Journal of the Neurological Sciences 404 (2019) 128–136

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Review Article

Homocysteine: A modifiable culprit of cognitive impairment for us to conquer?

Yifan Ji[a,b], Peiyuan Lyu[b,*], Wei Jin[b], Xiangyu Li[a,b], Xiaosha Li[a,b], Yanhong Dong[b]

[a] Graduate School, Hebei Medical University, Shijiazhuang 050017, China
[b] Department of Neurology, Hebei General Hospital, Shijiazhuang 050051, China

ARTICLE INFO

Keywords:
Homocysteine
Cognitive impairment
Dementia
B-vitamin
Observational studies
Randomized controlled trial

ABSTRACT

Background: Cognitive impairment, including mild cognitive impairment and its progressive deterioration to dementia, results in great hazards to the patient and the surrounding society. While some of the risk factors are unmodifiable, such as age, lower educational attainment, and genetic factors, another proposed one-homocysteine, an amino acid produced in the methylation cycle of protein metabolism is modifiable by cheap and easily accessible B-vitamins treatments in medical practice.

Objective and methods: To investigate the relationship between homocysteine and cognitive impairment, elucidate the underlying pathophysiological mechanisms and exploit any potential therapeutic values of homocysteine-lowering treatments in prevention and/or treatment in cognitive decline, we searched on the PUBMED databases surrounding around the physiological homocysteine metabolism, detrimental effects of abnormal homocysteine concentrations on the brain, and review observational and interventional experiments to date estimating the relationship between homocysteine and cognitive impairment with relatively powerful evidence.

Results: Intrinsic and environmental factors help maintain the normal homocysteine concentrations, and pathological homocysteine concentrations exert adverse effects mediated by cellular and vascular pathways. Although many observational studies have suggested a causal link between hyperhomocysteinemia and cognitive impairment, the majority of randomized controlled trials failed to observe marked benefits on cognition by homocysteine-lowering treatments using B-vitamins, partly arising from some design limitations including: not identifying individuals at earlier stages of cognitive impairment who are most likely to benefit, overlooking any latent safety hazards of multiple vitamin supplementation, lack of sensitive and domain-specific cognitive tests, and interference of other underappreciated factors.

Conclusion: More studies are required to better explain the related pathophysiological mechanisms, improve experimental methods, and investigate the preventive or/and therapeutic effects of homocysteine-lowering strategies on cognitive impairment.

1. Introduction

Dementia is an overwhelmingly major contributor to dependence and disability for elderly individuals worldwide [1,2]. It's been reported that, with a dramatic increase in the average life expectancy, the prevalence of dementia will double every 20 years, affecting 115.4 million people in 2050 [3] and causing an exponentially increasing financial burden on society [4]. Fortunately, researchers have proposed several promising options for primary and secondary prevention to diminish these disastrous outcomes [5]. Any treatments to prevent or slow dementia's progression, even to a slight degree, should thus be encouraged to lessen the tremendous harm of this disease. Homocysteine (Hcy), well recognized as a strong independent risk factor of dementia, has attracted great interest in the medical field. In spite of the current dispute over the effect on cognitive improvement of Hcy-lowering treatment using B vitamins (folic acid/B_9, cobalamin/B_{12}, and pyridoxine/B_6), partly arising from imperfectly designed trials and limited knowledge of the related pathophysiological mechanism, we should continue exploring the potential benefits of this strategy. To that end, in this paper we survey the biochemistry of Hcy metabolism, the data from the observational and interventional studies exploring the relationship between Hcy and cognitive impairment, and the potential mechanisms. We then rethink current clinical trials and put forward several suggestions to improve future research methodologies.

* Corresponding author at: 348 Heping West Road, Xinhua District, Shijiazhuang, Hebei Province, China.
E-mail address: peiyuanlu17@163.com (P. Lyu).

https://doi.org/10.1016/j.jns.2019.07.015
Received 6 January 2019; Received in revised form 10 July 2019; Accepted 11 July 2019
Available online 15 July 2019
0022-510X/

2. Hyperhomocysteinemia, a potential risk of cognitive impairment

The term hyperhomocysteinemia (HHcy) first appeared in medical research in McCully's 1969 study, which indicated that the metabolic effects of increased concentrations of Hcy, or its derivative, could induce arterial damage in children with homocystinuria arising from defects in the cbIC gene [6]. Subsequent clinical research showed that HHcy not only contributes to an increased risk of atherosclerotic sequelae, including cardiovascular disease (CVD) [7,8], carotid atherosclerosis [9], clinical stroke [10,11], but also acts as an independent risk factor of cognitive decline and irreversible dementia [12].

Plentiful large population-based studies have evaluated the cross-sectional relationship between total plasma homocysteine concentrations (tHcy) and cognitive function among subjects with various risk classifications: subjects with intact cognition [13]; those free of stroke [14] or dementia [15]; community-dwelling elderly subjects with a wide spectrum of cognitions, ranging from normal cognition to dementia [16,17]; and patients suffering from mild cognitive impairment (MCI), vascular dementia (VaD), histologically confirmed Alzheimer's disease (AD) [18,19].These studies confirmed the negative association between tHcy and cognitive performance, after controlling for possible confounding variables, a finding also supported by meta-analysis [20]. However, descriptive observational studies can be problematic when establishing whether HHcy is a causative factor or simply a result of dementia-related nutritional and vitamin deficiencies. Nevertheless, we can initially postulate a possible causative status for Hcy in relation to dementia based on reasonably convincing prospective cohort studies compared with cross-sectional type studies [12,21–25]. The Framingham Study [12] found that elevated homocysteine levels (eHcy) > 14 μmol/L nearly doubled the risk of AD over an eight-year follow-up period. Haan et al. [22], in a cohort (N = 1779) of the Sacramento Area Latino Study on Aging, reported that HHcy was positively associated with an increased 4.5-year combined incidence of dementia and cognitive impairment without dementia (CIND), (HR: 2.39; 95% CI: 1.11, 5.16), consistent with the conclusion of Regalia et al. [21], in a dementia-free cohort of 816 subjects over an average follow up of four years. But the longitudinal analysis in the Leiden 85-Plus Study [17] produced a negative outcome, possibly because of uncompleted control for covariates like renal function, indicated by serum creatinine level. An outline of published observational research with powerful evidence and relatively large sample sizes (N > 500) can be seen in Table 1.

Moreover, Mendelian randomization (MR) studies, which avoid the inherent limitations of residual confounding bias and reverse causality found in other observational studies, can more cogently estimate the hypothesized causal link between tHcy and dementia by providing the most subjective epidemiological evidence. For instance, Casas et al., in their meta-analysis of 111 studies comprising a total of 15,635 individuals [10], observed similar odds ratio with stroke for TT versus CC homozygotes of the MTHFR C677T polymorphism, compared with those obtained from different homocysteine concentrations conferred by the corresponding variants, suggesting a causal status for Hcy in relation to strokes. Likewise, a meta-analysis by Hu et al., evaluating the effect of eHcy conferred by MTHFR C677T on the risk of AD, was suggestive of a causal link between tHcy and the risk of AD [26]. Kuźma et al. showed limited evidence for this causal link in their systemic review of correlating MR studies [27], while Roostaei et al. [28] and Larsson et al. [29] got negative results. These discrepant outcomes, consistent with Smulders' discussion [30] on the veiled intrinsic limitation of MR experiments, are not sufficient to reject the Hcy hypothesis. Strictly speaking, more elaborately designed trials are needed to prove whether HHcy is one of culprits in cognitive impairment and to further explain the possible underlying mechanism.

Table 1
Observational studies of HHcy and AD/cognitive decline.

