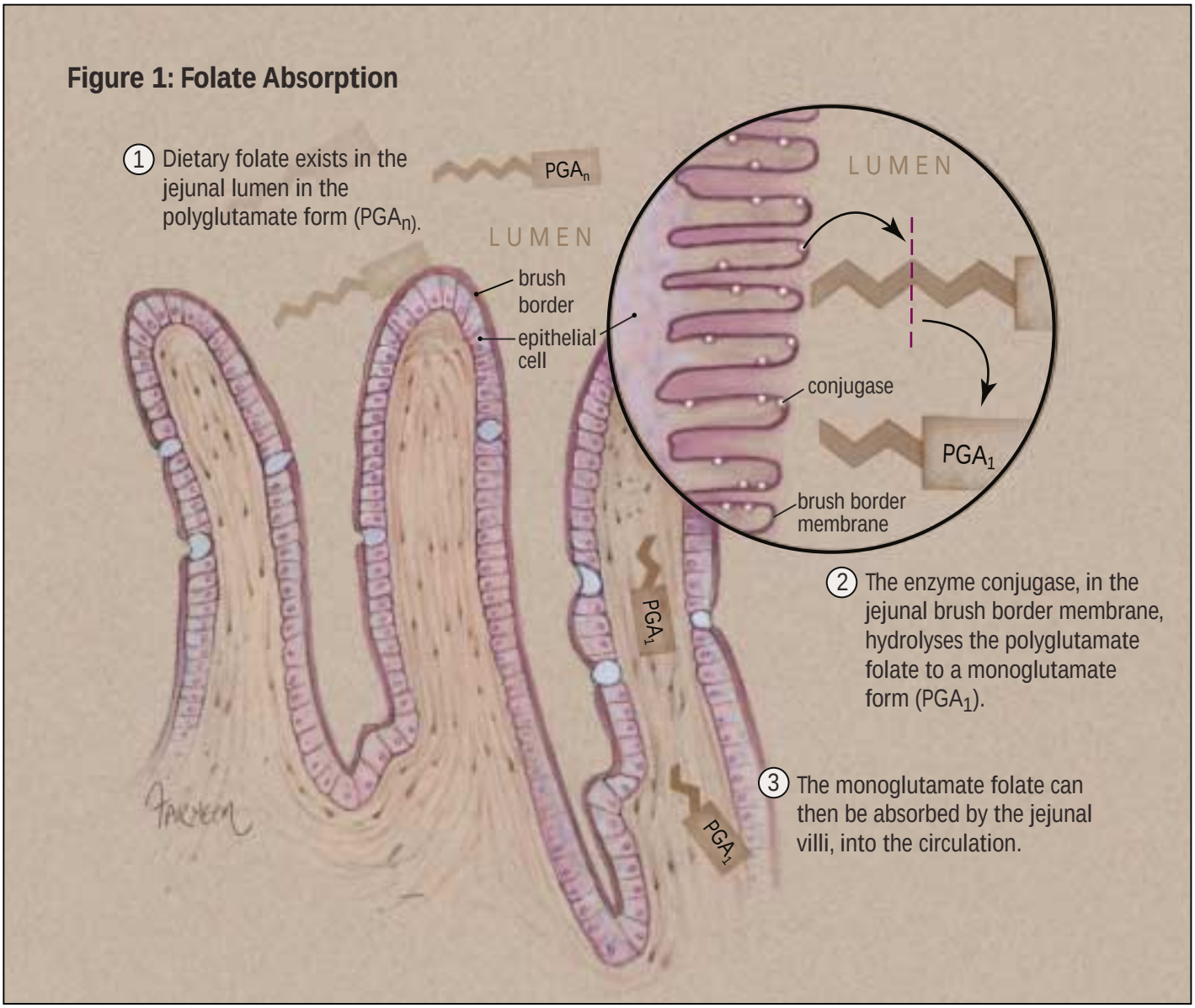
Figure 3 in the accompanying article on vitamin B_{12} deficiency details the role of folate in mammals (page 18). The primary intracellular function of folate in this metabolic pathway is to transfer one-carbon units in order to facilitate effective DNA synthesis. To achieve this coenzymatic activity, folate analogues must be in both a reduced form (i.e., tetrahydrofolate, THF) and a polyglutamated form (i.e., THF_n).[10] This metabolic pathway also leads to the production of S-adenosylmethionine (SAMe), which is thought to be essential to nervous system functioning.[8]

