



REVIEW

# Potential Neuroregenerative and Neuroprotective Effects of Uridine/Choline-Enriched Multinutrient Dietary Intervention for Mild Cognitive Impairment: A Narrative Review

Barry S. Baumel · P. Murali Doraiswamy · Marwan Sabbagh · Richard Wurtman

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## ABSTRACT

In mild cognitive impairment (MCI) due to Alzheimer disease (AD), also known as prodromal AD, there is evidence for a pathologic shortage of uridine, choline, and docosahexaenoic acid [DHA]), which are key nutrients needed by the brain. Preclinical and clinical evidence shows the importance of nutrient bioavailability to support the development and maintenance of brain structure and function in MCI and AD. Availability of key nutrients is limited in MCI, creating a distinct nutritional need for uridine, choline, and DHA. Evidence suggests that metabolic derangements associated with ageing and disease-related pathology can affect the body's ability to generate and

utilize nutrients. This is reflected in lower levels of nutrients measured in the plasma and brains of individuals with MCI and AD dementia, and progressive loss of cognitive performance. The uridine shortage cannot be corrected by normal diet, making uridine a conditionally essential nutrient in affected individuals. It is also challenging to correct the choline shortfall through diet alone, because brain uptake from the plasma significantly decreases with ageing. There is no strong evidence to support the use of single-agent supplements in the management of MCI due to AD. As uridine and choline work synergistically with DHA to increase phosphatidylcholine formation, there is a compelling rationale to combine these nutrients. A multinutrient enriched with uridine, choline, and DHA developed to support brain function has been evaluated in randomized controlled trials covering a spectrum of dementia from MCI to moderate AD. A randomized controlled trial in subjects with prodromal AD showed that multinutrient intervention slowed brain atrophy and improved some measures of cognition. Based on the available clinical evidence, nutritional intervention should be considered as a part of the approach to the management of individuals with MCI due to AD, including adherence to a healthy, balanced diet, and consideration of evidence-based multinutrient supplements.

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B. S. Baumel (✉)  
Department of Neurology, Miller School of  
Medicine, University of Miami, Miami, FL, USA  
e-mail: b.baumel@miami.edu

P. M. Doraiswamy  
Department of Psychiatry and Behavioral Sciences,  
Duke University School of Medicine, Durham, NC,  
USA

M. Sabbagh  
Lou Ruvo Center for Brain Health, Cleveland Clinic,  
Las Vegas, NV, USA

R. Wurtman  
Department of Brain and Cognitive Sciences,  
Massachusetts Institute of Technology, Cambridge,  
MA, USA

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## KEY SUMMARY POINTS

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In Alzheimer disease (AD) and mild cognitive impairment (MCI) due to AD, there is strong evidence for a pathologic shortage of uridine, choline, and docosahexaenoic acid (DHA).

While attention to improving nutrition is strongly recommended in the management of MCI, changes to normal diet alone cannot correct the shortage of uridine observed in the plasma and brains of individuals with dementia.

Uridine and choline work synergistically with DHA to increase phosphatidylcholine formation, and there is a compelling rationale to combine these nutrients to provide neuroprotection and promote neurogenesis.

Clinical evidence from randomized controlled trials suggests that the use of a uridine-, choline-, and DHA-enriched multinutrient product may have a role in the management of individuals with MCI due to AD.

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## DIGITAL FEATURES

This article is published with digital features, including a summary slide, to facilitate understanding of the article. To view digital features for this article go to <https://doi.org/10.6084/m9.figshare.13312946>.

## INTRODUCTION

According to the diagnostic criteria developed by the National Institute on Aging-Alzheimer's Association (NIA-AA) [1], mild cognitive impairment (MCI) may be differentiated from dementia by maintenance of functional

independence and the absence of significant impairment in social or occupational functions [2]. The NIA-AA criteria also define 'MCI due to Alzheimer's disease (AD)' to describe individuals who are symptomatic and have evidence of AD pathology prior to a diagnosis of dementia [1]. Individuals with MCI due to AD (the prodromal stage of AD, as defined using the International Working Group [IWG]-1 criteria [3]) are on a clinical pathway towards overt dementia. These individuals typically have mild cognitive and functional impairments, and pathologic changes shown by biomarkers [2–4]. Disease progression from MCI to AD is characterized by increasingly debilitating memory loss and cognitive impairment [5]. Worsening clinical symptoms correlate with a net loss of synapses [6], resulting from increased breakdown of existing synapses and reduced formation of new synapses [7]. These ominous pathophysiological changes begin even before the disease manifests clinically [6], and signal a need for early intervention [8, 9]. In MCI due to AD, there is an unmet medical need to stimulate the process of synapse formation (neuroregeneration) and to reduce neuronal loss and/or mitigate the adverse effects of neuronal breakdown products (neuroprotection) [10].

