# Vitamins Associated with Brain Aging, Mild Cognitive Impairment, and Alzheimer Disease: Biomarkers, Epidemiological and Experimental Evidence, Plausible Mechanisms, and Knowledge Gaps

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## ABSTRACT

The key to preventing brain aging, mild cognitive impairment (MCI), and Alzheimer disease (AD) via vitamin intake is first to understand molecular mechanisms, then to deduce relevant biomarkers, and subsequently to test the level of evidence for the impact of vitamins in the relevant pathways and their modulation of dementia risk. This narrative review infers information on mechanisms from gene and metabolic defects associated with MCI and AD, and assesses the role of vitamins using recent results from animal and human studies. Current evidence suggests that all known vitamins and some "quasi-vitamins" are involved as cofactors or influence ≥1 of the 6 key sets of pathways or pathologies associated with MCI or AD, relating to *1*) 1-carbon metabolism, *2*) DNA damage and repair, *3*) mitochondrial function and glucose metabolism, *4*) lipid and phospholipid metabolism and myelination, *5*) neurotransmitter synthesis and synaptogenesis, and *6*) amyloidosis and Tau protein phosphorylation. The contemporary level of evidence for each of the vitamins varies considerably, but it is notable that B vitamins are involved as cofactors in all of the core pathways or pathologies and, together with vitamins C and E, are consistently associated with a protective role against dementia. Outcomes from recent studies indicate that the efficacy and safety of supplementation with vitamins to prevent MCI and the early stages of AD will most likely depend on *1*) which pathways are defective, *2*) which vitamins are deficient and could correct the relevant metabolic defects, and *3*) the modulating impact of nutrient-nutrient and nutrient-genotype interaction. More focus on a precision nutrition approach is required to realize the full potential of vitamin therapy in preventing dementia and to avoid causing harm. *Adv Nutr* 2017;8:958–70.

Keywords: vitamins, brain, aging, Alzheimer, biomarkers, epidemiology, interventions, mechanisms, knowledge gaps

## Introduction

Aging of populations worldwide is increasing the numbers of people at high risk of degenerative diseases, including Alzheimer disease (AD), for which no cure exists (1–6). Mild cognitive impairment (MCI) is the prodromal stage of AD, and the risk for both increases with malnutrition (2–6). An understanding of which nutritional factors are associated with the risk of MCI and AD is essential in order to design appropriate preventive strategies based on dietary intervention. It is well recognized that prevention of AD requires intervention before or very early during the onset of MCI (5, 6). For this reason, biomarkers associated prospectively with eventual risk of MCI and AD are an important tool to determine the potential preventive effects of vitamins ingested either via nutrient-rich whole foods or as supplements. This review focuses on current knowledge regarding vitamins that have been associated with MCI and AD in animal models and in human epidemiological and interventional studies. It identifies important knowledge gaps and suggests new research directions, based on precision nutrition,

to achieve more successful outcomes with vitamin therapy for preventing dementia.

## Current Status of Knowledge

### Biomarkers associated with MCI and AD

Although specific tissues and organs may age in unique ways, fundamental hallmarks of aging that occur at the cellular and genome levels are common to all tissues; these include genomic instability at the chromosomal and DNA sequence levels; telomere attrition and dysfunction; shifts in epigenetic marks at the DNA and histone levels, which affect gene expression; and loss of mitochondrial function due in part to mitochondrial DNA mutations. These fundamental genomic deficits may then lead to increased cellular senescence, reduced capacity for stem cell regeneration, altered nutrient sensing, loss of proteostasis, and deleterious changes in cellular phenotypes and in intercellular communication (7, 8). Assuming that MCI and AD are manifestations of accelerated aging occurring systemically within the body, one could reasonably hypothesize that those at higher risk may already be exhibiting a heightened level of the above-mentioned hallmarks of aging in easily accessible peripheral tissues (e.g., blood, buccal cells, and urine) before the symptoms of MCI start to become evident.

Age-related changes that are biochemically more directly associated with MCI and AD have been identified and are progressively being better characterized as knowledge accumulates (1, 9, 10). The best-characterized temporal sequence of pathology indicative of increased risk of MCI and AD is reduced amyloid β-42 ($A\beta_{42}$) in cerebrospinal fluid (CSF); amyloid accumulation in the brain, which is detected by positron emission tomography using Pittsburgh compound B (PIB-PET); followed by accumulation of tau protein in CSF, after which brain atrophy and reduced uptake of glucose become evident in the brain, as detected by MRI and fluorodeoxyglucose positron emission tomography, respectively (1, 10). The accumulation of these biomarkers precedes the onset of cognitive impairment that is measured using a defined set of cognitive function tests specific for memory, attention, and executive function (1, 6).

Total tau, phosphorylated tau, neurofilament light protein, and $A\beta_{42}$ in CSF have the strongest diagnostic value (1, 9, 10), but CSF collection is a more invasive procedure than blood collection, requiring highly skilled personnel to achieve success and minimize adverse effects. Total tau and chitinase-3-like protein 1 (an inflammatory protein) in blood are also associated with MCI and AD risk and may be useful in a research setting. Tau, neurofilament light protein, and chitinase-3-like protein 1 are biomarkers of axonal damage. $A\beta_{42}$ is a toxic form of amyloid generated through the inappropriate processing of amyloid precursor protein (APP) and can accumulate intracellularly or extracellularly to a concentration that induces neuronal cell death and brain atrophy (11, 12).