Unlike dietary folate, synthetic folate is reduced directly to THF without the need for cobalamin as a cofactor. As a result, the administration of supplemental folate to a cobalamin-deficient patient may correct megaloblastic hematologic changes, but would still allow neurologic damage to progress.[8]

Clinical Features of Folate Deficiency

The clinical features of folate deficiency can be related back to its pathophysiology. As folate is responsible for DNA synthesis, the effect of inadequate folate stores are most pronounced on cell lines with high turnover, such as the hematologic system and gastrointestinal tract. As a result, the megaloblastic anemia of folate deficiency produces fatigue, palpitations and pallor. Gastrointestinal involvement can produce angular cheilitis and stomatitis. When neuropsychiatric features are present, it is traditionally thought that they represent the effects of coexistent cobalamin (vitamin B_{12}) deficiency rather than folate deficiency *per se*.[8,11] There is some evidence to suggest that folate

Figure 1: Folate Absorption



deficiency itself may produce cognitive impairment and mood disorders,[11,12] although this remains controversial.[11] The common link of hyperhomocysteinemia may explain the finding of dementia in subjects with either vitamin B_{12} or folate deficiency.

Laboratory Testing for Folate and Homocysteine

As mentioned above, red blood cell folate levels are thought to reflect total body stores better than serum levels, and are therefore most often used to screen for folate deficiency.[13] Even red blood cell measurements, however, may have limited sensitivity in detecting folate deficiency in pregnant or alcoholic subjects.[8] Because cobalamin is necessary for the normal transfer of methyltetrahydrofolate from the blood into cells, cobalamin deficiency can cause falsely lowered red blood cell folate levels and falsely elevated serum folate levels.[8,13] Furthermore, the red blood cell folate concentration can be misleading if the patient has received a recent blood transfusion.[13]

If folate deficiency is diagnosed, other tests to determine its cause may be necessary. For example, celiac disease (also called gluten-induced enteropathy) may be diagnosed via duodenal biopsy.[13] Although histopathology from duodenal biopsy is considered the gold standard for diagnosis, serum assays for antibodies against gliadin and endomysium have gained popularity due to their high predictive value and convenience compared to endoscopy.[14]

Homocysteine levels are typically elevated in subjects with folate deficiency. Hypovolemia, renal insufficiency and deficiency of either pyridoxine (vitamin B_6) or cobalamin can also cause elevations of homocysteine, and these should be ruled out before making the diagnosis of folate deficiency in a subject with elevated homocysteine levels but normal folate levels.[8] Following serial homocysteine levels can be valuable in assessing the response to treatment; metabolite levels usually normalize within seven to 14 days of treatment.[8]

Measurement of homocysteine can be extremely useful in establishing the presence of folate deficiency, especially in cases of subtle or atypical deficiency.[15] In healthy fasting individuals, total plasma homocysteine concentrations usually fall between 5 and 15mmol/L.[15,16] Abnormally high levels are arbitrarily categorized as moderate (16–30mmol/L), intermediate (30–100mmol/L) and severe (> 100mmol/L) hyperhomocysteinemia.[16] There is some debate over the upper reference limit for total plasma homocysteine. Some authorities feel that the formerly accepted value of 15mmol/L is too high in healthy subjects, and suggest that this value be dropped to as low as 10mmol/L.[15] Total plasma homocysteine is most commonly measured after a 12-hour fast. Oral methionine loading has been suggested as a means of identifying subjects who might have normal fasting homocysteine values but high post-load values.[15] However, oral methionine loading is inconvenient and reference values are not well established for this test. As a result, fasting samples are generally recommended instead.[15] Plasma samples must be sent on ice to the laboratory immediately to prevent an artefactual rise in levels.[15]

Should folate and/or homocysteine levels be assessed in all patients with suspected dementia? The Canadian Consensus Conference on Dementia (CCCD) guidelines suggest that folate measurement is an optional additional test that may be helpful in the diagnostic evaluation of dementia.[17] Interestingly, however, neither the CCCD[17] nor the updated American Academy of Neurology practice parameter on dementia[18] address the issue of homocysteine testing. Among the guideline writers' concerns may be that homocysteine assays are not available in many peripheral laboratories, and that fasting samples are needed. A recent editorial suggests that perhaps homocysteine levels should be added to the battery of blood tests that are routinely done in the initial work-up of dementia,[19] but there is currently little evidence to support this approach.

