

## Review

# The role of hyperhomocysteinemia and B-vitamin deficiency in neurological and psychiatric diseases

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## Abstract

Hyperhomocysteinemia (HHcy) is related to central nervous system diseases. Epidemiological studies show a positive, dose-dependent relationship between plasma total homocysteine (tHcy) concentration and neurodegenerative disease risk. tHcy is a marker of B-vitamin (folate, B<sub>12</sub>, B<sub>6</sub>) status. Hypomethylation, caused by low B-vitamin status and HHcy, is linked to key pathomechanisms of dementia; B-vitamin supplementation could potentially reduce neurological damage. In retrospective studies, the association between tHcy and cognition is impressive; there is also evidence that tHcy-lowering treatment could be effective in primary and secondary stroke prevention. Increased tHcy and low serum folate occur in patients with Parkinson's disease, especially those receiving L-dopa. There is also an association between HHcy and multiple sclerosis, and between B-vitamin status and depression. Studies also confirm a causal role for tHcy in epilepsy, and certain anti-epileptics enhance HHcy. B-vitamin status should be optimized by ensuring sufficient intake in patients with neuropsychiatric diseases. HHcy occurs commonly in the elderly and can contribute to age-related neurodegeneration. Treatment with folic acid, B<sub>12</sub> and B<sub>6</sub> lowers tHcy. For secondary and primary prevention from several neuropsychiatric disorders, it seems prudent to actively identify deficient subjects and ensure sufficient vitamin intake.

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## Introduction

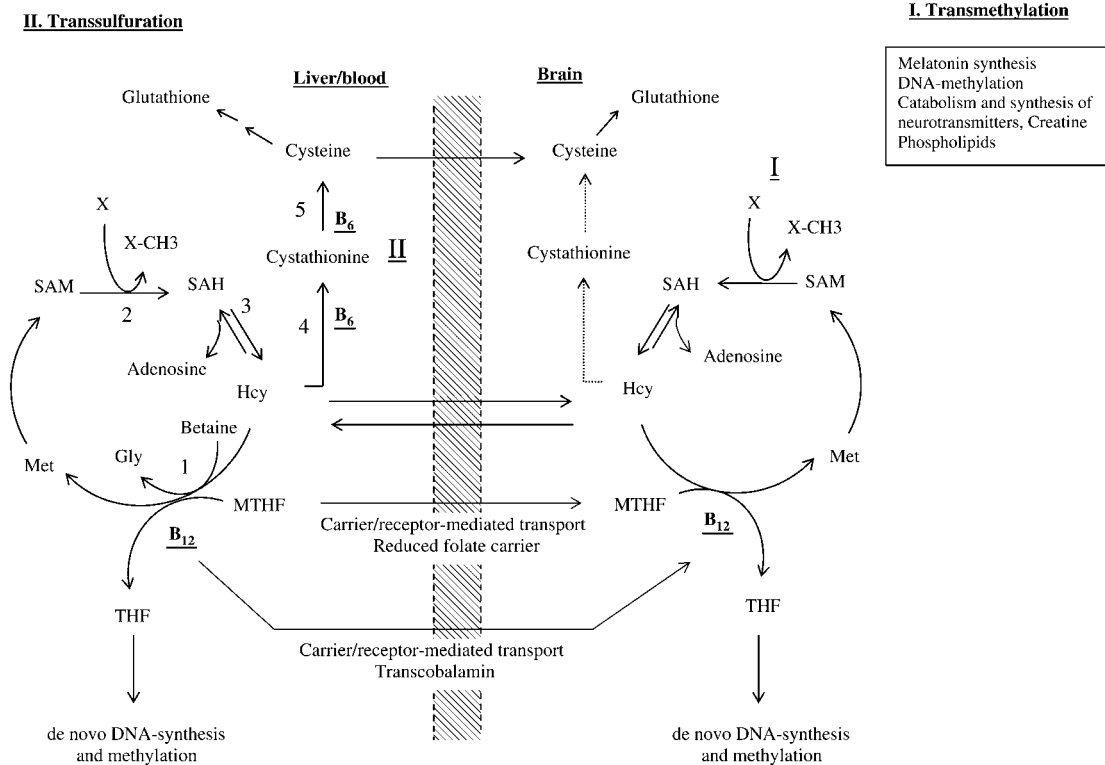
Neurological and psychiatric disorders are chronic diseases affecting patients across all age groups. The frequency of central nervous system (CNS) disorders is particularly high in the geriatric population; a high proportion of elderly people develop dementia, stroke, Parkinson's disease (PD) or depression. Nutritional status has increasingly gained attention as possibly contributing to the initiation or progression of many CNS diseases.

A causal link between hyperhomocysteinemia (HHcy) and CNS disorders was first described in patients with cystathionine  $\beta$ -synthase (CBS) deficiency (1). These patients suffer from mental retardation and cognitive dysfunction in addition to severely elevated plasma total homocysteine (tHcy) concentrations (tHcy >70  $\mu$ mol/L). Other inherited disorders related to homocysteine (Hcy) metabolism also cause impaired cognitive function, depression, cerebral seizures, myelopathy and polyneuropathy.

Because B-vitamins (folate, B<sub>12</sub>, B<sub>6</sub>) are important cofactors for Hcy catabolism, an elevated concentration of tHcy can indicate B-vitamin deficiency (2) (Figure 1). There is a relationship between serum concentrations of markers of vitamin B<sub>12</sub> deficiency and some cognitive domains (3). Furthermore, serum concentrations of B-vitamins are negatively related to deficits in neurocognitive tests in a healthy elderly population (4). It remains unclear whether tHcy causes these disorders, or if it is a surrogate marker for vitamin deficiencies and disturbed transmethylation reactions in the CNS.

Elevated concentrations of tHcy (>12.0  $\mu$ mol/L) and methylmalonic acid (MMA >271 nmol/L) (a metabolic marker for vitamin B<sub>12</sub> deficiency) are common in the neuropsychiatric population, even in the absence of hematological manifestations (5). Neuropsychiatric symptoms occur in patients with folate or vitamin B<sub>12</sub> deficiency and megaloblastic anemia (6). In one study, peripheral neuropathy was observed in 40% of vitamin B<sub>12</sub>-deficient subjects and affective disorders in 56% of those with folate deficiency (6). Additionally, spinal cord lesions, myelopathy and psychosis are reported in B<sub>12</sub>-deficient cases, which are reversible after initiation of vitamin treatment (7, 8).

In this review, we focus on the role of HHcy or B-vitamin deficiency in the development and progression of neuropsychiatric diseases. We also present recent results from vitamin intervention studies in neurological and psychiatric diseases.



