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# Hyperhomocysteinemia and Its Role in Cognitive Impairment and Alzheimer's Disease: Recent Updates from the Literature

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## Abstract

Homocysteine (Hcy) is a sulfur-containing non-essential amino-acid produced as the result of the metabolism of the essential amino-acid methionine. Hcy levels are influenced by several factors, including age, renal function, genetic polymorphisms, dietary habits and lifestyle conditions. Increased plasma level of Hcy is defined as Hyperhomocysteinemia (HHcy). HHcy has been linked to several pathological conditions; among them cognitive decline and neurodegenerative diseases are receiving increasing attention by scientific investigations. The aim of this review was to discuss the pathological mechanisms, mostly investigated by basic research and animal models that HHcy is able to trigger in the brain and reporting the latest studies that examine the association between HHcy and cognitive decline/dementia. Moreover, we included a review of the recent clinical trials investigating the efficacy of Hcy lowering therapies on cognitive outcomes.

**Keywords:** Homocysteine; Cognitive impairment; Alzheimer's disease; Dementia; Cognitive decline

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## Introduction

Homocysteine (Hcy) is a sulfur-containing non-essential amino-acid produced as the result of the metabolism of the essential amino-acid methionine. Optimal methionine metabolism is vital to a great number of biochemical processes in the body. Hcy is normally metabolized via two biochemical pathways: remethylation, which converts Hcy back to methionine via the folate cycle, which is catalyzed by the enzyme methylenetetrahydrofolate reductase (MTHFR) and the essential cofactor vitamin B12, and trans-sulfuration, which converts Hcy to cysteine (Cys) via cystathionine beta synthase (CBS) and the essential cofactor vitamin B6. Therefore, these two metabolic pathways balance the level of methionine and Hcy [1,2].

A high level of plasma Hcy is defined as Hyperhomocysteinemia (HHcy). The normal range of Hcy plasma levels varies with age. In any case, the clinically acceptable concentration should be <12-15 µmol/L. Several factors are able to influence the levels of Hcy. The dietary intakes of methionine, as well as folates and vitamin B12 levels are the principal determinants of the physiologic levels of Hcy [3]. Lifestyle conditions such as excessive coffee or alcohol consumption, cigarette smoking and physical inactivity as well as overweight seem to be associated to higher levels of Hcy [4,5], even if some authors reported that modifications in plasma Hcy levels were not associated with changes in dietary habits, physical activity, smoking status, coffee, tea, total alcohol or wine consumption [6]. Impaired renal and thyroid function can cause HHcy [7,8]. Renal failure (which is often associated with diabetes mellitus) could also explain, at least in part, the wide range of Hcy levels reported in diabetic patients, even if the link between Hcy and diabetes needs further investigation [9]. Moreover, genetic factors can influence Hcy concentrations. The MTHFR locus is mapped on chromosome 1 at the end of the short arm (1p36.6). This enzyme is crucial for the folate metabolism, as previously described. The common MTHFR C677T polymorphism, which results in the amino-acid product changing from alanine to valine, affects the activity of the enzyme and hence folate distribution. Homozygous mutated subjects have higher Hcy levels while the heterozygous mutated subjects have mildly raised Hcy levels when compared with the normal non-mutated controls [10,11]. Another MTHFR polymorphism has been described: the A1298C,

 

which changes a glutamate into an alanine residue. This mutation results in decreased MTHFR activity, which is more pronounced in the homozygous than heterozygous state. Anyway, neither the homozygous nor the heterozygous state is associated with higher plasma Hcy or a lower plasma folate concentration, which is evident with homozygosity for the C677T mutation. However, it has been reported an interaction between these two common mutations [12].