Pharmacologic approaches targeting synaptic dysfunction have been reviewed by other authors [10–14]. We wished to consider the challenge from a different perspective, looking at the importance of nutrient substrates involved in the metabolic pathways leading to synaptogenesis [15]. Evidence suggests that substrates needed simultaneously for the Kennedy/phosphatidylcholine (PC) pathway [16], namely uridine, choline, and docosahexaenoic acid (DHA), have important neuroregenerative and neuroprotective functions in the central nervous system (CNS) [17, 18]. In this review, we examine the evidence for a disease-related shortage in the bioavailability of uridine, choline, and DHA, and evaluate the potential for increasing brain levels of these nutrients to improve long-term outcomes in MCI due to AD. Other authors have highlighted the potential of dietary and nutritional intervention for MCI due to AD, while noting the limited evidence supporting effectiveness, particularly for single-

agent nutrients [19–22]. It is not our intention to recapitulate previous comprehensive reviews; instead, we focus on uridine and choline in MCI due to AD, and highlight the particular challenge of correcting the shortfall in uridine availability.

## METHODS

We searched the PubMed database in May 2020 using various combinations of the following search terms: ‘mild cognitive impairment’, ‘Alzheimer’s disease’, ‘prodromal Alzheimer’s disease’, ‘uridine’, ‘choline’, and ‘docosahexaenoic acid’. The primary focus of the search was to identify studies in human subjects with MCI. In addition, we included nonclinical studies investigating the effects of nutrient interventions on neuronal structure and function. We selected the most relevant articles based on our knowledge of the field. The specific objectives of the literature review were to assess evidence showing changes in the levels of uridine and choline in patients with MCI and AD; the neurologic consequences of nutrient shortages; the possible neuroregenerative and neuroprotective effects of increasing nutrient supply; and outcomes data from controlled clinical trials investigating single or multinutrient supplements in patients with MCI. We considered the available evidence supporting the hypothesis that a shortage of specific nutrients leads to an inability to increase neuronal membrane formation to counteract the net loss of synapses occurring in MCI.

### Compliance with Ethics Guidelines

This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors. All clinical trials cited in this review provided ethical declarations in the original publications and were conducted in compliance with the Declaration of Helsinki.