**Figure 1** Homocysteine metabolism.

SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; Hcy; homocysteine, Met; methionine, Gly; glycine, THF; tetrahydrofolate, MTHF; 5-methyltetrahydrofolate. Enzymes: 1, methionine synthase; 2, transmethylase; 3, SAH-hydrolase; 4, cystathionine  $\beta$ -synthase; 5, cystathionase.

## Homocysteine and its metabolism in the central nervous system

Hcy is a sulfhydryl containing amino acid arising as an intermediate during methionine metabolism. An important role of Hcy-methionine metabolism is to provide S-adenosylmethionine (SAM), the methyl donor for numerous biological reactions. SAM donates its methyl group to a methyl acceptor and is transformed to S-adenosylhomocysteine (SAH). SAH is hydrolyzed to Hcy by SAH-hydrolase. The SAH-hydrolase reaction is reversible, but favors SAH formation in the presence of increased tHcy.

The enzyme methionine synthase and its cofactor, methyl cobalamin, mediate remethylation of Hcy to methionine. Betaine-Hcy-methyltransferase (BHMT) catalyzes an alternative Hcy remethylation pathway and utilizes betaine as a methyl donor. This pathway accounts for only a minor part of Hcy catabolism, and BHMT is undetectable in the brain (9). In the transsulfuration pathway, Hcy is converted to cystathionine and subsequently to cysteine. Two vitamin B<sub>6</sub>-dependent enzymes, CBS and cystathionase, mediate this pathway.

Brain cells synthesize Hcy. Hcy metabolism in the brain is an important source of SAM (10). In experimental folate deficiency, neuronal cells produce more Hcy than cells incubated in folate-rich media (11). The role of folate in Hcy metabolism in the brain is further

confirmed from investigations on patients receiving antifolate treatment (methotrexate), where low serum folate and SAM and increased tHcy concentrations in serial cerebrospinal fluid (CSF) samples are observed (12).

In the liver, approximately 50% of the cysteine for glutathione synthesis is produced via the transsulfuration pathway. CBS expression and activity are observed in the brain (13). Limited cystathionase activity is detectable in only a few studies with a wide regional variation (14–16). Early experimental studies on isolated astrocytes from neonatal rat brain showed that cysteine and cystathionine can be converted into glutathione (17), indicating that cystathionase is active in rat brain. A recent study provided results supporting the presence of an intact transsulfuration pathway in the brain (18). Nevertheless, the contribution of this pathway to cysteine synthesis in hepatoma cells is greater than that in cultured human neurons and astrocytes (18).

The transport of Hcy in brain cells is mediated via a specific cellular receptor (19). The transport and exchange of Hcy within different brain regions and between the brain and the blood is not well studied. Blood-brain barrier integrity seems to be essential for Hcy distribution. Severe HHcy in patients with CBS deficiency is associated with a 10-fold increase in CSF-tHcy (20). Lowering plasma concentrations of tHcy with betaine causes a significant reduction of

CSF-tHcy, although betaine cannot be directly utilized for Hcy-remethylation in the brain. This suggests that Hcy can be transported across the blood-brain barrier in both directions. Increased concentrations of tHcy, or of its precursor SAH, in brain and CSF occurs in several neurological diseases (21, 22). Normally, plasma tHcy concentrations are 20- to 100-fold higher than in CSF (23). Studies also show that plasma tHcy concentrations are positively related to brain or CSF concentrations (23). Therefore, elevated plasma tHcy concentrations may reflect increased brain concentrations of this amino acid. Excess tHcy leads to increased SAH. SAH is a potent competitive inhibitor of many SAM-dependent methyltransferases in human brain. Brain hypomethylation is related to numerous functional and biochemical defects.

### Homocysteine is linked to neurodegenerative diseases

Neurodegenerative diseases include a wide group of disorders of various etiologies and clinical features. They all share one important feature, namely, structural and functional modifications in protein structures, resulting in deterioration of certain nerve cells or neurons. Dementia, Alzheimer's disease (AD), PD and stroke are examples of neurodegenerative diseases associated with HHcy.

### Dementias

Dementias are progressive illnesses with manifestations that include memory loss, cognitive dysfunction, attention deficit and other behavioral and emotional disturbances. The prevalence of dementias will double every 20 years to more than 80 million cases by 2040 (24). HHcy and AD could be linked (25) by stroke or microvascular disease, because 25% of dementia cases are attributed to stroke.

AD constitutes 60%–90% of all dementias. AD is characterized by depositions of extracellular senile plaques in brain, comprising amyloid  $\beta$  ( $A\beta$ ), lipids and other cellular components. AD patients typically have memory impairment and deterioration of language and other cognitive functions.  $A\beta$  is a 37–42 amino acid fragment originating from proteolysis of a precursor protein (amyloid precursor protein; APP). Another protein contributing to AD is tau protein. In healthy conditions, tau stabilizes neuronal microtubules. Structural modifications (hyperphosphorylation, glycosylation, truncation) of tau protein facilitate self-aggregation of this protein. For example, hyperphosphorylated tau protein self-aggregates and participates in neurofibrillary tangle (NFT) formation in the brain of patients with dementia. NFTs are pathological structural modifications that arise in the brain several decades before the onset of clinical symptoms of dementia. They cause impaired axonal transport, synaptic dysfunction and axonal degeneration.

The role of micronutrients in neuronal development and degeneration is well established (4, 26). B-vitamin

deficiency and HHcy are common in the elderly and coincide with a high incidence of AD at an older age. Data from prospective studies suggest a causal role for HHcy in the etiology of AD. Plasma concentration of tHcy is a strong predictor of cognitive decline with age (27). An independent association between folate or vitamin B<sub>12</sub> deficiency and dementia is also reported (28–30). Elevated plasma concentration of tHcy is associated with increased concentrations of tHcy and SAH in the brain (23). Patients with dementia have lowered CSF-SAM or increased CSF-tHcy or SAH (31). Patients with late onset AD have lower concentrations of CSF-folate than control subjects (32). Therefore, disordered Hcy metabolism can negatively influence some biological pathways in the brain and increase the risk of dementia.

Vascular dementia is the second most common cause of dementia in elderly people after AD. Vascular dementia (or multi-infarct dementia) constitutes 10%–40% of all dementias. Diffuse periventricular white matter abnormalities and lesions in the central lacunar result from atherosclerotic changes in small cerebral arteries and arterioles.