## Effects of HHCY in the brain

Several mechanisms have been proposed to explain the known Hcy neurotoxicity. Oxidative stress is generated during oxidation of free thiol group of Hcy. Several mechanisms have been hypothesized for Hcy-induced oxidative stress: auto-oxidation, inhibition of cellular antioxidant enzymes, nitric-oxide (NO)-synthase dependent generation of superoxide anion, disruption of extracellular superoxide dismutase from endothelial surfaces, activation of Nicotinamide-Adenine Dinucleotide Phosphate (NADPH)-oxidase [13]. Hcy induced oxidative stress may result in the production of reactive oxygen species with consequent oxidation of proteins, lipids and nucleic acids [14] which may lead to endothelial dysfunction and damage to vessel wall with subsequent platelet activation and thrombus formation [15]. It is also important to mention Hcy relation with glutathione (GSH) synthesis, which is one of the most important antioxidants in human cells. GSH biosynthesis is Cys dependent. It has been hypothesized that trans-sulfuration pathways could be less functional in the brain since a reduced expression of cystathionine γ-lyase in neurons [16] leads to a higher susceptibility of the brain itself to oxidative damage. Impaired syntheses of NO in the endothelium as well as increased production of asymmetric dimethylarginine and activated oxygen species are involved in the impairment of vasodilator effects of NO; this could impair brain circulation with the consequence of triggering or enhancing neurodegenerative processes [17].

The conversion of Hcy to Homocysteine thiolactone (HcyTLN) followed by protein structure modifications (homocysteinilation) largely contributes to Hcy toxicity. HcyTLN, an intramolecular thioester of Hcy, is synthesized by methionyl-tRNA synthetase in an error-editing reaction that prevents translational incorporation of Hcy into proteins. HcyTLN reacts with proteins by a mechanism involving homocysteinylation of protein lysine residues; this mechanism seems to lead to several detrimental effects like immune activation, autoimmune inflammatory response, excitotoxicity and apoptosis of neuronal cells, amyloid-beta (A-β) deposition as well as predisposition of N-homocysteinylatyed proteins to aggregation and precipitation [13,18].

Hcy may exert its toxic effects by acting on N-Metil-D-Aspartate (NMDA) receptor as a glutamate-receptor agonist. Excitation of NMDA receptors results in incongruous calcium influx to the intracellular compartment and this leads to neuronal toxicity, apoptosis and death of neuronal cells [19]. Hcy may also act like an excitatory neurotransmitter by competing with inhibitory neurotransmitters such as gamma-aminobutyric acid (GABA) [20].

As for the mechanisms by which HHcy can have a role in neurodegeneration, Irizarry et al. demonstrated a positive association between total Hcy levels and plasma Aβ levels. After covariate analysis of the data, the authors evidenced that the association was not explained by other factors like diagnosis, age, folate levels, vitamin B12, or APOE polymorphisms [21].

Research on animal models showed the possible mechanisms by which Hcy plays a significant role in the deposition of Aβ in the brain. Li et al. [22], using a mouse model of Alzheimer's disease (AD), observed that, when compared with controls, mice having high Hcy levels manifested an exacerbation of their behavioral deficits, and a significant increase in the amount of Aβ plaques and neurofibrillary tangles was demonstrated. The changes in Aβ were associated with an upregulation of the γ-secretase pathway, whereas the hyperphosphorylation of tau was secondary to a selective activation of the cyclin-dependent kinase 5. The same authors also demonstrated that a diet-induced high Hcy in mice favored the development of cerebral amyloid angiopathy by means of a reduction of Aβ clearance and transport within the brain [23]. Xie et al. [24] have investigated whether stress-induced HHcy could be correlated with the accumulation of Aβ in the brain of rats. The authors showed that both hippocampal and cerebral levels of Aβ were positively associated with the concentration of plasma Hcy. They also evidenced that chronic unexpected mild stress (CUMS) in rats was able to induce Amyloid Precursor Protein **(**APP) misprocessing by the up-regulation of Aβ precursor protein, β-site APP-cleaving enzyme, presenilin-1 (PS1), and the down- regulation of insulin-degrading enzyme (IDE), a protease able to remove Aβ. The authors suggested that Hcy is likely to be involved in chronic stress-evoked APP misprocessing. PS-1 could probably be the target of Hcy effects in stress situations and IDE could also partially be regulated by Hcy levels.

A recent article [25] suggested that Hcy could also have a direct neurotoxic effect on hippocampal neurons. The regression analyses presented by the authors showed that plasma total Hcy level was significantly associated with hippocampal volume in elderly people even after controlling the degree of global cerebral beta amyloid deposition and vascular burden as well as other potential confounders (age, gender, education, and apolipoprotein E ε 4 genotype).