Studies	Subjects	Design type	N	Age (years)	Cognitive assessment	Results
Seshadri et al. [12]	Nondemented participants	Prospective cohort (median follow-up 8 y)	1092	68–97	Dementia: DSM-IV,CDR, AD: NINCDS-ADRDA	eHcy was a strong, independent risk factor for the development of dementia and AD
Prins et al. [15]	Nondemented elderly	Cross section	1077	60–90	MMSE,Stroop test, Letter Digit Substitution Task, Verbal fluency, Paper and Pencil Memory Scanning Task, 15-word verbal learning test.	eHcy(> 14umol/L)was associated with cognitive decline, most marked for psychomotor speed, independent of structural brain changes on MRI
Ravaglia et al. [13]	cognitively normal community dwellers	Cross section	650	≥65	MMSE	eHcy (> 15 mol/L)was independently, inversely associated with cognitive impairment
Miller et al. [16]	Community-dwelling elderly	Cross section	1789	≥60	3MSE,Delayed recall, Object naming, Picture association, Verbal conceptual thinking, Verbal attention span, Pattern recognition	Hcy was a modest independent predictor of cognitive function in community-dwelling elderly Latinos
Wright et al. [14]	Stroke-free subjects	Cross section	2871	≥40	MMSE	eHcy was associated with lower mean MMSE scores for those > 65,independent of vascular risk factors
Ravaglia et al. [21]	Dementia-free elderly	Prospective cohort (average follow up of 4 y)	816	> 65	Dementia: DSM-IV, AD: NINCDS-ADRDA	eHcy was an independent predictor of the development of dementia and AD
Mooijaart et al. [17]	Participants in Leiden 85-Plus Study	cross-sectional and longitudinal analysis of prospective study (follow up of 4y)	599	85	MMSE for global cognition; Stroop test, letter digit coding test, word learning test	Hcy was inversely associated with cognitive performance but longitudinally, not with rate of cognitive decline
Haan et al. [22]	Community-residing elderly, from normal cognition to dementia	Prospective cohort (average follow up of 4.5 y)	1779	60–101	Dementia: DSM-III and NINCDS-ADRDA; CIND: failure (≤10th percentile) one additional cognitive test battery but did not meet the criteria for dementia	Hcy was an independent risk factor for both dementia and CIND

Note: DSM-IV = Criteria of the Diagnostic and Statistical Manual of Mental Disorders, fourth edition; CDR: Clinical Dementia Rating scale; NINCDS-ADRDA: National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's disease and Related Disorders Association; MMSE: Mini-Mental State Examination; CIND: cognitive impairment without dementia.

3. Possible mechanisms by which HHcy harms the brain

Clinical studies and animal experiments provide strong evidence regarding the possible underlying mechanism of Hcy-induced cognitive impairment. Firstly, population-based studies utilizing brain magnetic resonance imaging (MRI) showed an association between homocysteine and cortical and hippocampal atrophy [31–34], a marked pathological trait of the AD subtype. Researchers also revealed that tHcy exacerbates the histopathological process of AD: it potentiated brain Aβ formation and deposition, mediated by the activation of γ- secretase pathway, and increased insoluble tau and neurofibrillary tangle via Cdk5 or caspase3 activation in mice models [35,36]. Afterwards, plasma Hcy levels were positively correlated with white matter lesions [37,38] and lacunar infarcts [39,40], two cardinal subtypes of cerebral small vessel disease (CSVD), serving as vascular contributors to cognitive impairment and dementia (VCID). The related physiopathological mechanism may involve its detrimental effects on the endothelial function, which remains incompletely understood but can be conventionally explained by reduced nitric oxide (NO) bioavailability through the inactivation of endothelial NO synthase (eNOS) and increasing oxidative stress [41]. Recent studies also provide new mechanistic insight that endoplasmic reticulum stress and redox homeostasis may be involved in this process [42,43]. Thirdly, the neurotoxicity of Hcy interferes in neural proliferation and reduces neuronal differentiation at embryonic ages [44]. In fully developed nervous systems, Hcy exerts its adverse effects on neurons by oxidative stress injury, DNA damage, and neurotoxicity of upregulating N-Methyl-D-aspartate receptor (NMDAr) expression, leading to perturbing calcium homeostasis, mitochondrial dysfunction, neuronal autophagy overactivation, and apoptosis, accompanied by increased blood-brain barrier permeability [45–47].

4. Biochemistry of Hcy

4.1. Hcy metabolism

Hcy, a nonessential sulphur-containing intermediate of the methionine (Met) pathway involving multiple enzymes and cofactors, is synthesized from Met in the methylation pathways, where the activated S-adenosylmethionine (SAM), formed via phosphorylation of Met, serves as a one-carbon donor for methylation reactions of numerous substrates, including phospholipids, myelin, nucleic acids, choline, and catecholamines. It is subsequently hydrolysed to Hcy and adenosine by S-adenosylhomocysteine (SAH) hydrolase. Once formed, Hcy can be transformed and metabolized by one of two pathways. The first is remethylation to Met, which requires bioactive methylenetetrahydrofolate reductase (MTHFR) and methionine synthetase (MS), dependent on folate as a substrate and vitamin B12 as a cofactor, or by interaction with betaine catalysed by betaine homocysteine methyltransferase (BHMT). The second pathway involves transsulfuration to cysteine for catabolism, which requires cystathionine-beta-synthase (CβS) and pyridoxal −5′-phosphate, the vitamin B6 coenzyme (Fig. 1). However, in the brain, the transsulfuration pathway is not active and the betaine remethylation pathway is absent, since the only human tissues in which BHMT is expressed are the liver and kidneys [48]. So, we can summarize that Hcy metabolism is mainly determined by the nutrient status of folate and cobalamin.

4.2. Determinants of Hcy concentrations

Hcy exists in plasma as three presenting forms: protein (albumin)-bound, free circulating disulfide, and sulfhydryl; all three forms are reported as tHcy in laboratory tests. In the fields of nutrition, Patrick Holford regarded tHcy < 6 μmol/L to be the ideal status in his work-*The New Optimum Nutrition Bible*. According to the guidelines for prevention of stroke set by the American Heart Association/American Stroke Association Council on Stroke, a cutoff of 10 μmol/L is

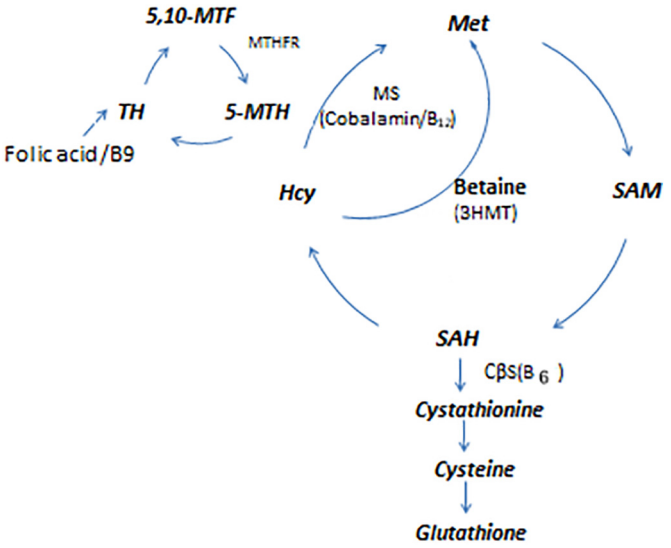

Fig. 1. Note: TH: tetrahydrofolate; 5, 10 -MTH: $N^{5,10}$- methylenetetrahydrofolate; 5-MTH: N^{5}-methyltetrahydrofolate; MTHFR: methyltetrahydrofolate reductase; MS: methionine synthetase; Met: methionine; SAM: S-adenosylmethionine; SAH: S-adenosylhomocysteine; CβS: cystathionine beta synthase; BHMT: betaine homocysteine methyltransferase.

recommended for use in identifying individuals who would benefit from an intervention with B- vitamins [49]. Therefore, we define eHcy as tHcy > 10 μmol/L. To be more precise, mild-to-moderate eHcy falls within a concentration range of 10—100 μmol/L, while a result of > 100 μmol/L represents severe eHcy, which typically occurs as a result of congenital errors in Hcy metabolism leading to homocystinuria, but also occurs in a few patients who have a serious lack of vitamin B12/folate and in some cases of severe renal disease [50].

Homocysteine concentrations, positively correlated with increasing age and male sex [51], are also considered to be etiologically involved with multiple factors: genetic or nutritional-related disturbance in the re-methylation or transsulfuration of Hcy metabolism, lifestyle, and other conditions [52,53]. This section provides a more detailed description of these factors.

4.2.1. Genetic aetiology

Functional polymorphic variants of the enzymatic gene of Hcy metabolism, including MTHFR, CβS, MTR(5-MethyltetrahydrofolateHomocysteineMethyltransferase)/MS, and MTRR (5-Methyltetrahydrofolate-Homocysteine Methyltransferase Reductase), are critical in maintaining nontoxic Hcy levels. The most intensively explored variant occurs in C677T in the gene encoding the MTHFR, which correlates with reduced enzymatic activity and renders the enzyme thermolabile, eventually inducing decreased production of N5-MTF, the methyl donor for Hcy's conversion to Met. Variants of MTR/MTRR are associated with deficits of methylcobalamin, the bioactive form of B12. And the homozygous CβS mutation, a key enzyme in the catabolic pathway of Hcy commonly manifesting as I276T, causes severely HHcy with an inborn error of Hcy metabolism, inducing severe cardiovascular outcomes.