An elevated plasma tHcy concentration is an independent risk factor for cerebrovascular damage and vascular dementia (33, 34). Additionally, HHcy is associated with carotid artery disease and macroangiopathic and microangiopathic CNS diseases (35). The Northern Manhattan Study found that a tHcy concentration of  $>15 \mu\text{mol/L}$  is a risk factor for cerebral ischemia (36), and HHcy is associated with an increased risk of vascular dementia [odds ratio, OR (95% confidence intervals, CI) 4.3 (1.3–14.7)] and AD [3.7 (1.1–13.1)].

Mild cognitive impairment (MCI) is an intermediate state between normal aging and dementia. MCI is defined as deficit in one or more cognitive domains, particularly memory, or a general mild decline in cognitive abilities, but which is insufficient to interfere with social and occupational functioning. Approximately 20% of patients with MCI develop AD or progressive dementia within 2 years of follow-up (37). Patients with MCI have an 8.6-fold higher OR of developing AD compared with subjects without memory impairment (37). Because the progression of dementia takes several decades, it would be important to identify and manage risk factors that facilitate the conversion of MCI into AD or dementia.

HHcy is associated with an increased risk of mild cognitive dysfunction [OR (95% CI) 3.1 (1.2–8.1)] (33). In the Framingham Study, a relationship between tHcy and cognitive function was found in older but not younger adults (38). Subjects with elevated concentrations of tHcy at baseline were more likely to develop dementia after several years compared with subjects with normal tHcy (38). Similar studies also confirm that higher concentrations of tHcy or MMA correlate with some measures of cognitive function in elderly subjects (3, 36). The correlation between serum concentrations of B-vitamins (B<sub>12</sub>, B<sub>6</sub>, folate) and cognitive function is documented even in healthy elderly subjects (39). The Hordaland Homocysteine

Study, a follow-up study extending over 6 years, showed that tHcy plasma concentration can predict memory decline with age in elderly people (40). Approximately 7%–8% of the variations in cognitive function between elderly people could be explained by plasma concentrations of tHcy (41).

Sporadic late-onset dementias are common and their risk factors are not well studied. Genetic predisposition plays a secondary role; acquired or environmental factors are likely to be more important. Several prospective studies show a significant correlation between baseline concentrations of tHcy and the incidence of dementia several years later. Therefore, plasma tHcy concentration is considered an early predictor of cognitive decline with age (42). This was confirmed for late-onset, but not early-onset dementia (43).

Numerous studies report a marked and a dose-dependent correlation between concentrations of tHcy or B-vitamins and cognitive decline (Table 1). In the Framingham Study, subjects with plasma concentrations of tHcy  $\geq 14$   $\mu\text{mol/L}$  had a 2-fold higher risk for dementia compared to those with concentrations  $< 14$   $\mu\text{mol/L}$  (38). The risk for dementia increased by 40% for each 5  $\mu\text{mol/L}$  increase in plasma tHcy (38). In the OPTIMA Study, the OR for cerebral white matter changes in patients with AD was 1.4 for a 5  $\mu\text{mol/L}$  increase in plasma tHcy (44). Furthermore, computed tomographic scans and MMSE scores were compared in AD patients at baseline and after 3 years (45). Greater radiological evidence of disease progression, as assessed by medial temporal lobe thickness, was observed among patients with tHcy levels  $> 11$   $\mu\text{mol/L}$  compared with those with tHcy  $\leq 11$   $\mu\text{mol/L}$ , who showed little atrophy (45). Collectively, these studies suggested a causal role for tHcy in the onset and progression of dementia.

On the other hand, there are several studies that failed to detect an association between tHcy and cognitive domains. In a longitudinal study on 679 elderly people, high tHcy levels ( $> 14$   $\mu\text{mol/L}$ ) were not associated with AD and were not related to a decrease in memory scores over time (46). The study confirmed that age and sex are significant confounders. After adjustment for these variables, a modest non-significant association between tHcy and AD was observed. The northern Manhattan community had higher baseline tHcy levels, included more subjects with diabetes, were older than subjects in the Framingham study (38) and included several ethnic groups (46). Therefore, the association between elevated tHcy and cognitive decline can be confounded by other factors, such as age, gender, ethnic origin or associated diseases.

### Effect of treatment on cognitive function

Because of the consistent, dose-dependent relationship between tHcy and cognitive decline, lowering plasma concentrations of tHcy may have desirable effects on the progression of the disease. There is interest in whether folate and/or vitamin B<sub>12</sub> supplementation may improve cognitive function or delay

the progression of cognitive decline, whether this effect is related to lowering tHcy or to an independent effect of the vitamins. Several intervention studies are underway with the aim of improving symptoms or disease progression in patients with dementia.

Several, but not all, vitamin intervention studies document improvements in some measures of cognitive function (Table 2). In a recent review including data from two vitamin trials, there was no evidence that folate and/or B<sub>12</sub> had significant effects on cognitive function (47). Likewise, another study found no improvement in cognitive function in elderly people with vascular events who were treated for 1 year with folic acid plus vitamin B<sub>12</sub>, even though the treatment effectively lowered plasma tHcy concentrations (48). Similar results were reported by Eussen et al. (49), where elderly people with vitamin B<sub>12</sub> deficiency were treated with vitamin B<sub>12</sub> and folic acid, or placebo for 24 weeks. In another study testing the effect of vitamins in elderly people at risk of dementia, cognitive function was not improved during 12 weeks of the trial (50). Trials that used vitamin B<sub>6</sub> for prevention or treatment of cognitive decline with age are limited and the evidence is insufficient to make any firm conclusions (51).

There are several major limitations for all available studies. Firstly, most included only a small number of subjects. Secondly, their duration is too short to achieve a marked improvement. Thirdly, because baseline tHcy concentrations are related to a decline in cognitive function with age, a halting of such decline would mean a protective effect. One fact that should also be recognized is that the turnover of cells in the nervous system is negligible, whereas blood cells divide very rapidly. Therefore, vitamin treatment is known to improve hematological symptoms, while neurological symptoms may take longer to improve and may be only partially reversible. This is unsurprising given that the progression of dementia may extend over several decades. Therefore, it is currently believed that ensuring sufficient B-vitamin intake might be more effective in disease prevention rather than in disease treatment.