Hyperhomocysteinemia

Homocysteine, a sulphur-containing amino acid, is a central intermediate in the metabolic pathways involving methionine, folate, cobalamin and pyridoxine (see Figure 3 in the accompanying article on page 18). When methionine is abundant, homocysteine undergoes trans-sulfuration, a process for which vitamin B_6 is an essential cofactor. When methionine levels are low, homocysteine is re-methylated to conserve methionine. This re-methylation process requires both folic acid and vitamin B_{12}.[16]

Causes of hyperhomocysteinemia are outlined in Table 2.[15,16] While genetic disorders characterized by severe hyperhomocysteinemia are rare, mild to moderate elevations caused by point mutations are fairly common. The "thermolabile" methyltetrahydrofolate reductase (MTHFR) C677T point mutation is estimated to be

Table 2
Causes of Hyperhomocysteinemia

Nutritional deficiencies
– folate
– vitamin B_{12}
– vitamin B_6
Genetic defects in homocysteine metabolism
– MTHFR* C677T substitution ("thermolabile" MTHFR mutation) (common; 10–13% of Caucasian population)
– congenital homocystinuria (homozygous CbS** deficiency) (rare)
– homozygous MTHFR deficiency (rare)
Hormonal influences
– male sex
– postmenopausal status
– corticosteroid use
Increasing age
Chronic renal insufficiency
Hypothyroidism

*MTHFR = methyltetrahydrofolate reductase
**CbS = cystathionine beta-synthase

present in 10–13% of the Caucasian population.[16] Other factors associated with high homocysteine levels include increasing age, renal insufficiency, hypothyroidism, male sex and postmenopausal status (suggesting that sex hormones have a role).[15,16,20] Because of the metabolic pathways involved, deficiencies in folate and vitamins B_6 and B_{12} also result in elevated homocysteine. According to large surveys, high homocysteine levels can be found in approximately 10% of the general population and in approximately 45% of those older than 60 years.[21] The most common cause of mild hyperhomocysteinemia, particularly in the elderly, appears to be dietary insufficiency of folate and / or vitamin B_{12}.[21]

Homocysteine and Dementia

Hyperhomocysteinemia was first proposed as a risk factor for severe atherothrombotic vascular disease over 30 years ago among young subjects with congenital homocystinuria.[16] Since then, evidence suggesting that homocysteine may play a key role in atherosclerosis has mounted.[1] More recently, observational studies have begun to link high concentrations of homocysteine to Alzheimer disease and other dementias.[2-7]

There are several potential mechanisms by which hyperhomocysteinemia might promote the development of dementia. Elevated homocysteine levels can cause endothelial cell dysfunction, smooth muscle cell proliferation, impaired fibrinolysis and increased oxidative stress. These factors might contribute to atherothrombotic cerebrovascular disease.[22] In addition, homocysteine and its metabolites may cause neurotoxicity via enhanced β-amyloid peptide production and NMDA receptor stimulation.[22]

Findings from several observational studies have supported the link between hyperhomocysteinemia and the risk of dementia.[2-7] Two small case-control studies found significantly higher homocysteine levels in patients with Alzheimer disease compared to levels in cognitively intact controls.[2,3] In a subsequent small, retrospective, cohort study involving 32 healthy elderly subjects, McCaddon's group reported that homocysteine was a predictor of cognitive decline independent of age, sex, education, renal function, B vitamin status, smoking and hypertension.[4] A larger case-control study also found an association between elevated homocysteine levels and Alzheimer disease.[5] Radiological evidence of disease progression (using serial computed tomography measurements of medial temporal lobe thickness) in this study was greater among those with higher initial homocysteine levels.[5] Lehmann, *et al.* studied 366 consecutive patients referred to a memory clinic, and categorized them by probable diagnosis (e.g., Alzheimer disease, vascular dementia, other dementias, "minor cognitive impairment" and subjective symptoms only).[6] The investigators found that homocysteine levels were inversely correlated with cognitive performance in the groups with Alzheimer disease, vascular dementia and "minor cognitive impairment".[6] A further analysis of the Rotterdam study cohort has suggested that elevated homocysteine levels are also associated with decreased cognitive performance in nondemented elderly subjects.[23]