Schaub et al. [26] performed an electrophysiological study on primary cultures of mouse hippocampal neurons using the whole-cell patch-clamp technique. They showed that chronic Hcy exposure caused modifications in the intrinsic electrophysiological properties of cultured hippocampal neurons as a mechanism of neurological symptoms of HHcy.

Furthermore, another mechanism by which Hcy could have a neurodegenerative effect in the brain is its toxic action on glial cells. Indeed, it has been demonstrated that this molecule could be a potent gliotoxic agent, able to induce death of human glial cells already at concentrations reached in brain during HHcy [27].

It has been hypothesized that HHcy could also act as endoplasmic reticulum **(**ER) stressor, by causing protein misfolding as a consequence of its interaction with protein disulfide bonds. The

ER is the principal site for protein synthesis and maturation in the cell. It has a number of jobs within the cell; these include the folding and transport of various proteins, specifically carrying them to the Golgi apparatus. Some other proteins, mostly the glycoproteins, move across the ER's membrane. ER stress can be induced when some proteins become unfolded or misfolded and tend to accumulate and/or aggregate. If not resolved, this phenomenon slows down protein synthesis, activates protein degradation pathways and eventually triggers apoptosis [28].

In recent years, it has also been suggested that HHcy can act through epigenetics pathways by alterations in DNA metilation caused by imbalance in the levels of biochemical components of Hcy cycle. These studies agree with the latest hypothesis that the pathogenesis of neuropsychiatric and neurodegenerative diseases could be associated to genome-environment interactions [29,30].

Finally, HHcy produces structural and functional changes in cerebral microcirculum, which is very sensitive to local levels of Hcy. Both dietary and genetic HHcy have been associated with hypertrophy of cerebral arterioles and increased arteriolar distensibility in mice [31]. Moreover, other Hcy induced mechanisms leading to microvascular modifications are: impairment in vascular endothelial cell function (by increasing oxidative stress and reducing NO activity and availability), resulting in vasoconstriction and thrombosis, plaque formation [32], basement membrane irregularities, disruption of blood-brain barrier [33] and ultrastructural changes in endothelial mitochondria [34].

## Hyperhomocysteinemia and cognitive functions

**HHCY and mild cognitive impairment:** Mild cognitive impairment (MCI) is an intermediate pathological condition between normal aging and definite dementia. It affects memory, language, thinking and judgment with greater symptoms when compared to normal age-related changes. Generally, these modifications are not severe enough to significantly interfere with basic activities of daily living. A minimal impairment in complex instrumental functions could be demonstrated. For the diagnosis of MCI, the patient must not meet the criteria for dementia [35]. MCI is a heterogeneous entity, with possible evolutions including AD, other types of dementia, and even reversion to normal cognitive functioning. The transition from MCI to dementia is usually gradual. Several studies evidenced that HHcy is associated with this transition [36].

Despite most of the small cross-sectional studies demonstrated an association between HHcy and cognitive impairment, longitudinal studies reported inconsistent results [37].

Kim et al. [38] investigated the association between plasma levels of folate, B12, Hcy with cognitive functions in 321 Korean elderly. Plasma Hcy levels were higher in patients with AD and MCI when compared to normal subjects ($p < 0.001$). The authors found out that plasma levels of these substances were associated with cognitive performances in MCI and AD elderly patients.

Seven hundred fifty-seven elderly aged 70 to 90 (a subsample of participants in the Sydney Memory and Ageing Study) without dementia were enrolled in a cross-sectional study [39] with the aim of comparing the risk profiles of MCI subtypes. The age and sex-adjusted multiple regression analysis evidenced that high Hcy was a risk factors for MCI (OR=1.07, 95% CI=1.03–1.11, $P<0.001$). Similar results have been evidenced by Moustafa et al. [40] who investigated the relationship between cognitive functions and Hcy levels in healthy subjects (Global Dementia Rating, CDR=0) and individuals with MCI (CDR=0.5) (total n=52). They found that Hcy levels were significantly higher in individuals with MCI than in healthy controls ($p<0.001$).