Untargeted approaches have also yielded several metabolomic and proteomic biomarkers in blood that indicate significant risk for AD. These include biomarkers associated with cell growth (insulin-like growth factor binding protein), pancreatic function (pancreatic polypeptide), stress (cortisol), 1-carbon metabolism (homocysteine), antioxidant function (superoxide dismutase), and kidney function (β-2 microglobulin) (13–15). Other studies showed strong associations of single FAs or combinations of specific SFAs, MUFAs, PUFAs, and ω-3 FAs with MCI (16–19). Such biomarkers, when combined with other risk factors such as age, sex, and *APOE* genotype may provide a stronger predictive potential for identifying those with an increased probability of MCI and AD.

We recently reviewed the various biomarkers in peripheral tissue that are associated with risk for MCI and AD (20). One of the increasingly investigated tissues are buccal cells because they are easily obtained in a minimally invasive manner. It has been hypothesized that buccal cell tissue could be a good candidate to reflect AD-related pathology if the disease was systemic, given that both brain and buccal cells are of ectodermal origin (21). In a pioneering study, we showed that buccal cells of patients with AD and patients with Down syndrome have increased levels of DNA damage, measured as micronuclei, reduced frequencies of dying cells, and a much reduced frequency of basal cells, which represents the regenerative capacity of the tissue. The combination of the low frequency of karyorrhexis cells (biomarker 1) and basal cells (biomarker 2) was strongly associated with AD [positive predictive value: 98%; negative predictive value: 77%; sensitivity: 82%; specificity: 97%; likelihood ratio: 25, OR: 140 (95% CI: 17, 1165); $P = 0.0001$ for biomarker 1 + 2 <41/1000 cells] (22, 23). The area under the receiver operating characteristic curve (AUROC) for the association of basal cell frequency with AD was 0.96 ($P < 0.0001$), for karyorrhexis cells it was 0.88 ($P < 0.0001$), and for both basal and karyorrhexis cells the AUROC was 0.91 ($P < 0.0001$). We observed that the lower frequency of basal cells and karyorrhexis cells in patients with AD may be explained by lower vitamin B-12 and higher homocysteine concentrations in plasma, respectively (22, 23).

The association with low basal cell frequency was further verified using the basal cell–specific cytokeratin 14 (CK14) marker. CK14 was significantly reduced in buccal cells of patients with MCI and AD, and its diagnostic risk value was better than that of plasma homocysteine (24). The diagnostic value for detecting a case of MCI based on the AUROC was 0.57, 0.90, and 0.93 for homocysteine, CK14, and the CK14-to-homocysteine ratio, respectively. The diagnostic value for detecting a case of AD based on the AUROC was 0.67, 0.77, and 0.79 for homocysteine, CK14, and the CK14-to-homocysteine ratio, respectively. Furthermore, it was also noted that CK14 was positively correlated with vitamin B-12 in the MCI and AD groups ($r = 0.51$ and 0.63, respectively). In addition, $A\beta_{1-42}$ was detectable in buccal cells and increased significantly in MCI and even further in AD relative to controls (21, 24, 25). Telomere shortening in buccal cells, like lymphocytes, was also associated with increased risk for dementia, but the association tended to be stronger with blood lymphocytes in our studies (26, 27). It is interesting

to note that some of the dietary and metabolic factors associated with protection against (e.g., Mediterranean diet and higher ω-3 FA intake) or aggravation of (e.g., high homocysteine and obesity) telomere shortening are also often linked in the same directions with AD risk (28–33).

## Which vitamin combinations might provide an effective metabolic tune-up to prevent brain aging and the early stages of MCI and AD? Does it depend on genotype?

It is essential to understand the biology of brain aging and the metabolic pathways involved, before attempting to work out which vitamin combination (nutriome) might be required to optimize (tune up) metabolic function in a manner that decelerates brain aging and the pathologies that lead to MCI and AD (34). Which metabolic pathways matter most can be thoroughly understood by examining *1*) genetic risk factors, *2*) changes in gene expression in the brain with age and with MCI or AD, *3*) using bioinformatics, to identify which metabolic pathways are affected by such genetic changes, and *4*) metabolic imbalances that indicate where metabolic blocks may be occurring. Once the metabolic pathways are identified, it becomes possible to establish which vitamins might be required as cofactors and substrates in these pathways and therefore reasonably have a putative role in preventing MCI and AD. These could then be considered as legitimate candidates for a metabolic tune-up to prevent brain aging and dementia. The aim would be to intervene with the appropriate vitamin intake combination well before the symptoms of MCI emerge; current evidence indicates AD-related brain pathology and atrophy commence several years before the onset of MCI (6, 35, 36).

Highly penetrant mutations have been identified that increase risk of dementia, and recent studies have shed some light on the protective effects of vitamins in these genetic backgrounds. The most important of these mutations occur in the *APP*, presenilin (*PSEN*) 1, and *PSEN2* genes, which cause AD through their role in the metabolism of the APP by shifting APP processing toward the generation of $A\beta_{1-42}$ and fibrillogenesis, leading to amyloid plaque formation (37). Such mutations have strong causative effects, but they are relatively rare (<1%) and are associated with early onset of AD. Dietary factors that influence APP processing have not yet been systematically defined, but several studies of mouse models with mutations in the *App* and *Psen* genes indicate the potential of folate, thiamine, nicotinamide, and vitamins C, D, and E to exert a substantial beneficial modifying effect on either APP processing, amyloidosis, or amyloid plaque formation (38–48).