The study by Seshadri and colleagues recently generated a great deal of interest in the relationship between homocysteine and dementia.[7] The investigators employed a retrospective cohort study design and used data from the Framingham Study. They made adjustments for many known dementia risk factors, including age, sex, apolipoprotein E genotype, educational status, history of stroke, smoking, alcohol, diabetes mellitus, blood pressure and body mass index. They also measured folate and B vitamin status. Of the 1,092 participants, 111 developed dementia during a median follow-up of eight years; Alzheimer disease accounted for 83 of these cases. The multivariable-adjusted relative risk of dementia was 1.4 for each increase of one standard deviation in the log-transformed homocysteine value. For subjects with homocysteine levels greater than 14mmol / L, this meant that the risk of Alzheimer disease was nearly doubled.[7]

One study that failed to find an association between hyperhomocysteinemia and dementia used a random sample of 702 community-dwelling respondents to the Rotterdam Study.[24] The mean duration of follow-up was 2.7 years. After adjustment for age, sex and education, there was no relationship found between total homocysteine and cognitive impairment. Potential explanations for the conflicting results in the papers by Seshadri, *et al.*[7] and Kalmijn, *et al.*[24] include differences in the duration of follow-up, in adjustments for possible confounding factors and in outcome definitions (diagnosis of dementia vs. "cognitive impairment" or "cognitive decline").

Several randomized controlled trials have been launched to evaluate the role of lowering homocysteine in the prevention of coronary events,[25,26] stroke[27] and venous thrombosis.[28] Randomized controlled trials designed to test the hypothesis that lowering homocysteine has an impact on the risk of developing dementia also are now underway.[29] One published open-label study has examined the effects of oral supplementation with vitamin B_{12} (1mg / day) and folate (5mg / day) on cognitive outcomes in 28 patients diagnosed with various dementias of mild-to-moderate severity.[30] Subjects in this study were categorized as having normal or elevated homocysteine levels (cut-off point 19.9mmol / L). In the 17 subjects with elevated homocysteine, there were significant improvements in cognitive testing after two months of supplementation. In contrast, there were no significant changes in test performance in those subjects without baseline

homocysteine levels > 19.9mmol/L.[30] Although unblinded and uncontrolled, this intriguing study supports the need for randomized controlled trials of homocysteine-lowering therapies to prevent and/or treat dementia.

Treatment of Folate Deficiency and Hyperhomocysteinemia

In subjects with folate deficiency, the efficacy of supplemental oral folic acid (1–5mg/day) depends on the identification and management of underlying pathology. For example, supplements will be of limited value if specific treatment for the malabsorption associated with celiac disease is not provided. Vitamin B_{12} deficiency must be excluded in all patients starting folic acid treatment to prevent occult progression of the neurological damage associated with B_{12} deficiency.[8]

Most trials that evaluate homocysteine-lowering therapies use a combination of folate, cobalamin and pyridoxine to achieve this goal. The optimal dose of each component has not been established, but doses range from 1–5mg of folate, 0.4–1mg of cobalamin and 0–50mg of pyridoxine.[25-30]

Conclusions

Deficiencies of folate and vitamin B_{12} are common in older adults. We have tried to present a rational approach to the evaluation of these conditions by reviewing their physiology and metabolism. Folate and B vitamins play an integral role in the metabolism of homocysteine. The role of homocysteine and vitamin deficiencies in various disease states is now being delineated. Observational studies have linked hyperhomocysteinemia with an increased risk of dementias such as Alzheimer disease. We now await interventional studies to see if the risk conferred by high homocysteine levels is modifiable by supplementation with folate and B vitamins. Given the current lack of effective dementia therapy, there is considerable excitement about the potential benefits of these inexpensive, well-tolerated agents. ◆