### Stroke

The association between HHcy and cerebrovascular diseases is impressive when compared with other diseases. HHcy is very common in patients with stroke. Silent brain infarction and brain atrophy are linked to HHcy and low B-vitamin status (52). The incidence of silent brain infarction increases with age, and studies show that elderly subjects with this lesion have significantly higher tHcy concentrations compared with those free of brain infarction (53). In the Rotterdam Study, it was estimated that each 1  $\mu\text{mol/L}$  increase in plasma tHcy concentration was associated with 6%–7% increase in the risk of stroke (54). In the Physicians Health Study, a slight increase (1.4-fold) in the risk of stroke was observed in subjects with tHcy  $> 12.7$   $\mu\text{mol/L}$  compared with subjects with tHcy lower than this limit (55). Similar results were reported by the Northern Manhattan Study, where the adjusted

**Table 1** Studies linking tHcy to cognitive dysfunction.

Prospective studies	Subjects	tHcy, vitamins	Tests	Results
McCaddon et al. (39)	32 healthy subjects (22 F), age > 65 years, 5 years follow-up	Median tHcy = 10.4 $\mu$ mol/L Folate = 14.5 nmol/L B <sub>12</sub> = 226 pmol/L	MMSE, ADAS-Cog	tHcy predicted cognitive decline in healthy elderly with a maximal effect on spatial copying skills
Seshadri et al. (38), Framingham Study	1092 non-demented subjects (667 F), mean age 76 years, 8 years follow-up	Mean tHcy = 13.1 $\mu$ mol/L 30% had tHcy > 14 $\mu$ mol/L Folate ~ 14.5 nmol/L B <sub>12</sub> ~ 324 pmol/L	Cases with dementia and AD was identified after 8 years	Relative risk 1.9 (1.3–2.8) for dementia and 1.9 (1.2–3.0) for AD. An increase in tHcy of 5 $\mu$ mol/L increased the multivariable-adjusted risk of AD by 40%.
Kado et al. (160), MacArthur Studies of Successful Aging	370 elderly subjects (age 70–79 years), 7 years follow-up	Mean tHcy at baseline = 11.3 $\mu$ mol/L Folate = 14.9 nmol/L, B <sub>12</sub> = 326 pmol/L, B <sub>6</sub> = 70 pmol/L	Standardized cognitive tests to assess language, memory, visuospatial, and conceptualization abilities	Subjects with folate in the bottom quartile (<3.15 ng/mL) had 1.6 higher risk of being in the worst quartile of cognitive performance after 7 years
Nurk et al. (40), The Hordaland Homocysteine Study	2189 subjects (age 65–67) years, subjects were re-investigated 6 years later	tHcy = 11.6 $\mu$ mol/L Folate = 7.5 nmol/L Vitamin B <sub>12</sub> = 348 pmol/L	Memory performance (Kendrick Object Learning Test)	Mean tHcy was higher and folate was lower in subjects with memory deficit at baseline. Baseline tHcy in the highest quintile, folate in the lowest quintile and vitamin B <sub>12</sub> in the lowest quintile predicted a higher risk for memory deficit after 6 years.
Retrospective, cross sectional studies	Subjects	tHcy, vitamins	Tests	Results
Clark et al. (161)	200 healthy post-menopausal women, aged 56–67 years	Mean tHcy = 10 $\mu$ mol/L	California Verbal Learning Test-II, Ten unrelated words, WAIS Letter-Number Sequencing	tHcy > 13 $\mu$ mol/L was associated with poor performance of combined verbal and working memory independent of age, and hormone therapy
Lewerin et al. (162)	209 free living subjects, mean age 76 years	Mean tHcy = 17.2 $\mu$ mol/L MMA = 0.22 $\mu$ mol/L Folate = 16.0 nmol/L B <sub>12</sub> = 325 pmol/L	Postural-Loocomotor-Manual test A battery of cognitive tests	tHcy and MMA correlated independently with movement and cognitive performance
Wright et al. (36), the Northern Manhattan Study	2871 stroke free subjects, age > 40 years	Mean tHcy = 10.2 $\mu$ mol/L Mean MMA = 214 nmol/L; 17% had MMA > 271 nmol/L	MMSE scores	MMSE scores were negatively related to tHcy in subjects > 65 years. 3.3 lower MMSE points in subjects with tHcy > 15 $\mu$ mol/L.
Elias et al. (163), The Maine-Syracuse Study	812 subjects free of dementia and stroke	Mean tHcy = 10.0 $\mu$ mol/L Folate = 38.5 nmol/L B <sub>12</sub> = 389 pmol/L	Visual spatial organization, working memory, scanning tracking, abstract reasoning	tHcy was negatively related, and vitamin B <sub>6</sub> was positively related to cognitive performance even after adjusting for possible confounding factors
Elias et al. (164), The Framingham Offspring Study	2096 dementia and stroke free subjects age > 40 years	Mean tHcy = 10.32 $\mu$ mol/L in 705 subjects aged 60–82 years	Multiple measures of cognitive function	tHcy was inversely related to 9 of 12 cognitive measures tested. This was significant in subjects who were 60 years or older.
Miller et al. (165), The Sacramento Area Latino Study	1789 elderly, age $\geq$ 60 years	Median tHcy = 9.8 $\mu$ mol/L	The modified MMSE test, six other cognitive tests	A modest, significant correlation was observed between tHcy and several measures of cognitive function
Ravaglia et al. (166)	650 healthy, non-demented elderly (age $\geq$ 65 years)	Mean tHcy was higher in subjects with lower MMSE scores (11.9 vs. 14.5 $\mu$ mol/L for scores > 28 vs. < 26)	MMSE test	tHcy is an independent and graded risk factor for cognitive decline with age

**Table 2** Summary of results from vitamin intervention studies and cognitive function.

Study	Number	Duration	Dose	Results
McMahon et al. (167)	276 dementia free healthy elderly subjects	2 years	Folate 1 mg, B <sub>12</sub> 0.5 mg, B <sub>6</sub> 10 mg/placebo	No effect on cognitive function
Stott et al. (48)	185 patients age ≥ 65 years, with vascular disease	1 year	Folic acid 2.5 mg + B <sub>12</sub> 0.5 mg B <sub>6</sub> 25 mg or B <sub>2</sub> 25 mg	No improvement
Eussen et al. (49)	195 elderly ≥ 75 years with vitamin B <sub>12</sub> deficiency	24 weeks	1 mg B <sub>12</sub> , 1 mg B <sub>12</sub> + 0.4 mg folic acid/ placebo	No improvement
Bryan et al. (168)	211 healthy women, age 20–92 years	35 days	Folate 0.75 mg Vitamin B <sub>12</sub> 15 µg Vitamin B <sub>6</sub> 75 mg/placebo	Improvement in some measures of memory function in older women; No effect on mood
Clarke et al. (50)	128 subjects, median age 75 years	12 weeks	Folate 2 mg + vitamin B <sub>12</sub> 1 mg	No effect on cognitive function
Lewerin et al. (162)	195 subjects, mean age 75 years	4 months	Placebo/folic acid 0.8 mg + B <sub>12</sub> 0.5 mg + B <sub>6</sub> 3 mg	Improvement in few measures of cognitive function
Hvas et al. (169)	140 patients with increased MMA	4 weeks	Placebo Vitamin B <sub>12</sub> (i.m.); 1 mg/week)	B <sub>12</sub> treatment improved one of 8 dimensions of health related quality of life scores
Reynolds (127)	26 folate-deficient epileptic patients	1–3 years	Folic acid 5 mg/day	Improvements in multiple cognitive and motor functions;
Coppen and Bailey (170)	127 patients with depression	10 weeks	Folic acid 0.5 mg/day	Folic acid enhanced the antidepressant action of fluoxetine
Coppen et al. (171)	75 patients on lithium therapy	1 year	Folic acid 0.2 mg/day	Clinical improvements
Vermeulen et al. (60)	158 healthy siblings of patients with premature vascular disease (mean age 46 years)	2 years	5 mg folic acid + 250 mg B <sub>6</sub> placebo	Improvement in cerebral and cerebrovascular microangiopathy
McCaddon (172)	7 subjects (4 F) with cognitive impairments, > 71 years		B-vitamins + N-acetylcysteine	Clinical improvements in all patients, objective improvements in cognitive scores in 5 out of 7 patients