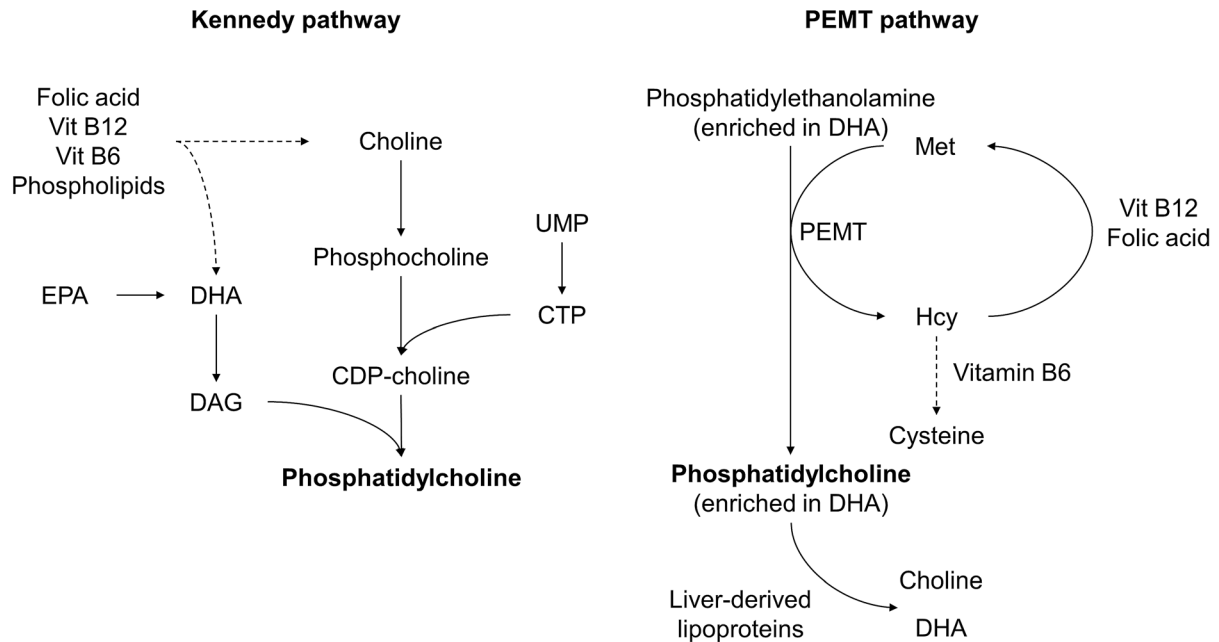
## URIDINE AND CHOLINE ARE CRUCIAL MOLECULES FOR BRAIN FUNCTION

### Uridine

Uridine is the major form of pyrimidine nucleoside taken up by the brain, where it is used in nucleic acids and for the synthesis of membrane constituents [18]. In addition, uridine is a biologically active molecule in the brain with apparent roles in several CNS functions including memory and neuronal plasticity (reviewed in [18]). The effects of uridine on brain structures and functions appear to be mediated by its effects in promoting neuronal membrane formation and through interactions with specific uridine-nucleotide receptors (brain P2Y2 receptors) that control neuronal differentiation [15, 18, 23]. It has been suggested that activation of P2Y2 receptors by uridine triphosphate (UTP), released as a neurotransmitter from presynaptic terminals [24], could have a neuroprotective effect in neurodegenerative diseases such as AD [25]. Furthermore, UTP may be converted to cytidine triphosphate (CTP), which is a key intermediate used in the Kennedy cycle to generate PC for the synthesis of neuronal membranes (Fig. 1) [15]. Considering the important role that uridine has in brain structure and functions, it is not surprising that shortages in uridine supply can lead to neurological symptoms [26].

### Choline and DHA

Choline is an essential micronutrient that is required for normal brain development and cognitive functions throughout life [27, 28]. Choline modulates the expression of key genes related to memory, learning, and cognitive functions via epigenetic mechanisms [27]. The central importance of the cholinergic system in the pathophysiology of dementia has been reviewed extensively [29]. Choline is a limiting precursor of the neurotransmitter acetylcholine (ACh) [27]. Cholinergic deficit is a hallmark of AD [29, 30], and changes may be evident from the early stages of disease [31]. However, in MCI



**Fig. 1** Pathways of phosphatidylcholine synthesis. *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid, *DAG* diacylglycerol, *UMP* uridine monophosphate, *CTP*

cytidine triphosphate, *CDP-choline* cytidine diphosphate-choline, *Hcy* homocysteine, *Met* methionine, *UMP* uridine monophosphate, *Vit* vitamin

and early AD, cognitive deficits are not directly associated with cholinergic system loss, and research suggests that compensatory upregulation of choline acetyltransferase (ChAT) activity could be important in mitigating the progression of MCI to AD [32].

Drug therapy to increase cholinergic neurotransmission is standard in the symptomatic management of AD [33] and may be used in some individuals with MCI, despite a lack of strong evidence [5]. An alternative approach to counter deficits in the cholinergic system could be to improve the supply of choline and other substrates. As a key substrate of metabolic pathways (Kennedy and phosphatidylethanolamine *N*-methyltransferase [PEMT]) involved in the generation of PC [34, 35], choline is needed, together with DHA and uridine, for the synthesis of neuronal membranes (Fig. 1) [17].

Phospholipid abnormalities, consistently affecting PC species with five or six double bonds, for example PC-DHA [28], are well documented in the brains [36–50] and cerebrospinal fluid (CSF) [51–53] of patients with AD, and these changes are reflected in the

plasma [54–70]. Disturbed phospholipid metabolism is evident early in the disease process and is observed in individuals with MCI [49, 50, 68, 71–73]. Studies in patients with AD have shown lower levels of PC-DHA, which is associated with faster cognitive decline than in control subjects [74–76], whereas the highest level of plasma PC-DHA was associated with a significant reduction in the risk of developing all-cause dementia in the Framingham Heart Study [77].