Other gene mutations increase risk of AD through alternative mechanisms. One of the more common of these mutations associated with AD risk is the *APOEε4* mutation, which occurs at a frequency of 2–5% in the homozygous state and 10–20% in the heterozygous state, depending on ethnicity. Relative to non–ε4 carriers, *APOEε4* carriers have a higher cholesterol concentration in their blood and show higher Aβ deposition in the form of senile plaques in the brain (49). Emerging evidence of the interactive effects

of the *APOEε4* genotype with specific vitamin status (e.g., vitamin B-12 deficiency) in modifying risk for AD suggests the possibility that *APOEε4* carriers may respond differently to micronutrient intervention relative to noncarriers. For example, a significant positive correlation between serum concentrations of vitamin B-12 and volume of brain regional gray matter was observed in *APOEε4* carriers with AD but not in noncarriers (50). Furthermore, in cross-sectional studies in the elderly in Norway and Singapore, researchers observed that cognitive decline was increased with low vitamin B-12 status, but only in those carrying the *APOE* ε4 allele (51, 52).

Other gene mutations that confer lower risk for AD but are common (15–50% frequency) include those related to cholesterol metabolism [ATP binding cassette subfamily A member 7 (*ABCA7*) and solute carrier family 24 member 4 (*SLC24A4*)], endocytosis [Ras and Rab interactor 3 (*RIN3*), phosphatidylinositol binding clathrin assembly protein (*PICALM*), and bridging integrator 1 (*BIN1*)], immune response [complement C3b/C4b receptor 1 (*CR1*), CD33 molecule (*CD33*), and inositol polyphosphate-5- phosphatase D (*INPP5D*)], and cytoskeletal function or axonal transport [CUGPB Elav-like family member 1 (*CELF1*) and NME/NM23 family member 8 (*NME8*)] (37). Mechanisms for some of these mutations are known; for example, ABCA7 deficiency alters the brain lipid profile and accelerates the processing of APP to amyloid β by increasing β-site APP cleaving enzyme 1 (BACE1) (53). However, whether vitamin status might substantially alter the impact of the genetic susceptibilities conferred by these mutations remains untested.

## Age-related changes in brain gene expression due to oxidative damage and DNA methylation of gene promoter methylation, and the role of vitamins in mitigating these effects

Apart from inherited gene mutations, it is also possible that normal genes associated with MCI or AD risk could be silenced by DNA damage in the promoter sequence or as a result of methylation of cytosine-guanine dinucleotide islands sites within it. Limited evidence shows differential DNA methylation of promoters between normal and AD brains for coding genes involved in myelination and microRNA genes that express microRNAs which control expression of genes required for the myelination process (54, 55). Furthermore, methylation of promoters of nucleolar ribosomal RNA (rRNA) genes are decreased in MCI and AD relative to healthy control brains (56, 57). This observation is consistent with other evidence that nuclear ribosomal RNA expression and nucleolar size is diminished in the cells of AD brains (58). The synthesis of rRNA is carefully tuned to match nutritional conditions such that rRNA gene expression is depressed when intracellular energy status is diminished (59). Furthermore, rodent studies suggest that maternal protein calorie malnutrition downregulates ribosomal DNA transcription in fetal tissues and that such effects might be carried over throughout the life span with the consequence that inhibition of rRNA gene expression could lead to neurodegeneration (60). The effects of vitamin deficiencies on

nucleolar structure and rRNA gene expression is barely explored, and the limited evidence available suggests that vitamin E deficiency can adversely affect nucleolar structure and function and reduce RNA transcription rate, similar to aging (61).

Oxidation of DNA in gene promoters is another important mechanism affecting gene expression in the aging brain. Lu et al. (62), using transcriptomic profiling, showed progressively reduced expression of key genes involved in synaptic plasticity, vesicular transport, calcium homeostasis, and mitochondrial function in frontal cortex brain tissue from human subjects aged $\geq$40 y. Furthermore, the promoters of these genes were selectively susceptible to oxidative damage as a result of reduced base-excision repair of oxidized DNA bases. The results of this study suggest that oxidative stress may reduce the expression of selectively vulnerable genes involved in learning, memory, and neuronal survival by damaging their promoters. In further studies, the same group showed that repressor element 1–silencing transcription factor (REST) provides protection against oxidative damage and amyloid β toxicity by repressing genes that promote cell death, thereby improving survival of neurons, and that expression of REST is lost in MCI and AD (63). These observations indicate the potential importance of dietary factors that protect against oxidative damage by supporting antioxidant defenses (e.g., vitamin C and vitamin E) and by improving efficiency of base-excision repair (zinc, magnesium, niacin, and folate) to remove oxidized guanine in DNA (64–69). However, the impact of dietary factors on the expression of REST remains unknown.

In this context, it is important to note the strong dependence of the human brain on vitamin C and folate; concentrations of these vitamins are much higher in the brain than in plasma (70, 71). Arguably, this could be mainly to prevent oxidative damage to DNA and optimize its repair. However, alternative explanations have emerged relating to regulation of promoter methylation since the 2009 discovery of the sixth deoxyribonucleoside 5-hydroxymethyldeoxycytidine in DNA, which is most abundant in neurons (72), and the concurrent discovery of the three 10–11 translocation (TET) enzymes, which not only synthesize but also oxidize 5-hydroxymethyldeoxycytidine in DNA, before removing glycosylase and repairing base excisions (73, 74). TET enzymes require vitamin C as a cofactor to demethylate DNA, whereas reduced folates are needed to maintain DNA methylation, to synthesize mitochondrial and nuclear DNA, and to perform DNA repair synthesis (75, 76). A study in knockout *Tet1* rodents suggest that neuronal Tet1 is critical for extinction of memory and regulation of the expression of key neuronal activity–regulated genes and neuronal plasticity (73).