hazard ratio for a tHcy level  $\geq 15$   $\mu\text{mol/L}$  compared with  $< 10$   $\mu\text{mol/L}$  was 2.01 (95% CI 1.00–4.05) for ischemic stroke (56). The Third National Health and Nutrition Examination Survey reported that subjects in the highest quartile for tHcy had an adjusted OR for stroke of 2.3 (95% CI 1.2–2.6) (57). Another report from the Rotterdam Study documented a significant independent relationship between plasma tHcy and silent brain infarcts, periventricular and subcortical white matter lesions measured by magnetic resonance imaging (MRI) (58). These brain lesions are usually an intracranial small vessel disease that causes areas of ischemic demyelination. A recent meta-analysis of available studies estimated that a 3  $\mu\text{mol/L}$  reduction in tHcy can cause 24% lower risk of stroke (59).

Investigations using magnetic resonance angiography and MRI showed that Hcy-lowering with folic acid and vitamin B<sub>6</sub> causes slight improvement in cerebrovascular and cerebral indices (60). The Vitamin Intervention for Stroke Prevention (VISP) trial tested whether high doses of B-vitamins (25 mg vitamin B<sub>6</sub>, 0.4 mg vitamin B<sub>12</sub> and 2.5 mg folic acid) to reduce tHcy levels would reduce the risk of recurrent stroke over 2 years as compared with lower doses (200  $\mu\text{g}$  B<sub>6</sub>, 6  $\mu\text{g}$  B<sub>12</sub> and 20  $\mu\text{g}$  folic acid) (61). Mean tHcy reduction in the high dose group was only 2  $\mu\text{mol/L}$  greater than in the low dose group. The effect of treatment on stroke risk was not significant during this trial. Analyzing data from a subgroup of VISP patients who were likely to benefit from treatment showed that lowering tHcy reduced the combined risk of stroke, coronary disease and death by 21% (62). In the Heart Outcomes Prevention Evaluation (HOPE) 2 Study, 5522 patients with vascular disease or diabetes were randomized to either receive placebo or folic acid (2.5 mg) plus 50 mg vitamin B<sub>6</sub> and 1 mg B<sub>12</sub> for 5 years; one of the primary outcomes was stroke. The risk of stroke was lower in the vitamin group compared with the placebo group [relative risk (95% CI)=0.75 (0.59–0.97);  $p=0.03$ ] (63). Data on stroke mortality in the US and Canada after folate fortification demonstrate a significant decrease in stroke mortality (annually 16,000 less stroke deaths) (64). This was not the case in England and Wales, where food folate fortification has not yet been introduced.

Taken together, systematic reviews of epidemiological and prospective studies reveal a consistent, positive, dose-dependent relationship between tHcy and stroke risk. On the other hand, the B-vitamins (folic acid, B<sub>12</sub>, B<sub>6</sub>) reduce or normalize plasma concentrations of tHcy. Therefore, B-vitamin supplementation holds promise for reducing the risk of stroke. Prospective trials designed to determine whether lowering tHcy reduces the risk of a first stroke are not yet available. The most recent guidelines from the American Stroke Association Stroke Council reiterate the importance of meeting current recommended daily intakes of the vitamins. Because there is as yet no data to recommend a specific treatment approach, patients with a known elevated concentration of tHcy may benefit from treatment with B-vitamins, given their safety and low cost (65).

## Parkinson's disease

PD is an age-related neurodegenerative disorder characterized by depletion of dopamine, dysfunction and death of dopaminergic neurons. Increased plasma concentration of tHcy and low folate occur in many PD patients (66, 67). The increase in plasma tHcy in PD patients depends on folate and B<sub>12</sub> status, genetic polymorphisms that influence tHcy and the treatment used to manage PD symptoms. Patients homozygous for the MTHFR C677T mutation may be more susceptible of HHcy. Moreover, folate status is an important determinant of HHcy in patients homozygous for this mutation (67). In a follow-up study (9.7 years), dietary intake of folate and vitamin B<sub>12</sub> were not related to the risk of developing PD (68). Nevertheless, dietary intake of the vitamins does not reflect vitamin status.

HHcy is common in L-dopa treated PD patients. L-Dopa replenishes depleted dopamine and is methylated by catechol-O-methyltransferase (COMT), a SAM-dependent enzyme. A significant increase in plasma concentration of tHcy occurs in PD patients after starting L-dopa treatment (69). This may reflect depletion of methyl groups required for its metabolism (70). L-Dopa increases concentrations of cerebral SAH in animals (70). Depending on the dose and frequency, L-dopa treatment causes a decrease of SAM between 36% and 76%, in addition to a marked increase of SAH (70). From this point of view, L-dopa may promote neurodegeneration in PD patients by depleting SAM or increasing SAH and tHcy. Higher demands for SAM in L-dopa-treated patients in the face of unchanged food intake of labile methyl groups (methionine, choline) would require greater de novo synthesis of endogenous SAM. This is supported by the findings that L-dopa induces the activities of COMT and methionine adenosyl transferase (71).

In vitro studies demonstrate that SAM or its precursor methionine may ameliorate the neurotoxicity of L-dopa to dopamine neurons by providing sufficient methyl groups for COMT (72). Studies using COMT inhibitors to avoid L-dopa-induced HHcy are conflicting (73–75). Folate and vitamin B<sub>12</sub> status are important modifiers of the effect of COMT inhibitors on tHcy levels in L-dopa-treated PD patients. Higher vitamin status may enhance the catabolism of tHcy in these patients. Folate and vitamin B<sub>12</sub> supplementation was successfully used for lowering tHcy in PD patients receiving L-dopa (76, 77). In one study concentrations of tHcy increased with age but did not differ across AD, MCI, cerebral amyloid angiopathy and PD patients (78). The elevated levels within the PD group were due to high tHcy in individuals taking L-dopa (78). Increasing tHcy was associated with worse cognition in the PD cases, but not in the other diagnostic groups (78).