4.2.2. Nutritional factors

The maintenance of normal Hcy concentrations depends on its methylation to Met and transsulfuration to cysteine, which requires adequate store of three dietary vitamins: folate, B_{12}/cobalamin, and B_6/pyridoxal phosphate, as shown in Fig. 1. Folic acid is found primarily in dark green vegetables, grains, and fruits. Its concentration can be assessed by three distinct laboratory markers. Serum/plasma folate, the first-line assessment of folate status, is used by the majority of laboratories. Red cell folate, not influenced by transient changes in dietary

intake, is a strong indicator of the preceding four months' folate status. Finally, specific folate species, 5-MTHF and Hcy, reflect cellular folate use [54]. Poor dietary intake, conditions conferring gastrointestinal malabsorption (which are especially pronounced in the elderly), increased folate turnover, and consumption of drugs (including anticonvulsants, methotrexate, oral contraceptives, pyrimethamine, trimethoprim, triamterene, and trimetrexate), contribute to the development of folate deficiency, the most common cause of eHcy. As estimated in a large population-based study of 3511 British people aged 65 years or more, the prevalence of folate deficiency (serum folate < 7 nmol/L and homocysteine > 20 mmol/L) was found to affect 5–20% of older adults [55]. The current US recommended dietary allowance (RDA) for average adults, set by the Food and Nutrition Board of the US institute of Medicine (IOM) [56], is 400 μg/day and 400-800 μg/day for women of childbearing age, according to the reaffirmed recommendation of the US Preventive Services Task Force (USPSTF) [57]. A mandatory fortification policy of folate-enriched grain products was carried out in many countries including the USA [56], where it was fully implemented by January 1998, and has practically lowered the prevalence of folate deficiency [58].

Vitamin B_{12} (B_{12}, cobalamin), an essential nutrient for humans, derives from animal source foods. Total plasma/serum B_{12} concentration alone may not reliably reflect B_{12} status, so three other available bioactive markers— holotranscobalamin (holo-TC), methymalonic acid (MMA), and tHcy— are also utilized for optimal B_{12} assessment. Strict vegetarianism, gastrointestinal malabsorption or resection, medications, and genetic anomalies are relatively common causes that place individuals at increased risk of cobalamin deficiency [59]. Changes in the above four biomarkers can reflect the severity of B_{12} deficiency, which is initially characterized by reduced plasma holo-TC with depletion of the plasma and cell stores, followed by additionally increased tHcy and MMA, and ultimately by recognizably clinical signs such as megaloblastic anemia and progressive neurological disease. For jurisdiction with initiation of folic-acid fortification by authority, Vitamin B_{12} deficiency is substantially prevalent, especially in the elderly [60,61], while folate deficiency is practically non-existent [61]. The IOM recommended a RDA level of 2.4 μg/day for the elderly (≥51y), and no Tolerable Upper Intake Level (UL) was set for no reported adverse effects of a high supplement of B_{12} [56].

Vitamin B_6 (B_6, pyridoxine) plays an obligatory role as a coenzyme in the bioactive form of pyridoxal 5′-phosphate (PLP) and occurs in a wide variety of foods. With considerable bioavailability of up to 75% in a mixed diet [56], a serious lack of B_6 is rarely observed, but moderate B_6 deficiency (PLP < 20 nmol/L) is relatively common in the general population, affecting approximately 24% of Americans [62]. Several foods (e.g., mushrooms, and some leguminous plants) contain natural B_6 antagonists [63]; in flaxseed, for example, 1-amino D-proline (1ADP), released from linatine, may exacerbate the prevalence of B_6 deficiency [64]. Other conditions, such as related congenital diseases, coeliac disease, hematodialysis, various drugs (e.g., isoniazid, penicillamine, and cycloserine) can also predispose individuals to B_6 deficiency [65]. The RDA for adults aged > 50 years is 1.7 mg/day for men and 1.5 mg/day for women, while a UL of 100 mg/day was suggested for possible toxic effects caused by high concentrations of vitamin B_6 [56].

Riboflavin (Vitamin B_2) acts as a cofactor for MTHFR and independently determines plasma tHcy, an effect modified by the C677T polymorphism [66]. This suggests that individuals with the MTHFR 677 TT genotype, especially those who also have a low folate status, rely more on sufficient riboflavin to offset the hypoactive enzymatic function, thus providing a breakthrough point at which we can intervene. Riboflavin mainly occurs in foods from animal source, such as milk, meat, and eggs, usually in the form of flavin mononucleotide (FMN) or flavin-adenine dinucleotide (FAD); it may also be produced in situ in a free state by intestinal microorganisms [67]. The RDA for adults of all ages is 1.3 mg/day for men and 1.1 mg/day for women; there is no conclusive evidence of any deleterious effects to set a UL for riboflavin [56].

4.2.3. Other factors

Lifestyle factors, such as coffee consumption, smoking, and alcohol consumption are positively associated with tHcy concentrations, while physical activity is probably not or weakly inversely associated with tHcy concentration. It should be noted that, for specific groups within the population, certain drugs (e.g., hormones, antiepileptic drugs, methotrexate, nitrous oxide, L –Dopa, lipid-lowering drugs, and vitamin B_6 antagonists) and diseases (kidney dysfunction, proliferating disease, rheumatoid arthritis, endocrine disorders, intestinal disease, and hypothyroidism), which can influence Hcy metabolism, may also act as determinants for Hcy concentrations [68].

5. How far have clinical trials for Hcy-lowering treatment come?

As shown above, a preponderance of epidemiological studies has concluded that eHcy is inversely correlated with cognitive performance, marking it as a potentially modifiable risk factor, worthy of extensive attention to uncover possible solutions to postpone or reverse age-related cognitive decline, or even reduce the risk of dementia, including AD. However, we can only establish the causative association between HHcy and dementia through sufficient human intervention studies [69], particularly randomized controlled trials (RCTs) which are recognized as the golden standard for the efficacy of any therapeutic agent. A certain number of interventional trials have investigated the effect of Hcy-lowering therapy with folate and/or B_{12} and B_6 supplementation, which interact to maintain a normal homocysteine concentration, on cognitive changes in participants with intact cognition, MCI, and clinically diagnosed or histologically-confirmed dementia, mainly AD. Taking into consideration the sample size, follow-up duration, availability of detailed baseline information about subjects including vitamin B levels, and accuracy of cognitive assessment, we identified several limited randomized, double-blind, and controlled trials [70–79] in PUBMED, using the keywords “vitamin cognitive homocysteine” and “homocysteine vitamin dementia” (Table 2). These studies were published from 2000 to 2018 and have relatively powerful statistical evidence. But disappointingly, results of the overwhelming majority of randomized trials have failed to demonstrate any obvious benefits of tHcy-lowering therapy on cognitive changes. Notable exceptions include the Folic Acid and Carotid Intimamedia Thickness (FACIT) trial, a three-year folic acid supplementation that found a significant benefit in memory, information processing speed, and sensorimotor speed [73]. Similarly, a research team from China observed improvement in cognition in their RCT of subjects with MCI [80–82]. The large degree of heterogeneity existing within these trials can't be overlooked and should prompt researchers to further consider the shortage of experimental designs.

6. Reflection on existing interventional studies

The majority of interventional trials exploring the clinically therapeutic potential of Hcy-lowering treatment on cognitive/AD outcomes showed negative results, which may indicate some intrinsic methodological problems [83,84]. Nevertheless, it's not advisable to deny the cause-effect relationship between HHcy and dementia without seeking to improve these methods. Here we discuss several possibilities for the discrepancy in the existing data and provide some suggestions for future research.

6.1. Recruitment of specific target populations

To minimize attrition, we ought to identity subjects more likely to get benefit from intervening measures, to avoid a higher sample size. The following counts need to be carefully reconsidered. In view of the

Table 2
RCTs investigating the effect of B vitamins supplementation on cognitive function.