Rogne et al. [41] included 103 subjects with MCI without known stroke or other apparent causative diseases and 58 controls in an observational study in order to determine whether total plasma Hcy, cardiovascular risk factors and volumetric analyses of cerebral magnetic resonance imaging were differently associated with MCI in subjects from families with aggregation of late-onset AD and MCI in subjects from families without late-onset AD. The results of this study showed that Hcy was significantly elevated in all cases when compared to controls, except for women with probable familial late-onset AD.

In contrast with the previous studies, Reitz et al. [42] in a longitudinal cohort study of individuals aged 65 years or older without MCI or dementia at baseline, evidenced that levels of Hcy, vitamin B 12 or folate did not significantly differ between subjects with and without MCI at baseline. Moreover, Hcy levels at baseline did not also differ between persons who remained free of MCI during the follow-up or persons who developed MCI during the course of the study. The results of longitudinal analyses evidenced a trend towards an association between higher Hcy levels and a lower risk of all-cause MCI in crude models, but this risk was attenuated with adjustment for ethnic group and APOE-4 genotype.

Nilsson et al. studied a population of 326 AD patients and 281 patients with MCI. In agreement with the previous study, they reported that MCI patients without cobalamin/folate deficiency or renal impairment showed normal levels of total plasma Hcy [43].

Results from trials assessing the impact of Hcy lowering treatment on conversion form MCI to dementia are still conflicting and inconclusive.

Blasko et al. [44] selected 81 subjects already showing MCI at baseline from the Middle European cohort of the Vienna Transdanube Aging Study (VITA) and followed their conversion to dementia five years later with respect to their vitamin B status, plasma Hcy levels, as well as their subjective information on vitamins intake. The self-reported combined use of folic acid and vitamin B12 for more than one year was associated with a lower conversion rate to dementia: conversion rate at 5 years was 20% in combination users, 75% in inconsistent users and 62.7% in non-users ($p=0.013$). Plasma levels of Hcy and vitamin B12, measured at baseline or at five years, were not associated with conversion. On the contrary, in a previous study on the same cohort the increase of Hcy over 2.5 years was significantly associated with conversion from cognitive health to AD. Moreover, subjects with

stable MCI over 2.5 years showed lower Δ-changes of Hcy when compared to converters to AD [36].

A randomized, double-blind controlled trial which included participants with MCI, aged ≥ 70 years, randomly assigned to receive a daily dose of folate, vitamin B12 and vitamin B6 or placebo for 2 years evidenced that supplementation with B vitamins was able to slow cognitive and clinical decline in people with MCI, in particular in those with elevated plasma Hcy levels [45].

Smith et al. [46] performed a randomized, double-blind controlled trial in order to investigate whether supplementation with B vitamins was able to slow the rate of brain atrophy in subjects with MCI. A total of 168 participants (85 in active treatment group; 83 receiving placebo) completed the MRI section of the trial. The results showed that the treatment effect was greater in those with the highest baseline level of total Hcy, with a reduction in atrophy rate of 53% in those in the top quartile of total Hcy (>13.0 μmol/L). There was no effect of treatment on atrophy in those subjects in the bottom quartile (≤ 9.5 μmol/L).

**HHCY and alzheimer's disease (AD):** AD is a neurodegenerative disease, which results from the deposition of Aβ peptides and a form of tau protein. The primary cause of this multifactorial disease is the accumulation of Aβ, a peptide cleaved from the APP by the stepwise enzymatic action of the beta-secretase (BACE-1) and gamma-secretase [47]. Regland et al. in 1990 first reported elevated Hcy levels in primary degenerative dementia patients [48].

Zhuo et al. [49] summarized the findings from observational and intervention trials with a minimal sample of 50 subjects published until 2011 on the association between HHcy and AD. Among retrospective studies, at least 12 reported higher Hcy levels in AD patients when compared to age-matched healthy controls. On the contrary, three studies reported no difference in plasma or cerebral spinal fluid Hcy levels between AD patients and controls. Among the 12 reported prospective studies, 10 found that HHcy was a risk factor for AD or cognitive decline, while only 2 did not report any association between HHcy and AD/cognitive decline.

In the previously reported study by Nillson et al. [43] the authors reported that HHcy in AD patients was caused by cobalamin/folate deficiency or renal impairment, therefore they hypothesized that HHcy was not involved in the pathogenesis of AD.