Experimental studies provide more information about potential mechanisms linking tHcy and folate deficiency to PD. In a PD mouse model, HHcy or folate deprivation exacerbated MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-induced dopamine depletion, neuronal degeneration and motor dysfunction

(79). Animal studies also confirmed that tHcy itself may enhance the progress of PD by damaging dopaminergic neurons (80).

One interesting study tested the association between plasma tHcy concentrations and depression, and motor and mental impairments in PD patients (81). Interestingly, PD patients who were also hyperhomocysteinemic (tHcy >14  $\mu\text{mol/L}$ ) had worse cognition and were more likely to be depressed compared with patients with tHcy <14  $\mu\text{mol/L}$  (81). There was no significant association between tHcy concentrations and scores of motor function. Therefore, it seems that higher concentrations of tHcy in PD patients are generally associated with poor progression of the disease. This is in accordance with an increased risk of vascular disease in PD patients with tHcy  $\geq 17.7 \mu\text{mol/L}$  (82). A prospective study on elderly women with PD showed that patients with high baseline tHcy (>21.0  $\mu\text{mol/L}$ ) are more likely to develop hip fracture during 4.9 years of follow-up (83). Because hip fracture is frequent in PD patients (84) and because HHcy is a risk factor for hip fracture (85, 86), the data suggest that reducing plasma tHcy may be an important preventive measure against hip fracture in PD patients. Other studies failed to detect an association between vitamin intake or plasma tHcy and the incidence or clinical course of PD (87, 88). However, these studies were limited by a low number of participants, the short-term of follow-up or by the fact that vitamin intake does not reflect vitamin status, especially in elderly subjects where malabsorption commonly occurs.

Taken together, numerous studies suggest that a high plasma tHcy concentration may be a risk factor predicting a worse progression of PD. Because HHcy can be a result of L-dopa treatment, a causal role for tHcy in the onset of PD has not yet been confirmed. There are several intervention studies in patients with PD (76, 77), but there is no information currently available on the clinical outcome in treated patients. In general, plasma concentrations of tHcy should be tested in PD patients and maintained at low levels, especially in patients receiving L-dopa.

## Homocysteine is linked to other neurological and neuropsychiatric diseases

### Multiple sclerosis

Multiple sclerosis (MS) is a chronic inflammatory CNS disease. Its etiology is unknown, but it causes gradual destruction of myelin and neurons. MS is a multifactorial disease with genetic, environmental and immunological components.

There is a possible association between HHcy and MS; an early study found low concentrations of folate and vitamin B<sub>12</sub> in CSF samples from MS patients (89). Other studies showed that CSF and plasma concentrations of tHcy are elevated in MS patients compared with control subjects (90, 91). Folate and

vitamin B<sub>12</sub> concentrations are lower in MS patients (90, 91). There is no relationship between tHcy concentrations and the stage of the disease (90). Other studies failed to detect a difference in tHcy or MMA concentrations between patients and control subjects (92, 93). Conflicting results in these studies might be related to differences in disease severity or treatment regimens; little is known about the effect of immunomodulating medications on Hcy metabolism.

Mechanisms linking tHcy to MS are not well investigated. On the one hand, data available from other inflammatory diseases show that Hcy may initiate and enhance the development of inflammation (94). Recent studies suggest an important role for post-translational modifications (including methylation) of myelin basic protein (MBP) in the pathogenesis of demyelination diseases (95). MBP comprises 35% of total myelin protein and is a strong candidate autoantigen in MS. Methylation of arginine 107 of MBP is one important post-translational modification of this protein (96). Arginine methylation plays a role in cell signaling and modulating interferon transcription (97). The role of methylation in myelination is suggested by studies showing that methylation of MBP by vitamin B<sub>12</sub> treatment reversed the myelinolysis of subacute combined degeneration of spinal cord (98).

Results from treatment studies with B-vitamins in MS patients are encouraging. In a placebo-controlled double-blind study, 138 MS patients were treated with i.m. vitamin B<sub>12</sub> for 24 weeks (99). Patients who received vitamin B<sub>12</sub> showed greater improvements in clinical scores compared to patients who received placebo (99).

The available evidence suggests that patients with chronic CNS inflammation should at least ensure sufficient dietary requirements for B-vitamins. Such patients should also be tested for tHcy and B-vitamin status and supplementation should commence for those with a low vitamin status. Available data are limited and a general treatment with B-vitamins for all MS patients cannot yet be recommended.

### Depression

A possible relationship between mental illness and methylation in the CNS was postulated several decades ago (100). Administration of methionine, especially in combination with a monoamine-oxidase inhibitor could cause a psychotic reaction in approximately 40% of schizophrenic patients (101).

There is some evidence linking one-carbon metabolism to depression. Several surveys show that folate deficiency is very common in patients with depressive disorders. In a cross-sectional study of 883 elderly Latino females (>60 years), only 1% were folate-deficient (serum folate <6.8 nmol/L) (102). Despite folate fortification, serum folate <25.24 nmol/L (i.e., in the 'low-normal' range) was associated with an OR for depressive symptoms of 2.04 (95% CI 1.38–3.02) (102). The association between vitamin B<sub>12</sub> deficiency and depression was observed in the Women's Health

and Aging Study of 700 community dwelling non-demented disabled women (age  $\geq 65$  years) (103). Metabolically significant vitamin B<sub>12</sub> deficiency was present in approximately 15% of non-depressed women, 17% of mildly depressed women and 27% of severely depressed women (103). According to the study, women with vitamin B<sub>12</sub> deficiency were 2.05 times as likely to be severely depressed than non-deficient women. Neither folate deficiency nor HHcy were associated with depression in this study (103). In another study, increased plasma MMA ( $> 400$  nmol/L) was associated with a high prevalence of depression; however, vitamin B<sub>12</sub> injection (1 mg/week for 1 month) did not improve symptoms (104). In a cross-sectional study on middle-aged men (46–64 years) from Finland, tHcy  $> 11.4$   $\mu\text{mol/L}$  was associated with a higher risk of being depressed (105). In the Rotterdam Study involving 112 subjects with depressive disorders, low vitamin B<sub>12</sub> ( $< 258$  pmol/L) or HHcy (tHcy  $> 15$   $\mu\text{mol/L}$ ) were related to an increased risk of depression (106). These data are in contrast to Morris et al., who found no association between tHcy and depression in a large cohort of a US population (age 15–39 years) prior to folate fortification (107). However, subjects with a lifetime diagnosis of major depression had lower blood folate than subjects with no history of depression (107). In a study of patients with severe depression, more than half of the patients had concentrations of tHcy  $> 11.9$   $\mu\text{mol/L}$  (108).