Trials	Subjects	Follow-up period	Intervention(daily)	Main outcome measures	Cognitive outcomes
Clarke et al. 2003 [70]	149 people with dementia or MCI.	12wk	Folic acid 2 mg plus B_{12} 1 mg or placebo	MMSE, ADAS-Cog, Bristol Activities of Daily Living Scale.	Vitamins group lowered plasma homocysteine concentration by 30%, but no effect on cognitive function was detected.
David et al. 2005 [71]	185 patients aged ≥65y with ischemic vascular disease, excluded if MMSE < 19	12wk	Folic acid 2.5 mg plus B_{12} 500u g or placebo; B_6 25 mg or placebo; Riboflavin 25 mg or placebo.	TICSm, Letter Digit Coding Test,	Folic acid plus B_{12} decrease homocysteine levels by≈33% while riboflavin or B_6 showed no significant effect; no difference between groups in cognitive scores over a 1-y period.
McMahon et al. 2006 [72]	276healthy participants≥65y. (Hcy ≥ 13umol/L)	2years	1000μg folic acid; 500μg B_{12}, 10 mg B_6 or placebo.	1.MMSE 2.Wechsler Paragraph Recall test 3.Category Word Fluency test 4.Rey Auditory Verbal Learning Test, trials I–V 5.Rey Auditory Verbal Learning Test, trial VII, 6.Raven's Progressive Matrices 7.Controlled Oral Word Association Test 8 Part B of the Reitan Trail Making Test	Plasma homocysteine decreased by 26% In active group; no significant difference in Cognitive scores between groups apart from Reitan Trail Making Test B where vitamin group took longer
Jane et al. 2007 [73]	818 people aged 50-70y with tHcy≥13umol/L and normal serum B-12	3years	800 μg folic acid or placebo	Word learning test, Concept shifting test, Stroop colour-word test, Verbal fluency test, Letter digit substitution test.	Plasma homocysteine levels decreased by 26% in folate group,3-year change in memory, information processing speed and sensorimotor speed were better in the folate group.
Aisen et al. 2008 [74]	409 mild to moderate AD patients with normal vitamin and homocysteine levels	18 months	5 mg folic acid, 1 mg B_{12}, 25 mg B_6.or placebo.	ADAS-cog; MMSE,CDR-SOB, ADCS-ADL, QOL-AD, NPI	Active group reduced plasma homocysteine by 26.3% but had no improvement on any of the cognitive tests, but involving higher rate of depression
Ford et al. 2010 [75]	299 community-representative hypertensive men aged≥75 y	2years	2 mg folic acid, 400μg B_{12}, 25 mg B_6 or placebo.	ADAS-cog, CDT, CVLT 1,CVLT 2, MMSE,DCT	No difference in the ADAS-cog change between two group, or before-after comparison in vitamins group.
Kwok et al. 2011 [76]	140 patients with mild to moderate AD or vascular dementia	2years	5 mg folic acid and 1 mg methylcobalamin, or placebo	MDRS, MMSE,CNPI, CSDD.	Supplementation significantly reduced plasma homocysteine levels, but showed no significant difference in any of cognitive scores, but a smaller d decline of MDRS(construction domain) compared to placebo group
De Jager et al. 2012 [77]	266participants with MCI, aged ≥70 y,	2years	0.8 mg folic acid, 0.5 mg B_{12} and 20 mg B_6.	MMSE, Category fluency, CLOX, CDR, IQCODE, HVLT-R.	The mean plasma total homocysteine of vitamin group was 30% lower relative to placebo group, active group showed significant benefit in global cognition, episodic memory and semantic memory
Chen et al. 2016 [78]	121 newly diagnosed AD patients with treatment of donepezil aged > 60 y	6 months.	1.25 mg folic acid or placebo	MMSE	Interventional group showed slightly increased MMSE scores compared to that in the control group.
Kwok. et al., 2017 [79]	271 diabetic non-demented outpatients aged ≥70 y with borderline vitamin B12.	27 months	methylcobalamin 1000 mg or placebo	CDR, MMSE, NTB total and 185 domain z-scores	Active group showed significantly reduction of homocysteine level, but no difference in changes in CDR or NTB z-scores.

Note: ADAS-cog: Alzheimer's Disease Assessment Scale; MMSE: Mini-Mental State Examination; CDR-SOB: Clinical Dementia Rating sum of boxes; ADCS-ADL: Alzheimer's Disease Cooperative Study Activities of Daily Living; NPI: Neuropsychiatric Inventory; QOL-AD: Quality of Life-AD; CDT: Clock Drawing Test; CVLT 1: California Verbal Learning Test List immediate free recall trials 1–5 total; CVLT 2: California Verbal Learning Test List A long-delay free recall; DCT: Digit Cancellation Test; TICS: Telephone Interview for Cognitive status; CDR: Clinical Dementia Rating scale; GDS: Geriatric depression scale; NTB: Neuropsychological test battery; HVLT-R: Hopkins Verbal Learning Test-revised with delayed recall; IQCODE: Informant Questionnaire on Cognitive Decline in the Elderly; CLOX: executive Clock drawing Task; MDRS: Chinese Mattis dementia rating scale; CNPI: Chinese version of the Neuropsychiatric Inventory; CSDD: Cornell scale for depression in dementia.

long progressive course of dementia, ranging from age-related cognitive impairment to MCI and finally to irreversible dementia, providing B vitamins to populations of intact cognition with no space to improve, or those at the terminal phase of diagnosed dementia, is likely to get a few benefits. Furthermore, observational studies demonstrate that only mildly elevated Hcy levels (> 14umol/L) rather than normal Hcy (< 14umol/L) denote a risk factor for AD. The RCT conducted by McMahon et al. [72], involving healthy elderly (≥65 years) participants with HHcy, defined as low as 13umol/L, along with an already quite normal cognitive performance and a mean MMSE score of 29.2, had negative results, as did Aisen and colleagues' trial [74] of high dose folate/B_6/B_{12} supplementation in individuals with mild to moderate AD.

In the USA, the folic acid fortification policy (fully implemented by January 1998) via folate-enriched grain products, designed for the prevention of neural tube defects (NTD), effectively lowered the prevalence of folate deficiency. In the Framingham Offspring cohort study, folate fortification was associated with doubling of plasma folate concentrations and reduced tHcy prevalence [85]. Numerous clinically interventional trials with B vitamins conducted after this fortification, in countries like the US and Australia, have shown limited potential in lowering the already reduced Hcy levels, thus attenuating the relation between Hcy and cognitive function. However, to date few heavily invested RCTs have explored the clinical value of lowering Hcy with B vitamins to improve cognitive improvement in developing countries like China without folic acid fortification. By using a large sample size with long duration (≥3 years), such studies could target people most likely to benefit from this strategy, as suggested by the previous positive results from the RCTs of a Chinese team [80–82]. Recognizing that folate reduces the risk of dementia in fortification-free jurisdictions has significant implications for preventing dementia, providing huge research opportunities. Therefore, researchers must target subjects with actual HHcy (> 14umol/L) at appropriate cognition-impaired stages in the right venue, before instituting their trials.

6.2. Latent safety issues in the current B-vitamin treatments

Clinical RCTs should explore the efficacy of any agents on the premise of ensuring security profiles for both formulation and dosage. When it comes to formulation, compared with folic acid and cyanocobalamin, the final bioactive forms of B_9 and B_{12}, (see Fig. 1), N5-MTHF and methylcobalamin, respectively, with lower stability but higher pharmaceutical costs, seem less useful as optimal intervention agents against HHcy, and may have uncertain effects. A special situation occurs in the case of individuals with renal failure, cyanocobalamin, as a cyanide-releasing substance is removed from human bodies in the form of thiocyanate, a process that consumes hydrogen sulphide, a recognized endothelium-derived relaxing factor analogous to nitric oxide [86], and such patients experienced worse outcomes in Koyama et al.'s study [87]. Therefore, the harm of cyanocobalamin for participants with significant renal impairment may obscure the beneficial effect of B vitamins on the other participants with healthy renal function, therefore the former populations should be excluded from studies, as proposed by Spence and Stampfer [88].

As for dosage, unlike B_{12}, evidence from present data is insufficient to rule out the hazard associated with excessive exposure to folic acid, especially coupled with B_{12} deficiency, and a UL of 1000 μg/day was set by the IOM. A combination of high folate and low vitamin B_{12} status during pregnancy may contribute to high gestational diabetes mellitus (GDM) risk [89] and small-for-gestational-age infants [90]. Among elderly people, it also predicts faster cognitive decline compared with those with normal folate levels coupled with poor B_{12} status [91], possibly due to the harm unmetabolized folic acid (UMFA)/5-MTH inflicts on the nervous system when impaired MS activity due to B_{12} deficiency impedes remethylation of Hcy to Met and retrieval of non-methylated folates [92,93]. A Canadian trial that included patients with diabetic nephropathy found that high doses of combined B-vitamins (folic acid, B_6, and B_{12}) resulted in worsening renal dysfunction and a higher rate of vascular events when compared with a placebo [94]. One possible explanation is that this strategy has no beneficial effect and may even increase the levels of asymmetric dimethylarginine (ADMA), a competitive inhibitor of eNOS to that interferes with the synthesis of NO, since the release of ADMA by endothelial cells can be blocked by SAM rather than B vitamins per se [95]. To ensure sufficient production of SAM, it is necessary for researchers to follow the cardinal principle of synchronized folate-cobalamin supplementation, referencing the corresponding IOM-recommended levels of vitamins mentioned above. Furthermore, more details about the mechanism for deleterious effects of high folate intake in conjunction with low B_{12} status warrant further explorations.

6.3. Appropriate cognitive assessment

Hcy-reducing treatment, the average duration of which is relatively short compared to the years or even decades of accumulated HHcy-induced detrimental effects on cognition, may produce subtle cognitive benefits. As a result, detecting it requires both a long follow-up period and very sensitive and domain-specific cognitive tests, instead of relying on global cognitive measures such as MMSE.