It is also interesting to note that another important role of reduced folates is to metabolize formaldehyde and its oxidized form formate, which has been shown to be elevated in MCI and AD and to inhibit spatial learning and spatial memory, resulting in topographic amnesia, which is commonly observed in AD (77–79). Formate is incorporated into 1-carbon metabolism via the activity of 10-formyltetrahydrofolate synthetase, which catalyses the reaction of formate with tetrahydrofolate to form 10-formyltetrahydrofolate, which is a precursor of the methyl donors 5,10-methylenetetrahydrofolate and 5-methyltetrahydrofolate required for nucleotide and methione synthesis, respectively (80). When folate and vitamin B-12 are deficient, the concentration of tetrahydrofolate is diminished; as a consequence, formate increases, as do the pathological consequences of its excess (80, 81).

Another approach that could inform which nutriome provides the best metabolic tune-up to prevent brain aging and the early stages of AD is to integrate transcriptomic data from patients with AD through a genome-wide computational human metabolic model in order to characterize the altered metabolism in AD, then deduce with metabolic modeling methods which nutrients can help to overcome bottlenecks. Research using this approach showed that the AD brain exhibits highly significant decreases in *1*) mitochondrial nutrient transport ($P = 4.8 \times 10^{-11}$), *2*) the activity of the carnitine shuttle ($P = 3.5 \times 10^{-18}$), and *3*) folate metabolism ($P = 3.8 \times 10^{-13}$), indicating the possibility that specific micronutrients relevant to mitochondrial and 1-carbon metabolism might provide some metabolic stress relief and possibly delay the onset of the later stages of AD (82).

The plausibility of mitochondrial and 1-carbon metabolism involvement is supported by evidence that folate deficiency increases the frequency of large deletions in mitochondrial DNA in rodent models (83, 84) and that mitochondrial DNA deletions increase with age in the temporal and frontal cortices and the putamen of human brain (85). An initial pilot study showed that mitochondrial DNA deletions were increased 15-fold in the frontal cortex of younger patients with AD (<75 y old) relative to age-matched healthy controls, but this trend was not evident in older cases and controls (86). A recent review of all studies published in the past 2 decades was inconclusive regarding the role of mitochondrial DNA deletions in the brain as a cause of AD because of inconsistencies between studies (87).

Decreases in mitochondrial transport may be partly explained by mutations in translocase of the mitochondrial outer membrane (TOM) 40 or blockage of translocase of the TOM by A$\beta_{1-42}$; TOM40 is the channel-forming subunit of the TOM complex that is essential for importing of protein precursors into mitochondria (88, 89). There is a lack of knowledge regarding the direct and indirect effects of vitamin deficiencies on the transport of proteins into mitochondria.

As is evident from basic biochemistry, several vitamins are required as cofactors for the biochemical reactions within the mitochondria—which include the citric acid cycle (thiamine, riboflavin, niacin, pantothenic acid, and biotin), the electron transport chain (riboflavin, niacin, and pantothenic acid), 1-carbon metabolism (riboflavin and vitamin B-6)—and for methylmalonyl-CoA mutase (vitamin B-12) (90). The latter is required to obtain energy in the form of succinyl-CoA from methylmalonyl-CoA, which is primarily derived from propionyl-CoA, a substance formed from the catabolism and digestion of isoleucine, valine, threonine, methionine, thymine, cholesterol, or odd-chain FAs. This suggests that correcting thiamine, riboflavin, niacin, pantothenic acid, vitamin B-6, biotin, vitamin

B-12, and folate deficiencies has the potential to boost mitochondrial metabolism in the aging brain, potentially optimizing ATP production efficiency.

## Associations of vitamins with MCI and AD deduced from epidemiological studies, interventions, and brain imaging

A more direct approach to investigate the association between micronutrients and MCI and AD is to study the correlation with brain atrophy, metabolic function (e.g., glucose metabolism), and accumulation of amyloid, which can be measured with brain MRI, fluorodeoxyglucose standardized uptake value ratio, and PIB-PET, respectively (1, 37).

Using MRI, Vogiatzoglou et al. (91) and Smith et al. (92) reported that the rate of brain atrophy in patients with MCI was significantly positively correlated with homocysteine concentration; homocysteine is a biomarker of either folate deficiency or vitamin B-12 deficiency, or both. In addition, brain atrophy in amnestic or nonamnestic patients with MCI was prevented by supplementation with folic acid (0.8 mg/d), vitamin B-12 (0.5 mg/d), and vitamin B-6 (20 mg/d) for 2 y (92). These beneficial effects were attributable to improved vitamin B-12 status and mainly found in those with homocysteine >11 μmol/L or plasma ω-3 FAs >390 μmol/L, and were reflected in improved cognition scores (93–95). The same investigators also observed a significant interactive effect of vitamin B-12 status with *APOE* genotype, such that those carrying the ε4 allele benefitted most from cognitive improvements associated with higher plasma concentrations of vitamin B-12 and lower concentrations of methylmalonic acid, the metabolic biomarker of vitamin B-12 deficiency (51). Furthermore, they observed that ω-3 FA status has to be optimal to achieve the full benefit of vitamin B supplementation. A clinical trial of B vitamins combined with ω-3 FAs is needed to determine whether it is possible to further improve the prevention of conversion from MCI to AD (36, 95). It is evident from these observations that interventions with B vitamins are unlikely to be successful in preventing cognitive decline and brain atrophy unless they are tailored to meet the specific needs of subgroups and individuals according to their vitamin B and ω-3 FA status, their metabolic profile, and their genetic susceptibility. This could explain the apparent lack of substantive evidence from meta-analyses of vitamin B homocysteine-lowering trials designed to prevent cognitive decline with aging or in MCI and AD (96–98). This conundrum needs to be resolved with better-designed controlled interventions performed in communities with cohorts showing the strongest associations between combined high homocysteine and low vitamin B-12 with AD risk, such as that recently reported in China [adjusted OR: 30.5 (95% CI: 9.7, 95.9); *P* < 0.0001] (99).