A prospective cohort study by Ford et al. [50] included 4227 men aged 70-89 years from the Health in Men Study cohort. Diagnosis of dementia was carried out using morbidity and mortality records and information on total Hcy, MTHFR gene status, lifestyle and clinical variables were obtained using postal and face-to-face assessments. Men diagnosed with dementia had a mean baseline Hcy concentration of 13.9 μmol/l compared with 12.6 μmol/l in men remaining free of dementia. Men with total Hcy concentrations greater than 15 μmol/l were also at greater risk of dementia during the follow-up period. The hazard of dementia nearly doubled (HR 1.94, 95% CI 1.51 to 2.50, p<0.001) with the doubling of total Hcy concentration, and this association remained significant after adjustment for confounding factors. The results of this study evidenced a significant association between total Hcy and the risk of dementia in older men. The hazard of dementia increases by 48% with a doubling of total Hcy concentration.

A longitudinal study of two cohorts of elderly Yoruba and African Americans [51] investigated the association between levels of Hcy and incident dementia. The results obtained after adjusting for age, education, ApoE genotype, smoking, and time of enrollment, evidenced that the higher quartiles of Hcy were associated with a non-significant increase in dementia risk in the Yoruba and a similar but non-significant relationship between higher Hcy levels and dementia risk has been found in the African Americans.

Hooshmand et al. [52], in an autopsy study on 265 individuals aged ≥ 85 years, observed an association between elevated baseline Hcy and increased neurofibrillary tangles count at the time of death. Higher Hcy was not significantly related to increase Aβ burden, but participants with longer time between the baseline Hcy measurement and death tended to have a higher Aβ burden. Moreover, results from post-mortem brain MRI showed that higher Hcy levels were associated with higher medial temporal atrophy and periventricular white matter hyperintensity scores.

Nazef et al. [53] performed a case control-study in and Algerian population of 41 AD patients and 46 non-demented controls. Univariate logistic regression analysis showed a significant increase of total Hcy (p=0.008) and a significant decrease of vitamin B12 (p=0.012) in AD group vs. controls; in multivariate logistic regression analysis total Hcy (p=0.007, OR p=1.376) appeared as an independent risk factor predictor of AD.

Cervellati et al. [54] enrolled 294 elderly subjects with the aim of investigating whether LOAD and Vascular Dementia (VAD) might be associated with a distinct profile of oxidative stress peripheral markers, including Hcy. Results evidenced that higher Hcy levels (≥ 17.0 μmol/L) were associated with higher risk of VAD diagnosis compared with both controls and MCI, but the risk of LOAD diagnosis was not associated with higher levels of Hcy.

A review and meta-analysis by Meng et al. [55] examined whether midlife vascular risk factors were associated with increased risk of incident AD. Smoking and Hcy resulted associated with an increased risk of AD.

As for trials on the effect of Hcy lowering treatments on the risk of AD, a recent prospective case-control study [56] investigated the effect of Cerefolin®/Cerefolin-NAC® (CFLN), on cognitive impairment in a population of Alzheimer's disease and related disorders (ADRD) patients with either HHcy treated with CFLN (N=34) for some portion of follow up period, or no HHcy+ No-CFLN (N=82). CFLN contains distinct bioactive forms of B vitamins in addition to N-acetyl-cysteine. The results showed that HHcy+CFLN group significantly slowed cognitive decline when compared to the No-HHcy+No-CFLN group. The effect of slowing cognitive decline was observed in learning and memory, constructional praxis, and visual-spatial executive function, and was more significant in patients with milder baseline severity and with CFLN treatment durations of at least one year. Longer CFLN treatment duration, milder baseline severity, and magnitude of

Hcy reduction from baseline were all significant predictors of effect.

The same study group investigated if using Cerefolin®/ CerefolinNAC® (CFLN: L- methylfolate, methylcobalamin, and N-acetyl-cysteine) for HHcy in patients with AD or cognitive impairment due to cerebrovascular disease (CVD) (total n=67) could slow regional brain atrophy in the same population of the previous study. All subjects were treated with stable doses of memantine and a cholinesterase inhibitor (ChEI). Results showed that CFLN was associated with significantly slowed hippocampal and cortical atrophy rates in ADRD patients with HHcy [57].