In particular, we must choose scientific and rational methods for evaluating cognition in line with the hypothesis: Current experimental data does not seem to support the positive effects of B-vitamin supplementation on nonpathological cognitive ageing – the very slow cognitive decline observed during the normal aging process [96]. A reasonable hypothesis worthy of exploration should center around the therapeutic significance of B-vitamin supplementation for individuals experiencing measurable cognitive decline and its value for prevention prior to the onset of irreversible stages of dementia [69]. This means that, in an ideal experimental setting, researchers must prove there is slower cognitive decline in the intervention group compared with that of the non-intervention group, with a target population of individuals with pre-dementia or MCI with eHcy, to test the above hypothesis. The “cognitive changes” implicated in the hypothesis itself requires an integrated system for the assessment of cognition, including detailed information at both baseline and from regular follow-ups. It makes sense, therefore, why meta-analyses with prospective hypotheses, such as those reported by Clarke et al. [97] and Setién et al. [20], and which are usually lacking in strict inclusion/exclusion rules, and sensitive or appropriate cognitive tests, get disappointing results for the preventive and/or therapeutic values of this Hcy-lowering treatment. Moreover, comprehensive cognitive assessment through a combination of evaluation of clinical symptoms, neuropsychiatric tests, and neuroimaging, although difficult to obtain for researchers, can provide more objective details of cognitive changes. Studies using MRI scans in VITACOG [98,99], which showed that B-vitamin supplementation could slow the atrophy of specific brain regions associated with cognitive decline and the AD process, have set good examples for us to follow.

6.4. Influence of other associated factors

Cognitive decline and the development of dementia are complex processes induced by varied environmental elements and endogenous status, so it's not scientific or rational to expect delays in disease progression by lowering Hcy alone. Apart from comprehensive control of putative risk factors for dementia and Alzheimer's disease, including age, sex, APOE (apolipoprotein) genotype, plasma B vitamins and creatinine levels, depression, and vascular risk factors, to form practical clinical therapy, other associated factors, which may modulate the treatment's effect on cognition, need to be thoroughly considered. For instance, exploratory analysis [100] revealed the interplay between Hcy and ω-3 PUFAs (omega-3 polyunsaturated fatty acids) on the cortical β- amyloid, which beyond a certain threshold level is considered to

produce dementia-causing effects, in older adults at risk of dementia. This is in line with previous reports that ω-3 PUFAs may modify the effects of an Hcy-lowering strategy with B vitamins on brain atrophy [101] and cognitive decline among individuals with MCI [102] and AD [103], meriting further research on whether similar effects can be found in the conversion from MCI to dementia.

Since Hcy was originally found to be a risk factor for CVD, more research focused there than on the AD field. Therefore, some lessons drawn from CVD studies may help shed light on the association between Hcy and AD. Similar to what we have observed in AD, only observational trials support Hcy as a risk factor for CVD, while RCTs overall showed an equivocal effect of Hcy-reducing treatments on cardiovascular outcomes. Are there any common factors that could explain the failure of Hcy-lowering treatments for both CVD and AD? Interestingly, a post hoc subanalysis of VITATOPS [104], restricted to 1463 patients not receiving antiplatelet therapy, showed reduced risk of stroke (HR:0.6; 95% CI:0.50–0.95) [105], revealing a significant interaction between antiplatelet medications and assignment to B vitamins. This effect was later corroborated by another retrospective analysis of VISP [106], discovering the differential effect of B-vitamin therapy by antiplatelet use on risk of recurrent stroke. Also encouragingly, Park et al. [107], in their meta-analysis evaluating 4643 high vascular risk subjects not taking antiplatelets, reported a significant reduction (29%) in overall stroke risk by lowering Hcy with B vitamins. The interaction between B vitamins and antiplatelet medication may be related to their endothelial effect [106], which also contributes to the detrimental influence of Hcy on cognitive impairment, as described above. Additional studies are needed to elucidate whether this interaction effect can also be observed in AD patients.

7. Conclusion and perspective

Dementia is a chronic neurodegenerative disease resulting from a complex interaction of multiple factors. Unremitting efforts have been undertaken to explore any possible modifiable factors to minimize its devastating damage to patients and their families. Consistent evidence suggests Hcy is an independent risk factor of cognitive impairment and Alzheimer's disease, but two important questions remain. First, is Hcy the potential culprit or just a biomarker? Second, is it modifiable to prevent MCI or slow the cognitive decline rate in the terminally irreversible phase of dementia? Given the heavy financial and mental burden caused by dementia and the easily available, money-saving superiority of the Hcy-lowering therapy using B vitamins, more cautious consideration and better-designed randomized controlled trials are needed before jumping to the conclusion that there is no clinically therapeutic value in this strategy. Based on the discussion provided here, only with more research concentrating on the pathological mechanism, utilizing elaborately designed interventional studies, can we establish Hcy as a modifiable culprit of dementia, and subsequently bring hope to dementia patients and those who care for them.

Funding source

This work was supported by the National Natural Science Foundation of China (No.81701082) and the Natural Science Foundation of Hebei Province of China (No.H2017307012).

Declaration of Competing Interest

The authors report no conflicts of interest regarding the publication of this report.

References

[1] R.M. Sousa, C.P. Ferri, D. Acosta, et al., Contribution of chronic diseases to disability in elderly people in countries with low and middle incomes: a 10/66 Dementia Research Group population-based survey, Lancet 374 (9704) (2009) 1821–1830, https://doi.org/10.1016/S0140-6736(09)61829-8.
[2] C.L. Satizabal, A.S. Beiser, V. Chouraki, et al., Incidence of dementia over three decades in the Framingham Heart Study, N. Engl. J. Med. 374 (6) (2016) 523–532, https://doi.org/10.1056/NEJMoa1504327.
[3] M. Prince, R. Bryce, E. Albanese, et al., The global prevalence of dementia: a systematic review and metaanalysis, Alzheimers Dement. 9 (1) (2013) 63–75, https://doi.org/10.1016/j.jalz.2012.11.007 e2.
[4] M.D. Hurd, P. Martorell, A. Delavande, et al., Monetary costs of dementia in the United States, N. Engl. J. Med. 368 (14) (2013) 1326–1334, https://doi.org/10.1056/NEJMsa1204629.
[5] D.E. Barnes, K. Yaffe, The projected effect of risk factor reduction on Alzheimer's disease prevalence, Lancet Neurol. 10 (9) (2011) 819–828, https://doi.org/10.1016/S1474-4422(11)70072-2.
[6] K.S. McCully, Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis, Am. J. Pathol. 56 (1) (1969) 111–128.
[7] J.C. Chambers, O.A. Obeid, H. Refsum, et al., Plasma homocysteine concentrations and risk of coronary heart disease in UK Indian Asian and European men, Lancet 355 (9203) (2000) 523–527, https://doi.org/10.1016/S0140-6736(99)93019-2.
[8] D. Farhadi, Plasma homocysteine levels and mortality in patients with coronary artery disease, N. Engl. J. Med. 337 (1997) 1632–1633 22. 1632; author reply.
[9] W. Wu, Y. Guan, K. Xu, et al., Plasma homocysteine levels predict the risk of acute cerebral infarction in patients with carotid artery lesions, Mol. Neurobiol. 53 (4) (2016) 2510–2517, https://doi.org/10.1007/s12035-015-9226-y.
[10] J.P. Casas, L.E. Bautista, L. Smeeth, et al., Homocysteine and stroke: evidence on a causal link from mendelian randomisation, Lancet 365 (9455) (2005) 224–232, https://doi.org/10.1016/S0140-6736(05)17742-3.
[11] C. Homocysteine Studies, Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis, JAMA 288 (16) (2002) 2015–2022, https://doi.org/10.1001/jama.288.16.2015.
[12] S. Seshadri, A. Beiser, J. Selhub, et al., Plasma homocysteine as a risk factor for dementia and Alzheimer's disease, N. Engl. J. Med. 346 (7) (2002) 476–483, https://doi.org/10.1056/NEJMoa011613.
[13] G. Ravaglia, P. Forti, F. Maioli, et al., Homocysteine and cognitive function in healthy elderly community dwellers in Italy, Am. J. Clin. Nutr. 77 (3) (2003) 668–673, https://doi.org/10.1093/ajcn/77.3.668.
[14] C.B. Wright, H.S. Lee, M.C. Paik, et al., Total homocysteine and cognition in a triethnic cohort: the Northern Manhattan Study, Neurology 63 (2) (2004) 254–260, https://doi.org/10.1212/01.wnl.0000129986.19019.5d.
[15] N.D. Prins, T. Den Heijer, A. Hofman, et al., Homocysteine and cognitive function in the elderly: the Rotterdam Scan Study, Neurology 59 (9) (2002) 1375–1380, https://doi.org/10.1212/01.wnl.0000032494.05619.93.
[16] J.W. Miller, R. Green, M.I. Ramos, et al., Homocysteine and cognitive function in the Sacramento Area Latino Study on Aging, Am. J. Clin. Nutr. 78 (3) (2003) 441–447, https://doi.org/10.1093/ajcn/78.3.441.
[17] S.P. Mooijaart, J. Gussekloo, M. Frolich, et al., Homocysteine, vitamin B-12, and folic acid and the risk of cognitive decline in old age: the Leiden 85-Plus study, Am. J. Clin. Nutr. 82 (4) (2005) 866–871, https://doi.org/10.1093/ajcn/82.4.866.
[18] R. Clarke, A.D. Smith, K.A. Jobst, et al., Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease, Arch. Neurol. 55 (11) (1998) 1449–1455.
[19] P. Quadri, C. Fragiacomo, R. Pezzati, et al., Homocysteine, folate, and vitamin B-12 in mild cognitive impairment, Alzheimer disease, and vascular dementia, Am. J. Clin. Nutr. 80 (1) (2004) 114–122, https://doi.org/10.1093/ajcn/80.1.114.
[20] E. Setien-Suero, M. Suarez-Pinilla, P. Suarez-Pinilla, et al., Homocysteine and cognition: a systematic review of 111 studies, Neurosci. Biobehav. Rev. 69 (2016) 280–298, https://doi.org/10.1016/j.neubiorev.2016.08.014.
[21] G. Ravaglia, P. Forti, F. Maioli, et al., Homocysteine and folate as risk factors for dementia and Alzheimer disease, Am. J. Clin. Nutr. 82 (3) (2005) 636–643, https://doi.org/10.1093/ajcn.82.3.636.
[22] M.N. Haan, J.W. Miller, A.E. Aiello, et al., Homocysteine, B vitamins, and the incidence of dementia and cognitive impairment: results from the Sacramento Area Latino Study on Aging, Am. J. Clin. Nutr. 85 (2) (2007) 511–517, https://doi.org/10.1093/ajcn/85.2.511.
[23] B. Hooshmand, A. Solomon, I. Kareholt, et al., Associations between serum homocysteine, holotranscobalamin, folate and cognition in the elderly: a longitudinal study, J. Intern. Med. 271 (2) (2012) 204–212, https://doi.org/10.1111/j.1365-2796.2011.02484.x.
[24] K.L. Tucker, N. Qiao, T. Scott, et al., High homocysteine and low B vitamins predict cognitive decline in aging men: the Veterans Affairs Normative Aging Study, Am. J. Clin. Nutr. 82 (3) (2005) 627–635, https://doi.org/10.1093/ajcn.82.3.627.
[25] B. Hooshmand, A. Solomon, I. Kareholt, et al., Homocysteine and holotranscobalamin and the risk of Alzheimer disease: a longitudinal study, Neurology 75 (16) (2010) 1408–1414, https://doi.org/10.1212/WNL.0b013e3181f88162.
[26] Q. Hu, W. Teng, J. Li, et al., Homocysteine and Alzheimer's disease: evidence for a causal link from mendelian randomization, J. Alzheimers Dis. 52 (2) (2016) 747–756, https://doi.org/10.3233/JAD-150977.
[27] E. Kuzma, E. Hannon, A. Zhou, et al., Which risk factors causally influence dementia? A systematic review of mendelian randomization studies, J. Alzheimers Dis. 64 (1) (2018) 181–193, https://doi.org/10.3233/JAD-180013.
[28] T. Roostaei, D. Felsky, A. Nazeri, et al., Genetic influence of plasma homocysteine on Alzheimer's disease, Neurobiol. Aging 62 (2018), https://doi.org/10.1016/j.neurobiolaging.2017.09.033 (243 e7-243 e14).
[29] S.C. Larsson, M. Traylor, R. Malik, et al., Modifiable pathways in Alzheimer's disease: mendelian randomisation analysis, BMJ 359 (2017) j5375, , https://doi.