Another reason for past failures with vitamin interventions could be unexpected or previously unknown nutrient-nutrient and nutrient-gene interactions. The fact that nutrient intake combinations may have specific effects on health outcomes is not surprising given the evidence that nutrient-nutrient interactions affect fundamental pathologies such as DNA damage—sometimes in unexpected ways. For example, our studies of dietary nutrient intake and DNA damage in healthy Australians showed that micronucleus frequency in lymphocytes (a biomarker of chromosome breakage or loss), associated with increased risk for cancer, cardiovascular disease, diabetes, dementia, cognitive dysfunction, and microcephaly (100–104), is significantly affected by *1*) interactions of folate with riboflavin and calcium, such that micronuclei are increased when riboflavin is increased in a low folate background and when both folate and calcium intakes are low (105, 106), and *2*) interactions between polymorphisms in folate metabolism genes such as methionine synthase (*MTR*; A2756G), methylene tetrahydrofolate reductase (*MTHFR*; C677T), and reduced folate carrier (A80G) (107, 108). It was evident from genotype combination analyses that the frequency of micronuclei in lymphocytes was highest in those with the combined *MTR* (2756) AA and reduced folate carrier (79) GA or AA genotype and in those with the TT genotype for the C677T *MTHFR* polymorphism (107, 108).

In this regard there is an increasing concern about the cognitive effects of mandatory folic acid fortification in a low vitamin B-12 background, because of the observation in the United States and Australia that older people with low plasma concentrations of vitamin B-12 and high concentrations of folate in the blood have a substantially higher risk of cognitive impairment compared to those with low blood folate and low plasma vitamin B-12 (36, 109–111) (**Figure 1**). This also raises concerns as to whether the shift to plant-based diets may be jeopardized by folic acid fortification, because vitamin B-12 status in those choosing to shift to vegetarianism may decline substantially unless staple foods are more prevalently supplemented with vitamin B-12. These concerns also raise the urgent need to consider inclusion of vitamin B-12 together with folic acid in fortification programs to minimize risk of vitamin B-12 deficiency and mitigate cognitive deficits induced by high folic acid intake in the elderly (112). The plausibility that excessive intake of folic acid may contribute to cognitive decline is supported by 2 other observations: *1*) cognitive decline in the presence of a high plasma folate concentration is observed mainly in those homozygous for the 19-bp deletion in the dihydrofolate reductase gene, which codes for the enzyme that reduces folic acid to the active THF form required for participation in 1-carbon metabolism (113) and *2*) excess folic acid in animal models inhibits *Mthfr* and *Mtr* expression and promotes thymidylate synthase expression, favoring DNA nucleotide synthesis over homocysteine methylation and the generation of methionine and the methyl donor s-adenosyl methionine required for neurotransmitter synthesis (114, 115). Whether this observation in animal models applies to humans remains undetermined. Nevertheless, these observations indicate the risks inherent in the use of single-vitamin supplementation without considering interactions with other vitamins and genotype that affect the same metabolic pathways. The much higher complexity of nutrient-nutrient interactions and nutrient-genotype interactions across multiple interconnected pathways may vary greatly between individuals when using multinutrient supplementation. This could partly explain the relative
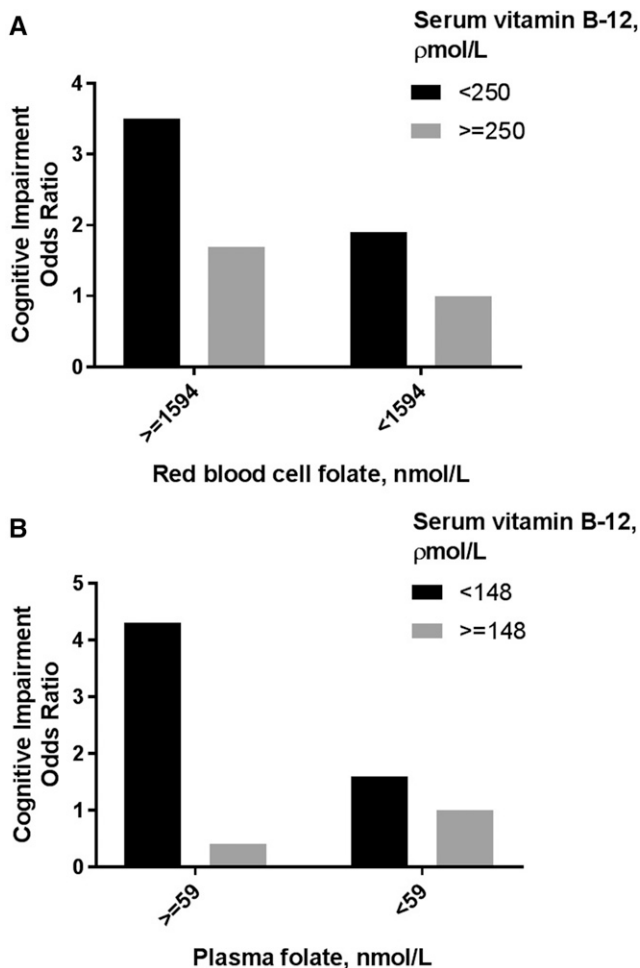
**FIGURE 1** High blood folate concentration in combination with low serum vitamin B-12 concentration is associated with a higher risk for cognitive impairment. The graphs show results from a study performed in Australia (109) (A) and a study performed in the United States (110) (B). Data were adapted from references 109 and 110 with permission.