**HHCY and cognitive decline in the elderly:** Hcy levels usually increase with age [58]; this can be explained by the reduction of plasma folate and B12 observed in the elderly, as well as by age-related increase of oxidative stress that might be the cause of HHcy [59]. Several researchers analyzed the influence of HHcy on cognitive performances in older populations finding an association between Hcy levels and cognitive performances [60-63].

A meta-analysis by Nie et al. [64] investigated the association between HHcy and the risk of cognitive decline including 14 studies with a total number of 15908 healthy subjects (mean age in all studies was 60 or greater). Most of the studies were performed in European and American countries. For the analysis, the studies were divided in two groups according to different follow-up periods (more than 5 years and less than 5 years). Results evidenced a pooled RR of 1.53 (95% CI, 1.23-1.91; p=0.0002) for patients with HHcy compared to subjects without HHcy. Subgroup analysis showed that pooled RRs were 1.51 (95% CI, 1.10-2.05; p=0.01) for more than five-year follow- up studies and 1.56 (95% CI, 1.13-2.14; p=0.007) for less than five-year follow-up studies. Therefore, this meta-analysis evidenced that HHcy may act as a risk predictor of cognitive decline.

Kong et al. [65] performed a cross-sectional study including 662 community dwellers and nursing home residents in Tianjin, China aged 55-93 with the aim of investigating the association between total Hcy and cognitive function in middle-aged and elderly persons. The cognitive function of the subjects was measured by two methods. Cognitive performances were assessed in all subjects by Mini-Mental State examination (MMSE) followed by computerized Basic Cognitive Aptitude Test (BCAT) in part of the subjects to further evaluate their cognitive functions. The prevalence of HHcy resulted to be 45.4%. The results showed that plasma serum total Hcy concentration was negatively correlated with total scores of BCAT.

Bonetti et al. [66] performed a cross-sectional study on elderly individuals aged ≥ 65 (N=318; 44 normal cognition, 127 with cognitive impairment, 147 with dementia) with the aim of determining the association between Hcy and cognitive function, taking into consideration the effect of B group vitamin (BGV) deficiency. The authors divided the sample into four groups according to plasma Hcy (high vs. normal) and BGV (normal vs. deficient) levels. Multivariate logistic regression analysis showed that HHcy (>15 µmol/L) was associated with a significantly greater risk of dementia, independent of folate and vitamin B12 deficiency, age, sex, education, stroke history, and cerebral atrophy (OR=1.98, 95% CI=1.13–3.48, P=0.02). Unexpectedly, the greatest prevalence of dementia (61.3%) and the worst cognitive and functional performance were found in individuals with high Hcy but normal BGV levels.

A recent research investigated cognitive functions in postmenopausal women with different Hcy levels [67]. A total of 170 healthy post-menopausal women aged 50-65 years were recruited. The assessment of cognitive function was made with the use of diagnostic equipment CNS Vital Signs (Polish version). Neurocognitive index (NCI) was calculated on the basis of five domains: memory, psychomotor speed, reaction time, attention, and cognitive plasticity. The results evidenced that the better neurocognitive index was obtained by women with low Hcy levels in comparison with those with HHcy (p=0.0017). The domains which were most influenced by Hcy levels were executive functioning, complex attention, cognitive flexibility, and memory. Moreover, the authors demonstrated that ε4/ε4 genotype was the most common (15.5%) in women with HHcy than in groups of patients with low (0%) or normal (1.9%) Hcy levels.

A research group from Taiwan [68] enrolled 225 subjects with normal serum levels of B12 and folate. The participants underwent neuropsychological assessment in order to investigate the role of Hcy in cognitive functions. Hcy levels were significantly higher in the elderly group (≥ 65 years) when compared to the younger group. After adjusting for confounders, only the Digit Symbol Substitution (DSS) score was significantly lower in subjects with HHcy (homocysteine >12 µmol/L) than those with Hcy ≤ 12 µmol/L in the elderly group. Results did not show an association between HHcy and other neuropsychological assessments. The authors hypothesized that the effect of Hcy level on DSS score are age-dependent and that DSS score could be an independent marker of cognitive impairment in response to HHcy in the elderly regardless of diagnosis of dementia.