org/10.1136/bmj.j5375.
[30] Y.M. Smulders, H.J. Blom, The homocysteine controversy, J. Inherit. Metab. Dis. 34 (1) (2011) 93–99, https://doi.org/10.1007/s10545-010-9151-1.
[31] T. Shimomura, F. Anan, T. Masaki, et al., Homocysteine levels are associated with hippocampus volume in type 2 diabetic patients, Eur. J. Clin. Investig. 41 (7) (2011) 751–758, https://doi.org/10.1111/j.1365-2362.2010.02464.x.
[32] K. Maesato, T. Ohtake, Y. Mochida, et al., Correlation of hippocampal atrophy with hyperhomocysteinemia in hemodialysis patients: an exploratory pilot study, PLoS One 12 (4) (2017) e0175102, , https://doi.org/10.1371/journal.pone.0175102.
[33] M.J. Firbank, S.K. Narayan, B.K. Saxby, et al., Homocysteine is associated with hippocampal and white matter atrophy in older subjects with mild hypertension, Int. Psychogeriatr. 22 (5) (2010) 804–811, https://doi.org/10.1017/S1041610210000499.
[34] T. den Heijer, S.E. Vermeer, R. Clarke, et al., Homocysteine and brain atrophy on MRI of non-demented elderly, Brain 126 (Pt 1) (2003) 170–175, https://doi.org/10.1093/brain/awg006.
[35] N. Shirafuji, T. Hamano, S.H. Yen, et al., Homocysteine increases Tau Phosphorylation, truncation and oligomerization, Int. J. Mol. Sci. 19 (3) (2018), https://doi.org/10.3390/ijms19030891.
[36] J.G. Li, J. Chu, C. Barrero, et al., Homocysteine exacerbates beta-amyloid pathology, tau pathology, and cognitive deficit in a mouse model of Alzheimer disease with plaques and tangles, Ann. Neurol. 75 (6) (2014) 851–863, https://doi.org/10.1002/ana.24145.
[37] J.L. Hsu, W.H. Chen, C.H. Bai, et al., Microstructural white matter tissue characteristics are modulated by homocysteine: a diffusion tensor imaging study, PLoS One 10 (2) (2015) e0116330, , https://doi.org/10.1371/journal.pone.0116330.
[38] C.C. Lee, S.W. Hsu, C.W. Huang, et al., Effects of homocysteine on white matter diffusion parameters in Alzheimer's disease, BMC Neurol. 17 (1) (2017) 192, https://doi.org/10.1186/s12883-017-0970-7.
[39] L.C. Rutten-Jacobs, M. Traylor, P. Adib-Samii, et al., Association of MTHFR C677T genotype with ischemic stroke is confined to cerebral small vessel disease subtype, Stroke 47 (3) (2016) 646–651, https://doi.org/10.1161/STROKEAHA.115.011545.
[40] R.P. Kloppenborg, M.I. Geerlings, F.L. Visseren, et al., Homocysteine and progression of generalized small-vessel disease: the SMART-MR Study, Neurology 82 (9) (2014) 777–783, https://doi.org/10.1212/WNL.0000000000000168.
[41] Z. Cheng, X. Jiang, M. Pansuria, et al., Hyperhomocysteinemia and hyperglycemia induce and potentiate endothelial dysfunction via mu-calpain activation, Diabetes 64 (3) (2015) 947–959, https://doi.org/10.2337/db14-0784.
[42] X. Wu, L. Zhang, Y. Miao, et al., Homocysteine causes vascular endothelial dysfunction by disrupting endoplasmic reticulum redox homeostasis, Redox Biol. 20 (2018) 46–59, https://doi.org/10.1016/j.redox.2018.09.021.
[43] X.C. Wang, W.T. Sun, C.M. Yu, et al., ER stress mediates homocysteine-induced endothelial dysfunction: modulation of IKCa and SKCa channels, Atherosclerosis 242 (1) (2015) 191–198, https://doi.org/10.1016/j.atherosclerosis.2015.07.021.
[44] K. Kobus-Bianchini, G.F. Bourckhardt, D. Ammar, et al., Homocysteine-induced changes in cell proliferation and differentiation in the chick embryo spinal cord: implications for mechanisms of neural tube defects (NTD), Reprod. Toxicol. 69 (2017) 167–173, https://doi.org/10.1016/j.reprotox.2017.02.011.
[45] R.S. Beard Jr., J.J. Reynolds, S.E. Bearden, Hyperhomocysteinemia increases permeability of the blood-brain barrier by NMDA receptor-dependent regulation of adherens and tight junctions, Blood 118 (7) (2011) 2007–2014, https://doi.org/10.1182/blood-2011-02-338269.
[46] Y. Zhao, G. Huang, S. Chen, et al., Homocysteine aggravates cortical neural cell injury through neuronal autophagy overactivation following rat cerebral ischemia-reperfusion, Int. J. Mol. Sci. 17 (8) (2016), https://doi.org/10.3390/ijms17081196.
[47] I.S. Ovey, M. Naziroglu, Homocysteine and cytosolic GSH depletion induce apoptosis and oxidative toxicity through cytosolic calcium overload in the hippocampus of aged mice: involvement of TRPM2 and TRPV1 channels, Neuroscience 284 (2015) 225–233, https://doi.org/10.1016/j.neuroscience.2014.09.078.
[48] S.L. Sunden, M.S. Renduchintala, E.I. Park, et al., Betaine-homocysteine methyltransferase expression in porcine and human tissues and chromosomal localization of the human gene, Arch. Biochem. Biophys. 345 (1) (1997) 171–174, https://doi.org/10.1006/abbi.1997.0246.
[49] R.L. Sacco, R. Adams, G. Albers, et al., Guidelines for prevention of stroke in patients with ischemic stroke or transient ischemic attack: a statement for healthcare professionals from the American Heart Association/American Stroke Association Council on Stroke: co-sponsored by the Council on Cardiovascular Radiology and Intervention: the American Academy of Neurology affirms the value of this guideline, Stroke 37 (2) (2006) 577–617, https://doi.org/10.1161/01.STR.0000199147.30016.74.
[50] A.H. Hainsworth, N.E. Yeo, E.M. Weekman, et al., Homocysteine, hyperhomocysteinemia and vascular contributions to cognitive impairment and dementia (VCID), Biochim. Biophys. Acta 1862 (5) (2016) 1008–1017, https://doi.org/10.1016/j.bbadis.2015.11.015.
[51] A. de Bree, W.M. Verschuren, H.J. Blom, et al., The homocysteine distribution: (mis)judging the burden, J. Clin. Epidemiol. 54 (5) (2001) 462–469.
[52] D. Girelli, S. Friso, E. Trabetti, et al., Methylenetetrahydrofolate reductase C677T mutation, plasma homocysteine, and folate in subjects from northern Italy with or without angiographically documented severe coronary atherosclerotic disease: evidence for an important genetic-environmental interaction, Blood 91 (11) (1998) 4158–4163.