The most important metabolic disturbance reported in affective disorders is abnormal monoamine (serotonin, dopamine, noradrenaline) metabolism. Because of the role of folate in maintaining methylation status, disturbed methylation may underlie mood disorders (109). Recent clinical and experimental studies link folate, SAM and monoamine metabolism, probably via the biopterin pathway (110). Patients with elevated tHcy had significantly lower concentrations of CSF-folate, CSF-SAM and CSF-monoamine metabolites compared with patients with normal tHcy. In addition, vitamin B<sub>6</sub> deficiency may be associated with depression (111), probably because of the role of pyridoxal 5'-phosphate in the tryptophan-serotonin pathway.

Early reports documented an antidepressive effect of SAM (112). SAM is as effective as tricyclic antidepressants in treating depression, particularly endogenous depression (113). SAM appears to lift mood faster than antidepressants. Moreover, SAM administration elevates CSF-SAM. Folic acid and SAM are frequently used as complementary treatments in depression (112, 114, 115). Folate deficiency can also be a result of poor diet in patients with mood disorders. Nevertheless, whether folate deficiency is primary or secondary in depression, folate administration enhances recovery of the mental state (116). On the basis of available data, oral doses of folic acid (0.8–2.0 mg daily) and vitamin B<sub>12</sub> (1 mg daily) are recommended to improve outcome in depression (117, 118).

## Epilepsy

Convulsive seizures are common in patients with homocysteinuria, suggesting that disturbed Hcy metabolism perhaps contributes to these epileptic episodes. Hcy and other sulfhydryl-containing metabolites (cysteine, homocysteic acid, homocysteine thiolactone) are N-methyl-D-aspartate (NMDA)-receptor agonists, and thereby can cause epileptic episodes. Homocysteine thiolactone administered to animals causes convulsions, and this compound has excitatory CNS effects (119).

These observations suggest a causal role for Hcy in epilepsy. Nevertheless, it should be recognized that HHcy may result from medications commonly used to treat patients with epilepsy. Between 13% and 40% of patients treated with antiepileptics develop elevated concentrations of tHcy (120). Concentrations of tHcy  $> 10.4$   $\mu\text{mol/L}$  occur in approximately 16% of children receiving long-term antiepileptic drugs (121). Furthermore, multidrug treatment and long duration of the therapy enhance the risk for HHcy (121). Elevated concentrations of tHcy and bone loss were found in patients taking antiepileptic drugs (122). Because of the role of tHcy in bone loss (123), HHcy in patients treated with antiepileptics might increase the risk of osteoporosis.

Antiepileptics increase Hcy concentrations via an effect on B-vitamins metabolism (121, 124). First, antiepileptics may impair folate absorption and gastrointestinal transport by altering gastrointestinal pH. Second, these drugs may induce folate catabolizing hepatic enzymes, such as cytochrome P450 (125). Antiepileptic drugs that do not induce P450 are not associated with a low folate concentration. The effect of valproate on folate is probably related to inhibiting glutamate formyl transferase and changing the balance between various folate forms (126). Third, folate status and intake before and during treatment affects the magnitude of HHcy. Valproate may induce methionine synthase and MTHFR activities in the liver and can inhibit serine hydroxymethyltransferase activity, thus causing lower folate status and increased concentrations of tHcy.

Oral folic acid supplementation (1 mg/day) in children with tHcy  $> 10.4$   $\mu\text{mol/L}$  significantly lowers tHcy after 6 and 12 weeks (121). Treating folate-deficient epileptic patients with 5 mg folic acid daily for 1–3 years markedly improves cognition, mood and social behavior (127). As discussed previously, folate deficiency is associated with depression. Unsurprisingly then, patients on anticonvulsants often develop depressive symptoms (128, 129).

Importantly, young epileptic women have low serum and red blood cell folate levels that are associated with poor pregnancy outcome (130, 131). Therefore, it is important to ensure that all pregnant women on antiepileptics should receive sufficient folate, especially during the first trimester. There is strong evidence that tHcy concentrations should be measured in patients taking antiepileptics (121).

Folate supplementation is recommended for all subjects on chronic treatment with such medications.

### **Pathological effect of hyperhomocysteinemia in the central nervous system**

#### **Morphological and functional changes in the brain**

Elevated tHcy concentrations increase the risk of micro- and macrovascular disease and thereby promote cognitive decline. HHcy and other related metabolic disorders cause structural changes in the brain. Silent brain infarcts and white matter lesions are frequently seen on brain MRI in elderly and are associated with an increased risk of stroke or dementia. Several cross-sectional studies document a positive relationship between tHcy and white matter hyperintensity (58) and brain atrophy. The volume of grey matter in the brain is positively related to blood tHcy concentration (52, 132).

In the Rotterdam Scan Study, the OR for silent brain infarction in subjects with tHcy > 13.7  $\mu\text{mol/L}$  was 2.5 (95% CI 1.4–4.5) compared with subjects with tHcy < 8.6  $\mu\text{mol/L}$  (58). The OR for white matter hyperintensity for the same group was 2.3 (95% CI 1.3–4.2) (58). One study demonstrated a negative association between folate and white matter hyperintensity in elderly patients with psychiatric disorders (133). The association between tHcy and MRI-visible microangiopathy was confirmed by most (58, 134), but not all studies (135), despite the association between tHcy and cognitive function. Additionally, an association between tHcy and white matter hyperintensity exists in AD patients (44). Wong et al. recently observed a similar relationship between tHcy and white matter hyperintensity in patients with cerebral small vessel disease (136). Nevertheless, the authors found no significant relationship between tHcy and silent brain infarcts, cerebral atrophy or psychometric performance (136). This is in contrast to the Northern Manhattan Study where tHcy correlated with microvascular lesions in the white matter (137). This association might relate to endothelial dysfunction caused by HHcy, excitotoxic and apoptotic mechanisms and also affect the integrity of myelin.

The relationship between tHcy and brain atrophy was observed in the Rotterdam Scan Study that included 1077 non-demented elderly subjects (138). Moreover, tHcy concentrations were independently related to smaller hippocampal width on MRI in healthy elderly subjects (139). In another study including younger subjects (60–64 years), the association between tHcy and brain atrophy was not significant (140). Nevertheless, this could be related to the fact that other factors associated with aging (i.e., oxidative stress) synthesize the brain to be damaged by HHcy.