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References

1. Eikelboom JW, Lonn E, Genest J Jr, et al. Homocyst(e)ine and cardiovascular disease: a critical review of the epidemiologic evidence. Ann Intern Med 1999;131:363-75.
2. Joosten E, Lesaffre E, Riezler R, et al. 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 1997;52:M76-9.
3. McCaddon A, Davies G, Hudson P, et al. Total serum homocysteine in senile dementia of Alzheimer type. Int J Geriatr Psychiatry 1998;13:235-9.
4. McCaddon A, Hudson P, Davies G, et al. Homocysteine and cognitive decline in healthy elderly. Dement Geriatr Cogn Disord 2001;12:309-13.
5. Clarke R, Smith AD, Jobst KA, et al. Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease. Arch Neurol 1998;55:1449-55.
6. Lehmann M, Gottfries CG, Regland B. Identification of cognitive impairment in the elderly: homocysteine is an early marker. Dement Geriatr Cogn Disord 1999;10:12-20.
7. Seshadri S, Beiser A, Selhus J, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med 2002;346:476-83.
8. Snow CF. Laboratory diagnosis of vitamin B12 and folate deficiency: a guide for the primary care physician. Arch Intern Med 1999;159:1289-98.
9. Bailey LB. New standard for dietary folate intake in pregnant women. Am J Clin Nutr 2000;71:1304S-7S.
10. Tefferi A, Pruthi RK. The biochemical basis of cobalamin deficiency. Mayo Clin Proc 1994;69:181-6.
11. Martin DC. B12 and folate deficiency dementia. Clin Geriatr Med 1988;4: 841-52.
12. Reynolds EH. Folic acid, ageing, depression, and dementia. BMJ 2002;324:1512-5.
13. Hoffbrand V, Provan D. ABC of clinical hematology: macrocytic anemias. BMJ 1997;314:430-3.
14. McMillan SA, Haughton DJ, Biggart JD, et al. Predictive value for coeliac disease of antibodies to gliadin, endomysium, and jejunum in patients attending for jejunal biopsy. BMJ 1991;303:1163-5.
15. Rasmussen K, Moller J. Total homocysteine measurement in clinical practice. Ann Clin Biochem 2000;37:627-48.
16. Hankey GJ, Eikelboom JW. Homocysteine and vascular disease. Lancet 1999;354: 407-13.
17. Patterson CJ, Gauthier S, Bergman H, et al. The recognition, assessment and management of dementing disorders: conclusions from the Canadian Consensus Conference on Dementia. Can Med Assoc J 1999;160:1S-15S.
18. Knopman DS, DeKosky ST, Cummings JL, et al. Practice parameter: diagnosis of dementia (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. Neurology 2001;56:1143-53.
19. Diaz-Arrastia R. Hyperhomocysteinemia: a new risk factor for Alzheimer disease? Arch Neurol 1998;55:1407-8.
20. Hussein WI, Green R, Jacobsen DW, et al. Normalization of hyperhomocysteinemia with L-thyroxine in hypothyroidism. Ann Intern Med 1999;131:348-51.
21. Selhub J, Jacques PF, Rosenberg IH, et al. Serum total homocysteine concentrations in the third National Health and Nutrition Examination Survey (1991-1994): population reference ranges and contribution of vitamin status to high serum concentrations. Ann Intern Med 1999;131:331-9.
22. Loscalzo J. Perspective: homocysteine and dementia. N Engl J Med 2002;346: 466-8.
23. Prins ND, Den Heijer T, Hofman A, et al. Homocysteine and cognitive function in the elderly: the Rotterdam Scan Study. Neurology 2002;59:1375-80.
24. Kalmijn S, Launer LJ, Lindemans J, et al. Total homocysteine and cognitive decline in a community-based sample of elderly subjects: the Rotterdam Study. Am J Epidemiol 1999;150:283-9.
25. Schnyder G, Roffi M, Flammer Y, et al. Effect of homocysteine-lowering therapy with folic acid, vitamin B(12), and vitamin B(6) on clinical outcome after percutaneous coronary intervention: the Swiss Heart study: a randomized controlled trial. JAMA 2002;288: 973-9.
26. MacMahon M, Kirkpatrick C, Cummings CE, et al. A pilot study with simvastatin and folic acid/vitamin B12 in preparation for the Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH). Nutr Metab Cardiovasc Dis 2000;10:195-203.
27. Spence JD, Howard VJ, Chambless LE, et al. Vitamin Intervention for Stroke Prevention (VISP) trial: rationale and design. Neuroepidemiology 2001;20:16-25.
28. Willems HP, den Heijer M, Bos GM. Homocysteine and venous thrombosis: outline of a vitamin intervention trial. Semin Thromb Hemost 2000;26:297-304.
29. Stott D. Pilot study: trial of homocysteine reduction in the prevention of vascular events and dementia in the elderly (THRIVED). Cochrane Central Register of Controlled Trials. Accession Number CN-00342772.
30. Nilsson K, Gustafson L, Hultberg B. Improvement of cognitive functions after cobalamin/folate supplementation in elderly patients with dementia and elevated plasma homocysteine. Int J Geriatr Psychiatry 2001;16:609-14.