[53] L.A. Kluijtmans, I.S. Young, C.A. Boreham, et al., Genetic and nutritional factors contributing to hyperhomocysteinemia in young adults, Blood 101 (7) (2003) 2483–2488, https://doi.org/10.1182/blood.V101.7.2483.
[54] A. Sobczynska-Malefora, D.J. Harrington, Laboratory assessment of folate (vitamin B9) status, J. Clin. Pathol. 71 (11) (2018) 949–956, https://doi.org/10.1136/jclinpath-2018-205048.
[55] R. Clarke, J. Grimley Evans, J. Schneede, et al., Vitamin B12 and folate deficiency in later life, Age Ageing 33 (1) (2004) 34–41, https://doi.org/10.1093/ageing/afg109.
[56] Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline, The National Academies Collection: Reports funded by National Institutes of Health, 1998 Washington (DC).
[57] U.S.P.S.T. Force, K. Bibbins-Domingo, D.C. Grossman, et al., Folic acid supplementation for the prevention of neural tube defects: US preventive services task force recommendation statement, JAMA 317 (2) (2017) 183–189, https://doi.org/10.1001/jama.2016.19438.
[58] C.M. Pfeiffer, C.L. Johnson, R.B. Jain, et al., Trends in blood folate and vitamin B-12 concentrations in the United States, 1988 2004, Am. J. Clin. Nutr. 86 (3) (2007) 718–727, https://doi.org/10.1093/ajcn/86.3.718.
[59] L.H. Allen, J.W. Miller, L. de Groot, et al., Biomarkers of nutrition for development (BOND): vitamin B-12 review, J. Nutr. 148 (suppl_4) (2018) 1995S–2027S, https://doi.org/10.1093/jn/nxy201.
[60] R. Green, L.H. Allen, A.L. Bjorke-Monsen, et al., Vitamin B12 deficiency, Nat. Rev. Dis. Primers 3 (2017) 17040, https://doi.org/10.1038/nrdp.2017.40.
[61] A. Barnabe, A.C. Alessio, L.F. Bittar, et al., Folate, vitamin B12 and Homocysteine status in the post-folic acid fortification era in different subgroups of the Brazilian population attended to at a public health care center, Nutr. J. (2015), https://doi.org/10.1186/s12937-015-0006-3 14. 19.
[62] M.S. Morris, M.F. Picciano, P.F. Jacques, et al., Plasma pyridoxal 5′-phosphate in the US population: the National Health and Nutrition Examination Survey, 2003-2004, Am. J. Clin. Nutr. 87 (5) (2008) 1446–1454, https://doi.org/10.1093/ajcn/87.5.1446.
[63] H.J. Klosterman, Vitamin B6 antagonists of natural origin, J. Agric. Food Chem. 22 (1) (1974) 13–16.
[64] S. Mayengbam, S. Raposo, M. Aliani, et al., A Vitamin B-6 antagonist from flaxseed perturbs amino acid metabolism in moderately Vitamin B-6-deficient male rats, J. Nutr. 146 (1) (2016) 14–20, https://doi.org/10.3945/jn.115.219378.
[65] P.T. Clayton, B6-responsive disorders: a model of vitamin dependency, J. Inherit. Metab. Dis. 29 (2–3) (2006) 317–326, https://doi.org/10.1007/s10545-005-0243-2.
[66] S. Hustad, P.M. Ueland, S.E. Vollset, et al., Riboflavin as a determinant of plasma total homocysteine: effect modification by the methylenetetrahydrofolate reductase C677T polymorphism, Clin. Chem. 46 (2000) 1065–1071 8 Pt 1.
[67] M.M. O'Brien, M. Kiely, K.E. Harrington, et al., The North/South ireland food consumption survey: vitamin intakes in 18-64-year-old adults, Publ. Health Nutr. 4 (5A) (2001) 1069–1079.
[68] A. De Bree, W.M. Verschuren, D. Kromhout, et al., Homocysteine determinants and the evidence to what extent homocysteine determines the risk of coronary heart disease, Pharmacol. Rev. 54 (4) (2002) 599–618.
[69] A. McCaddon, J.W. Miller, Assessing the association between homocysteine and cognition: reflections on Bradford Hill, meta-analyses, and causality, Nutr. Rev. 73 (10) (2015) 723–735, https://doi.org/10.1093/nutrit/nuv022.
[70] R. Clarke, G. Harrison, S. Richards, et al., Effect of vitamins and aspirin on markers of platelet activation, oxidative stress and homocysteine in people at high risk of dementia, J. Intern. Med. 254 (1) (2003) 67–75.
[71] D.J. Stott, G. MacIntosh, G.D. Lowe, et al., Randomized controlled trial of homocysteine-lowering vitamin treatment in elderly patients with vascular disease, Am. J. Clin. Nutr. 82 (6) (2005) 1320–1326, https://doi.org/10.1093/ajcn/82.6.1320.
[72] J.A. McMahon, T.J. Green, C.M. Skeaff, et al., A controlled trial of homocysteine lowering and cognitive performance, N. Engl. J. Med. 354 (26) (2006) 2764–2772, https://doi.org/10.1056/NEJMoa054025.
[73] J. Durga, M.P. van Boxtel, E.G. Schouten, et al., Effect of 3-year folic acid supplementation on cognitive function in older adults in the FACIT trial: a randomised, double blind, controlled trial, Lancet 369 (9557) (2007) 208–216, https://doi.org/10.1016/S0140-6736(07)60109-3.
[74] P.S. Aisen, L.S. Schneider, M. Sano, et al., High-dose B vitamin supplementation and cognitive decline in Alzheimer disease: a randomized controlled trial, JAMA 300 (15) (2008) 1774–1783, https://doi.org/10.1001/jama.300.15.1774.
[75] A.H. Ford, L. Flicker, H. Alfonso, et al., Vitamins B(12), B(6), and folic acid for cognition in older men, Neurology 75 (17) (2010) 1540–1547, https://doi.org/10.1212/WNL.0b013e3181f962c4.
[76] T. Kwok, J. Lee, C.B. Law, et al., A randomized placebo controlled trial of homocysteine lowering to reduce cognitive decline in older demented people, Clin. Nutr. 30 (3) (2011) 297–302, https://doi.org/10.1016/j.clnu.2010.12.004.
[77] C.A. de Jager, A. Oulhaj, R. Jacoby, et al., Cognitive and clinical outcomes of homocysteine-lowering B-vitamin treatment in mild cognitive impairment: a randomized controlled trial, Int. J. Geriatr. Psychiatr. 27 (6) (2012) 592–600, https://doi.org/10.1002/gps.2758.
[78] H. Chen, S. Liu, L. Ji, et al., Folic acid supplementation mitigates Alzheimer's disease by reducing inflammation: a randomized controlled trial, Mediat. Inflamm. 2016 (2016) 5912146, , https://doi.org/10.1155/2016/5912146.
[79] T. Kwok, J. Lee, R.C. Ma, et al., A randomized placebo controlled trial of vitamin B12 supplementation to prevent cognitive decline in older diabetic people with borderline low serum vitamin B12, Clin. Nutr. 36 (6) (2017) 1509–1515, https://doi.org/10.1016/j.clnu.2016.10.018.