inefficacy of Souvenaid [a complex of ω-3 FAs, the nucleotide uridine monophosphate, phospholipids, B complex vitamins (pyridoxine, cyanocobalamin, and folate), choline, vitamin E, and the micronutrient selenium] in preventing cognitive decline in the early stages of AD (116).

The homocysteine and B vitamins in cognitive impairment intervention (92) illustrated the importance of combining brain neuroimaging with nutritional studies, an approach that is also yielding valuable insights on the impact of a wider spectrum of vitamins and other nutrients in cross-sectional studies. For example, a cross-sectional neuroimaging pilot study investigated dietary nutrient intake and brain biomarkers of AD in at-risk but otherwise cognitively normal individuals (117). In that investigation, fluorodeoxyglucose positron emission tomography and PIB-PET were used to measure glucose uptake and metabolism, and amyloid deposition in the brain, respectively. Glucose uptake and metabolism in the brain were correlated positively with higher intake of folate and β-carotene and were reduced with more saturated fat;

on the other hand, amyloid deposition in the brain seemed to be substantially reduced in those with higher intake of vitamin D, vitamin B-12, and the ω-3 PUFA EPA. Using a similar approach, but also including gray matter volume measurements and focusing on nutrient intake patterns, the same group of researchers showed that intake of a nutrient combination that was protective against AD was associated with higher consumption of fresh fruit and vegetables, whole grains, fish, and low-fat dairy products, and lower intake of sweets, fried potatoes, high-fat dairy products, processed meat, and butter in cognitively normal individuals (118).

The above associations of folate, β-carotene, vitamin D, vitamin B-12, and ω-3 FA with a healthier brain imaging profile are supported by 1) results from a meta-analysis of 43 prospective cohort studies, showing that the relative risk for dementia was decreased with dietary patterns that reflected a Mediterranean diet [RR: 0.69 (95% CI: 0.57, 0.84); $P < 0.0001$], higher intake of vitamin B-6, folate, and vitamin B-12 [RR: 0.72 (95% CI: 0.54, 0.96); $P = 0.026$], vitamin E [RR: 0.80 (95% CI: 0.65, 0.98); $P = 0.034$], unsaturated FAs [RR: 0.84 (95% CI: 0.74, 0.95); $P = 0.006$], vitamin C [RR: 0.89 (95% CI: 0.74, 1.06); $P = 0.192$], and flavonoids [RR: 0.97 (95% CI: 0.65, 1.46); $P = 0.896$] (119) (**Figure 2**) and 2) the outcome from a meta-analysis of 106 investigations of plasma vitamin concentration in patients with AD, showing significantly lower values of vitamin B-12 ($-12\%$), vitamin A ($-14\%$), vitamin E ($-18\%$), folate ($-21\%$), vitamin D ($-27\%$), and vitamin C ($-33\%$) than those in healthy controls (120) (**Figure 3**). However, it is important to note that studies may not always be consistent across populations and cohorts, because dementia may still emerge in cohorts that are not deficient in the above-mentioned vitamins or do not have additional risk factors such as obesity, hyperhomocysteinemia, or inflammation, as was, for example, reported in a recent small cross-sectional study in Norway (121).

## The effects of less investigated vitamins and "quasi-vitamins"

Less is known about the effects of other vitamins on MCI and AD risk, but results from initial studies suggest the possibility that vitamin B-1 (thiamin), vitamin B-3 (niacin), vitamin K, and other essential nutrients such as inositol, choline, and carnitine may also contribute to the prevention of dementia.

Thiamine-dependent enzymes (transketolase, pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and branched-chain α-ketoacid dehydrogenase) play a critical role in glycolysis and the Krebs cycle, and deficiencies in the activity of these enzymes may contribute to reduced glucose metabolism, as evident in the brain of patients with dementia (122, 123). Preclinical models of thiamine deficiency and human thiamine deficiency (Korsakoff syndrome) exhibit memory deficits, neuritic plaques, and hyperphosphorylation of tau (123). In addition, dietary supplementation with benfotiamine, a more bioavailable analog of thiamine, enhanced the spatial memory of APP/PSEN1 mutant mice and reduced amyloid plaque and phosphorylated tau in their brains (124). Furthermore, a recent