In contrast, the relationship between tHcy and brain atrophy was not confirmed in the PATH Through Life Study (140), despite the significant association with increased microvascular lesions. Other pathological effects of HHcy in the brain include damage to the blood-brain barrier (141). This is consistent with an

intervention trial showing improvement in the permeability of the blood-brain barrier (142).

Cross-sectional studies showing an association between tHcy and brain atrophy or leukoaraiosis do not provide evidence for a causal relationship between tHcy and brain damage. Nevertheless, treatment with folic acid and vitamin B<sub>6</sub> lowers tHcy and improves cerebrovascular and cerebral indices (60), suggesting that intervention with B-vitamins can protect the brain by improving cerebrovascular function.

#### **Mechanisms of total homocysteine neurotoxicity**

Observations in CBS-deficient patients suggest that Hcy plays a direct neurotoxic role in the CNS. Others have confirmed these observations, and also supplied evidence for an independent role for vitamins. Hcy is toxic to neuronal cells in vitro (143–145). Animal studies show that CBS knockout mice (Cbs<sup>-/+</sup> or Cbs<sup>-/-</sup>) have severe neurological damage in addition to an increase in tHcy concentrations by approximately 2–50-fold in comparison to wild type mice (141, 146, 147). These animals show alterations in neuronal plasticity, suffer from severe retardation and die early (148). Animals exposed to Hcy accumulate this compound in the brain (149), suffer from restricted growth, neural or cognitive dysfunction (149, 150) and exhibit impaired brain energy metabolism (151). Moreover, HHcy is implicated in neural plasticity and neurodegenerative disorders in human studies (152).

Hcy is an endogenous glutamate receptor agonist that acts on NMDA and non-NMDA receptor subtypes (153). The effect of Hcy on NMDA receptors increases calcium influx and causes apoptosis or changes in cell signaling. Furthermore, Hcy induces neurological dysfunction via oxidative stress (154). This effect can explain the enhanced production of reactive oxygen species, oxidative deactivation of nitric oxide and lipid peroxidation (155). Antioxidant treatment restores several toxic effects of Hcy (155).

HHcy is associated with increased concentrations of tHcy and SAH and disturbances in the transmethylation of novel biochemical pathways in the brain. Brain hypomethylation results in DNA-hypomethylation (156) and changes in expression of genes encoding presenilin I, a secretase enzyme that degrades A $\beta$ . Moreover, available data suggest that alterations in SAM metabolism or SAM/SAH ratio inhibit dephosphorylation of tau protein thus enhancing the risk of dementia (157). One important issue to be considered on the pathogenetic mechanism of HHcy is that elevated tHcy can cause damage to the vascular system thus causing the mental illness (158). The association between the risk of stroke and HHcy is one example. Other mechanisms linking Hcy to neuronal damage are reviewed elsewhere (159).

#### **Final comments on vitamin intervention studies in neurological diseases**

There are currently no clear guidelines for the dose, duration and combination of vitamin treatment for

**Table 3** Treatment studies in neurological and neuropsychiatric diseases.

Disease	Patients	Dose	Duration	Clinical effects	Reference
Stroke	3361 patients with cerebral infarction, mean age 66 years	25 mg B <sub>6</sub> , 0.4 mg B <sub>12</sub> , 2.5 mg folic acid/day or 200 µg B <sub>6</sub> , 6 µg B <sub>12</sub> , 20 µg folic acid/day	2 years	A graded association between baseline tHcy and the risk of stroke was found. However, moderate reduction of tHcy had no significant effect on the risk of stroke within 2 years of follow-up.	(61)
Stroke	2155 patients with cerebral infarction, mean age 66 years (exclusion criteria: patients with low B <sub>12</sub> and those with very high B <sub>12</sub> at baseline, B <sub>12</sub> malabsorption, B <sub>12</sub> supplementation, renal dysfunction)	25 mg B <sub>6</sub> , 0.4 mg B <sub>12</sub> , 2.5 mg folic acid/day or 200 µg B <sub>6</sub> , 6 µg B <sub>12</sub> , 20 µg folic acid/day	2 years	A 21% reduction in the combined risk of (stroke, coronary disease, death)	(62)
-	141 healthy siblings (mean age 46.0 years) of patients with premature atherosclerotic disease	5 mg folic acid + 250 mg of vitamin B <sub>6</sub> /day or placebo	2 years	Non-significant improvement in magnetic resonance angiography (MRA) (carotid stenosis: carotid and/or vertebral elongation) and magnetic resonance imaging (MRI) (white matter abnormalities: cerebral atrophy)	(60)
MS	138 patients	i.m. vitamin B <sub>12</sub> (1 mg/week) placebo	24 weeks	Improvement in clinical scores	(99)
Epilepsy	19 patients with Hcy > 10.4 µmol/L	Oral folic acid/1 mg/day or placebo	12 weeks	Not tested	(121)
Epilepsy		Folic acid 5 mg/day	1-3 years	Improvement in cognition, mood and social behavior	(127)
Depression, schizophrenia	41 patients with acute psychiatric disorders and folate deficiency	Methyl folate 15 mg/day or placebo in addition to a standard therapy	6 months	Depressed and schizophrenic patients showed significant improvement in clinical social tests	(116)
Depression, dementia	140 subjects with MMA > 400 nmol/L	1 mg B <sub>12</sub> /i.m./week or placebo	1 month	Vitamin B <sub>12</sub> did not improve cognitive function or symptoms of depression after 3-months of follow-up	(104)
Parkinson's disease	20 PD patients treated with L-dopa for at least 1 year, 35 controls	0.5 mg B <sub>12</sub> + 5 mg folic acid/day/orally	5 weeks	tHcy lowered from 17.9 to 10.5 µmol/L, no clinical effects were tested	(76)

CNS disorders. The American Stroke Association Stroke Council recommended sufficient daily intake of the vitamins in general, and treatment for subjects with known elevated concentration of tHcy in particular (65). Although it is possible that tHcy elevation that occurs during the course of the neurological disease can represent an epiphenomenon, it should be important to ensure sufficient vitamin intake for primary and secondary prevention. Folate treatment for vitamin B<sub>12</sub>-deficient subjects may delay the diagnosis of B<sub>12</sub> deficiency and cause irreversible CNS damage. Therefore, addition of vitamin B<sub>12</sub> to folate treatment is strongly recommended. Doses and duration of vitamin supplementation should be individually determined; malabsorption is a major limiting factor for the bioavailability of the vitamins in elderly people. Tables 2 and 3 show some examples on vitamin dose and responses to treatment in neurological diseases.

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