[80] F. Ma, Q. Li, X. Zhou, et al., Effects of folic acid supplementation on cognitive function and Abeta-related biomarkers in mild cognitive impairment: a randomized controlled trial, Eur. J. Nutr. (2017), https://doi.org/10.1007/s00394-017-1598-5.
[81] F. Ma, T. Wu, J. Zhao, et al., Folic acid supplementation improves cognitive function by reducing the levels of peripheral inflammatory cytokines in elderly Chinese subjects with MCI, Sci. Rep. 6 (2016) 37486, https://doi.org/10.1038/srep37486.
[82] F. Ma, T. Wu, J. Zhao, et al., Effects of 6-month folic acid supplementation on cognitive function and blood biomarkers in mild cognitive impairment: a randomized controlled trial in China, J. Gerontol. A Biol. Sci. Med. Sci. 71 (10) (2016) 1376–1383, https://doi.org/10.1093/gerona/glv183.
[83] M. Butler, V.A. Nelson, H. Davila, et al., Over-the-counter supplement interventions to prevent cognitive decline, mild cognitive impairment, and clinical alzheimer-type dementia: a systematic review, Ann. Intern. Med. 168 (1) (2018) 52–62, https://doi.org/10.7326/M17-1530.
[84] S. Andrieu, N. Coley, P. Aisen, et al., Methodological issues in primary prevention trials for neurodegenerative dementia, J. Alzheimers Dis. 16 (2) (2009) 235–270, https://doi.org/10.3233/JAD-2009-0971.
[85] P.F. Jacques, J. Selhub, A.G. Bostom, et al., The effect of folic acid fortification on plasma folate and total homocysteine concentrations, N. Engl. J. Med. 340 (19) (1999) 1449–1454, https://doi.org/10.1056/NEJM199905133401901.
[86] R. Wang, Is H2S a stinky remedy for atherosclerosis? Arterioscler. Thromb. Vasc. Biol. 29 (2) (2009) 156–157, https://doi.org/10.1161/ATVBAHA.108.180190.
[87] K. Koyama, A. Yoshida, A. Takeda, et al., Abnormal cyanide metabolism in uraemic patients, Nephrol. Dial. Transplant. 12 (8) (1997) 1622–1628, https://doi.org/10.1093/ndt/12.8.1622.
[88] J.D. Spence, M.J. Stampfer, Understanding the complexity of homocysteine lowering with vitamins: the potential role of subgroup analyses, JAMA 306 (23) (2011) 2610–2611, https://doi.org/10.1001/jama.2011.1834.
[89] J.S. Lai, W.W. Pang, S. Cai, et al., High folate and low vitamin B12 status during pregnancy is associated with gestational diabetes mellitus, Clin. Nutr. 37 (3) (2018) 940–947, https://doi.org/10.1016/j.clnu.2017.03.022.
[90] P. Dwarkanath, J.R. Barzilay, T. Thomas, et al., High folate and low vitamin B-12 intakes during pregnancy are associated with small-for-gestational age infants in South Indian women: a prospective observational cohort study, Am. J. Clin. Nutr. 98 (6) (2013) 1450–1458, https://doi.org/10.3945/ajcn.112.056382.
[91] M.S. Morris, J. Selhub, P.F. Jacques, Vitamin B-12 and folate status in relation to decline in scores on the mini-mental state examination in the framingham heart study, J. Am. Geriatr. Soc. 60 (8) (2012) 1457–1464, https://doi.org/10.1111/j.1532-5415.2012.04076.x.
[92] M.S. Morris, P.F. Jacques, I.H. Rosenberg, et al., Circulating unmetabolized folic acid and 5-methyltetrahydrofolate in relation to anemia, macrocytosis, and cognitive test performance in American seniors, Am. J. Clin. Nutr. 91 (6) (2010) 1733–1744, https://doi.org/10.3945/ajcn.2009.28671.
[93] J. Selhub, M.S. Morris, P.F. Jacques, et al., Folate-vitamin B-12 interaction in relation to cognitive impairment, anemia, and biochemical indicators of vitamin B-12 deficiency, Am. J. Clin. Nutr. 89 (2) (2009), https://doi.org/10.3945/ajcn.2008.26947C 702S–6S.
[94] A.A. House, M. Eliasziw, D.C. Cattran, et al., Effect of B-vitamin therapy on progression of diabetic nephropathy: a randomized controlled trial, JAMA 303 (16) (2010) 1603–1609, https://doi.org/10.1001/jama.2010.490.
[95] R.H. Boger, S.M. Bode-Boger, K. Sydow, et al., Plasma concentration of asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, is elevated in monkeys with hyperhomocyst(e)inemia or hypercholesterolemia, Arterioscler. Thromb. Vasc. Biol. 20 (6) (2000) 1557–1564.
[96] M.C. Morris, C.C. Tangney, A potential design flaw of randomized trials of vitamin supplements, JAMA 305 (13) (2011) 1348–1349, https://doi.org/10.1001/jama.2011.383.
[97] R. Clarke, D. Bennett, S. Parish, et al., Effects of homocysteine lowering with B vitamins on cognitive aging: meta-analysis of 11 trials with cognitive data on 22,000 individuals, Am. J. Clin. Nutr. 100 (2) (2014) 657–666, https://doi.org/10.3945/ajcn.113.076349.
[98] G. Douaud, H. Refsum, C.A. de Jager, et al., Preventing Alzheimer's disease-related gray matter atrophy by B-vitamin treatment, Proc. Natl. Acad. Sci. U. S. A. 110 (23) (2013) 9523–9528, https://doi.org/10.1073/pnas.1301816110.
[99] A.D. Smith, S.M. Smith, C.A. de Jager, et al., Homocysteine-lowering by B vitamins slows the rate of accelerated brain atrophy in mild cognitive impairment: a randomized controlled trial, PLoS One 5 (9) (2010) e12244, , https://doi.org/10.1371/journal.pone.0012244.
[100] C. Hooper, P. De Souto Barreto, N. Coley, et al., Cross-sectional associations of total plasma homocysteine with cortical beta-amyloid independently and as a function of omega 3 polyunsaturated fatty acid status in older adults at risk of dementia, J. Nutr. Health Aging 21 (10) (2017) 1075–1080, https://doi.org/10.1007/s12603-017-0989-x.
[101] F. Jerneren, A.K. Elshorbagy, A. Oulhaj, et al., Brain atrophy in cognitively impaired elderly: the importance of long-chain omega-3 fatty acids and B vitamin status in a randomized controlled trial, Am. J. Clin. Nutr. 102 (1) (2015) 215–221, https://doi.org/10.3945/ajcn.114.103283.
[102] A. Oulhaj, F. Jerneren, H. Refsum, et al., Omega-3 Fatty Acid Status Enhances the Prevention of Cognitive Decline by B Vitamins in Mild Cognitive Impairment, J. Alzheimers Dis. 50 (2) (2016) 547–557, https://doi.org/10.3233/JAD-150777.
[103] V. Solfrizzi, P. Agosti, M. Lozupone, et al., Nutritional interventions and cognitive-related outcomes in patients with late-life cognitive disorders: a systematic review, Neurosci. Biobehav. Rev. 95 (2018) 480–498, https://doi.org/10.1016/j.neubiorev.2018.10.022.
[104] G.J. Hankey, J.W. Eikelboom, Q. Yi, et al., Antiplatelet therapy and the effects of B vitamins in patients with previous stroke or transient ischaemic attack: a post-hoc subanalysis of VITATOPS, a randomised, placebo-controlled trial, Lancet Neurol. 11 (6) (2012) 512–520, https://doi.org/10.1016/S1474-4422(12)70091-1.
[105] J.F. Meschia, C. Bushnell, B. Boden-Albala, et al., Guidelines for the primary prevention of stroke: a statement for healthcare professionals from the American Heart Association/American Stroke Association, Stroke 45 (12) (2014) 3754–3832, https://doi.org/10.1161/STR.0000000000000046.
[106] B. Arshi, B. Ovbiagele, D. Markovic, et al., Differential effect of B-vitamin therapy by antiplatelet use on risk of recurrent vascular events after stroke, Stroke 46 (3) (2015) 870–873, https://doi.org/10.1161/STROKEAHA.114.006927.
[107] J.H. Park, G. Saposnik, B. Ovbiagele, et al., Effect of B-vitamins on stroke risk among individuals with vascular disease who are not on antiplatelets: A meta-analysis, Int. J. Stroke 11 (2) (2016) 206–211, https://doi.org/10.1177/1747493015616512.