**FIGURE 2** Results of a meta-analysis of 43 prospective cohort studies showing dietary factors associated with a reduced RR for dementia (119). The numbers in brackets in the figure are the 95% CIs. Data were adapted from reference 119 with permission.
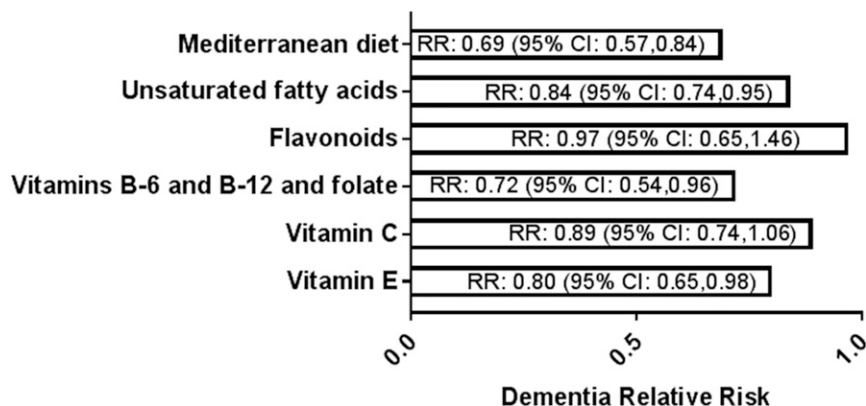
case-control study of humans showed that blood thiamine diphosphate concentration was significantly reduced in patients with AD relative to controls and had an AUROC of 77.4%, sensitivity of 78.1%, and specificity of 77.2% (125). A subsequent study reported that thiamine phophatases are increased in the blood of patients with AD, which may explain the low thiamine diphosphate concentration in AD (126).

Niacin plays an important role in carbohydrate and energy metabolism through its critical involvement as a cofactor in the Krebs cycle for conversion of acetyl-CoA generated from proteins, fats, and carbohydrates to ATP. Furthermore, niacin is important as a cofactor in myelination and DNA repair (68, 127, 128). Niaspan (a prolonged-release formulation of niacin) promoted synaptic plasticity and axon growth in a rodent model of stroke (129). These potential neuroprotective effects of niacin are also supported by a prospective cohort study in 3718 elderly aged $\geq$65 y which showed that the RR (95% CI) of AD in those in the second, third, fourth, and fifth quintiles of niacin intake was 0.3 (0.1–0.6), 0.3 (0.1–0.7), 0.6 (0.3–1.3), and 0.3 (0.1–0.7), respectively, when compared with those in the first quintile of intake during a 6-y follow-up, and that cognitive decline was diminished with higher niacin intake (130).

An increasing body of evidence suggests a role for vitamin K in brain physiology via its participation in sphingolipid metabolism and biological activation of the vitamin K–dependent growth arrest-specific 6 protein (Gas6), which may protect against neuronal apoptosis induced by amyloid $\beta$ or oxidative stress (131–133). Four human studies reported an association of low vitamin K intake or low blood concentrations of vitamin K with cognitive impairment or AD (134–137). Studies of dietary intake among older adults indicated that 1) those with serious subjective memory complaints had a lower mean dietary vitamin K intake than those with normal memory (298.0 compared with 393.8 μg/d; $P = 0.005$) (134), 2) mean vitamin K intake in patients at an early stage of AD, on a person-day basis, was 63 μg/d, compared with 139 μg/d in control subjects (135), and 3) dietary phylloquinone intake was positively associated with better cognitive function (136). A single study of measurements of vitamin K-1 (phylloquinone) and vitamin K-2 (menaquinone) in plasma showed significantly reduced plasma

vitamin K-1 concentrations in patients with mild and severe AD compared with healthy controls, but no difference in plasma vitamin K-2 between cases and controls (137). So far, to my knowledge, no intervention studies have been reported to determine whether depletion or supplementation with vitamin K affected cognitive function or prevented brain atrophy.

Evidence of a role for "quasi-vitamins" in providing protection against dementia is available: 1) myo-inositol and scyllo-inositol were observed to have a capacity to inhibit $\beta$-secretase-1, which is required to convert APP to amyloid-$\beta$ or amyloid-$\beta$ oligomerization (138, 139), 2) choline has a role as precursor of the neurotransmitter acetylcholine, as a methyl donor in 1-carbon metabolism, and as a critical component of membrane phospholipids, and evidence shows that specific choline-containing phospholipids such as CDP-choline and choline alphoscerate improve the cognitive abilities of patients affected by neurodegenerative diseases (140, 141), and 3) carnitine functions to transport acetyl
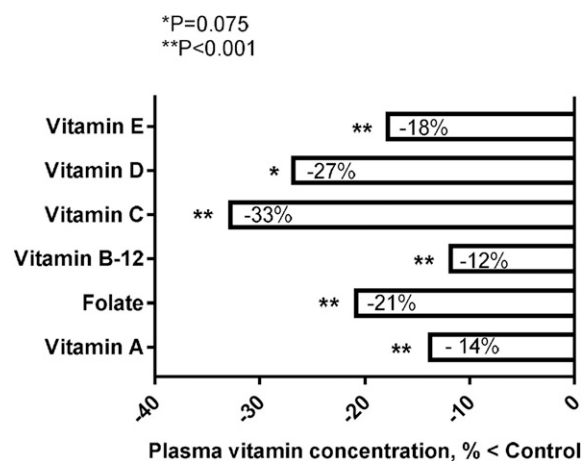


**FIGURE 3** Results of a meta-analysis of 106 investigations showing statistically significant reductions in the concentration of 6 key vitamins in plasma of patients with AD relative to healthy controls (120). The meta-analysis only included studies that used established criteria for identifying AD cases and cognitively intact controls. Data were adapted from reference 120 with permission. AD, Alzheimer disease.