With regards to trials using Hcy lowering therapies aimed at preventing or treating cognitive impairment, several studies obtained negative results, but there have also been encouraging results from specific groups of patients (based on age, severity of disease and B-vitamin deficiency status) [69].

Clarke et al. [70] performed a meta-analysis using data combined from 11 large trials in 22,000 participants with the aim of investigating the effects of treatment with B vitamins when administered for several years compared with placebo, on composite domains of cognitive function, global cognitive function, and cognitive aging. Treatment with B vitamins was associated with a 28.4% and 26.1% reduction in plasma concentrations of Hcy in cognitive-domain trials and global cognition trials, respectively. Allocation to B vitamins had no significant effect on the changes in the domain-specific scores for memory, speed, executive function, or the domain-composite score. Compared with the placebo group, allocation to B vitamins had no significant effect on MMSE-type score in the 20,431 individuals with data on cognitive function. In conclusion, the present meta-analysis evidenced that Hcy lowering by dietary

supplementation with B vitamins for approximately 5 years did not have an impact on cognitive aging in older people with or without vascular disease.

A recent article by Mc-Caddon et al. [71] critically reviewed the evidence raised by the previous meta-analysis in relation to Sir Austin Bradford Hill's criteria for assessing "causality". The authors suggested that no conclusion could be made on the effects of Hcy-lowering treatments on cognitive decline, since the trials examined did not include individuals who were experiencing such decline. In accordance with this assertion, the authors stated that it is worth screening for Hcy levels in individuals with early signs of age-related cognitive decline as it is inexpensive and relatively non-toxic B vitamin therapy could be beneficial.

## Conclusion

HHcy may be caused by several factors including age, renal failure, genetic polymorphisms, dietary habits and lifestyle conditions. It has been demonstrated that high levels of Hcy might be able to cause neuronal damage through different mechanisms: induction of oxidative stress, impaired synthesis of NO in the endothelium with structural and functional changes in cerebral microcirculum, homocysteinilation, excitation of NMDA receptors, upregulation of the γ-secretase pathway with enhanced production of Aβ and selective activation of the cyclin-dependent kinase 5 with consequent hyperphosphorylation of tau, reduction of Aβ clearance and transport within the brain by down-regulation of IDE, induction of ER stress and epigenetics modifications.

Some authors have also hypothesized a direct toxic action of Hcy on neuronal and glial cells.

Several studies investigated the association between HHcy and the risk of cognitive decline and AD. Most of retrospective and cross-sectional studies showed an evidence of increased risk of cognitive decline in patients with high levels of Hcy when compared to matched controls, but this kind of investigations are not able to determine whether HHcy can act as a causative factor of cognitive decline/dementia or if it is a result of the disease. Most of prospective studies, performed in different populations, also showed that high levels of Hcy were associated with a higher risk of cognitive decline. On the other hand, intervention trials with Hcy lowering treatments such as supplementation of vitamin B12, B6, folic acid and antioxidants showed inconsistent results in terms of cognitive outcomes. It seems that the threshold level of Hcy could play an important role. Indeed, more positive responses have been observed in trials with higher baseline levels of Hcy [45,46]. Moreover, the timing of intervention would play an important role as a greater effect may be obtained with early stage supplementation that can act before neuronal death has already happened. On the other side, vitamin supplementation could be an "add on" therapeutic strategy in dementia patients, as the pharmacological approach to manifest disease should be multifactorial.

With population aging, cognitive decline and dementia have become important issues for health and socio-economic systems. As a disease modifying therapy is still not available for clinicians, further investigations in the field of preventive strategies could be of great importance.

Dosing vitamin B12, folate and Hcy should routinely be performed in middle aged/elderly people and in subjects with early signs of cognitive impairment. Subjects with HHcy should receive dietary and lifestyle indications in order to try to lower Hcy levels. The clinicians will then evaluate the opportunity of prescribing a supplementation therapy, especially for moderate/severe HHcy, after having examined the risk/benefit balance for each patient.

In conclusion, further and better designed clinical trials and good animal models of HHcy would be useful in order to establish a conclusive opinion on the link between HHcy and the decline of cognitive functions.

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