groups from FAs into mitochondria for ATP generation, has a role in acetylcholine synthesis, and has reported restorative effects on mitochondrial function, physical activity, and memory in older rats (142, 143). Furthermore, a double-blind placebo-controlled trial showed that 1-y supplementation with acetyl-carnitine protected against decline in 13 of 14 measures of cognitive and functional performance in MCI and AD (144)

## Summary and Conclusions

Thirteen vitamins and 3 quasi-vitamins play a substantial role in ≥1 of 6 relevant core pathways or pathologies associated with AD (**Figure 4**). The level of evidence for each of these vitamins varies considerably (**Figure 5**), and in most cases it may be insufficient to make specific recommendations. Although it is notable that some vitamins, such as folate and vitamin B-12, are involved as cofactors in ≥5 core pathways or pathologies, it remains challenging to identify and test a combination of vitamins that would best contribute to the prevention of dementia in a precise and predictable manner at the individual level. Furthermore, several outstanding questions remain:

- Which vitamins and which pathways should be prioritized for further research?
- Should more effort be invested in replication studies, especially for some of the vitamins for which mechanistic plausibility exists but only minimal preliminary data are available from humans?
- The homocysteine and B vitamins in cognitive impairment intervention suggest that supplementation with just 3 B vitamins targeted to a relevant pathway (i.e., 1-carbon metabolism) may contribute to slowing cognitive decline in the early stages of dementia. Could effects be improved further by including other vitamins targeting other pathways, or would this be counterproductive?
- Efficacy is likely to be modest unless dosages are adequate and combinations are personalized based on need, metabolic

| Vitamins and "Quasi-Vitamins" | One-carbon metabolism HCY MMA | DNA oxidation DNA repair DNA synthesis DNA damage | Mitochondrial function, ATP synthesis, Glucose metabolism | Lipid and phospholipid metabolism and/or myelination | Neurotransmitter synthesis and/or synaptogenesis | Amyloidosis and/or Tau phosphorylation |
|---|---|---|---|---|---|---|
| Vitamin A | | ■ | | | | |
| Thiamine | | | ■ | | | ■ |
| Riboflavin | ■ | | ■ | | | |
| Niacin | | ■ | | | | |
| Choline | ■ | | | ■ | ■ | |
| Pantothenic acid | | | ■ | ■ | | |
| Vitamin B-6 | ■ | ■ | | | | |
| Biotin | | | ■ | | ■ | |
| Inositol | | | | | | ■ |
| Folate | ■ | ■ | ■ | | ■ | ■ |
| Vitamin B-12 | ■ | ■ | ■ | | ■ | ■ |
| Vitamin C | | ■ | | | | |
| Vitamin D | | | | | ■ | ■ |
| Vitamin E | | | | ■ | | |
| Vitamin K | | | | ■ | | |

**FIGURE 4** The 6 metabolic pathways or pathologies associated with Alzheimer disease risk and the various vitamins that are required as cofactors in these pathways or that influence the severity of brain pathology (black cells). HCY, homocysteine; MMA, methylmalonic acid.

**FIGURE 5** An overview of the current level of evidence of the association of low dietary intake or low blood concentration of various vitamins with risk for AD or cognitive dysfunction, brain pathology (e.g., brain atrophy, low glucose metabolism, and amyloid plaques), and DNA damage. Dark gray shading indicates substantial evidence, light gray shading indicates limited evidence, and no shading indicates insufficient or no evidence. AD, Alzheimer disease; Cog, cognitive function; conc., concentration.

| Association with low dietary intake | | | Vitamins or "Quasi-vitamins" | Association with low blood conc. | | |
|---|---|---|---|---|---|---|
| DNA Damage | Brain Pathology | AD Risk Low Cog | | AD Risk Low Cog | Brain Pathology | DNA Damage |
| | | | Vitamin A | | | |
| | | | Thiamine | | | |
| | | | Riboflavin | | | |
| | | | Niacin | | | |
| | | | Choline | | | |
| | | | Pantothenic acid | | | |
| | | | Vitamin B-6 | | | |
| | | | Biotin | | | |
| | | | Inositol | | | |
| | | | Folate | | | |
| | | | Vitamin B-12 | | | |
| | | | Vitamin C | | | |
| | | | Vitamin D | | | |
| | | | Vitamin E | | | |
| | | | Vitamin K | | | |

phenotype, and genotype. Which combinations and doses should be used and for which subgroups?

- When and for how long should we intervene? The timing of intervention should be as early as possible before symptoms of MCI become evident, but how soon before remains unclear. Is 2 y the minimum intervention period to see a reliable effect on reversal of brain pathology and cognitive decline? Will it be enough to predict that if a benefit occurs in an individual or subgroup it will be reliably sustained in the long-term?
- Detrimental interaction effects are also possible, suggesting the importance of including biomarkers of safety in intervention trials. Which biomarkers should be used to ensure that no harm is done? Would biomarkers of DNA damage be sufficient for this purpose, or would a more comprehensive pathology assessment be required?

In conclusion, future research should focus much more on a precision nutrition approach. Multiple omics technologies and bioinformatics, in combination with brain imaging and cognitive function tests, will be required to both design and test multivitamin combinations (nutriomes) that are tailored appropriately to match the metabolic phenotype and genotype of individuals and subgroups.

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