## AVAILABILITY OF KEY NUTRIENTS IS LIMITED IN MCI, CREATING A DISTINCT NUTRITIONAL NEED FOR URIDINE AND CHOLINE

Previously, two systematic meta-analyses have shown that patients with AD have significantly lower plasma and brain levels of specific nutrients, including DHA and choline-containing lipids, compared with age-matched controls with normal cognitive function [78, 79]. Studies have shown that levels of uridine are lower in

the plasma and/or brains of patients with AD compared with age-matched healthy controls [80–88]. These changes occur in very mild AD even in the absence of protein/energy malnutrition [82]. In addition, metabolomic analyses have shown that increased brain cysteine levels associated with decreased uridine can characterize mild AD [80]. The authors suggested that a reduction in uridine in the CSF of patients with AD could mediate reduced synaptic plasticity and neuronal deficits [80].

Metabolomic analyses have also shown significant changes in neurotransmitter metabolism in the ACh pathway in CSF from individuals with AD [88], and in choline and tryptophan pathways in early AD [89]. High levels of homocysteine are observed in patients with AD, which can impair choline synthesis by interfering with the activity of the PEMT pathway [74]. Therefore, metabolic disturbances affecting the PEMT pathway can reduce the syntheses of PC and ACh.

One cross-sectional study examined levels of uridine, choline, folate, homocysteine, and other substrates in the blood and CSF of 148 individuals with MCI (age  $66 \pm 8$  years, 37% female, mini-mental state examination [MMSE] 26.7) compared with 148 healthy, matched controls (age  $59 \pm 8$  years, 38% female, MMSE 28.3) [83]. The analysis showed that subjects with MCI had significantly lower levels of uridine than controls both in the blood (mean  $\pm$  standard deviation  $3.64 \pm 1.25$  vs  $4.08 \pm 1.50$ , respectively;  $P < 0.05$ ) and in the CSF ( $2.90 \pm 0.60$  vs  $3.07 \pm 0.59$ , respectively;  $P < 0.05$ ). Subjects with MCI also had lower blood and CSF folate, and higher CSF homocysteine concentrations than control subjects (all  $P < 0.05$ ) [83]. Blood and CSF levels of choline were not significantly different between MCI and control groups [83]. The study also included a cohort of 150 patients with AD (age  $66 \pm 7$  years, 37% female, MMSE 20.5). While patients with AD had lower levels of CSF uridine and blood choline (and higher CSF homocysteine) than control subjects, the study showed no differences in blood and CSF levels of these nutrients between subjects with MCI and those with AD [83]. This finding supports the notion that changes in nutrient status start early in the

course of AD [82]. The study also showed that blood levels of uridine, choline, betaine, folate, and homocysteine positively correlated with CSF levels in all groups [83]. However, the authors noted weaker correlations between blood and CSF levels of uridine and folate in subjects with AD than in control subjects, which they suggested could indicate decreased uptake into the brain [83]. The brain cannot synthesize choline, and plasma choline does not freely cross the blood–brain barrier [27]. The availability of choline to the brain may therefore be restricted by age-related changes in transport of plasma choline across the blood–brain barrier [27, 90, 91].

From a clinical perspective, it is important to know whether shortages in these key nutrients correlate with the severity of memory loss and cognitive impairment. A cross-sectional study of elderly subjects, aged 70–74 years, selected independently of their cognitive status, showed that low levels of choline in the plasma are associated with poor cognitive performance [92]. A National Health and Nutrition Examination Survey (NHANES) study found that inadequate intake of micronutrients including choline was significantly associated with lower working memory performance in healthy elderly subjects (aged  $\geq 60$  years) [93]. A prospective study involving a total of 551 individuals with subjective cognitive decline (SCD;  $n = 219$ , age  $61 \pm 8$  years, 47% female), MCI ( $n = 135$ , age  $66 \pm 8$  years, 40% female), or AD-type dementia ( $n = 197$ , age  $67 \pm 8$  years, 50% female) looked at potential nutritional markers associated with clinical progression (defined as progression of SCD to MCI or dementia, progression of MCI to dementia, an increase of  $\geq 1$  point on clinical dementia rating scale or admission to a nursing home or death in subjects with AD, or self-reported progression of cognitive symptoms in all groups) [94]. Clinical progression was observed in 25 (11%) subjects with SCD, in 45 (33%) with MCI, and in 100 (51%) with AD. Preliminary results showed that clinical progression was associated with higher levels of low-density lipoprotein cholesterol in subjects with SCD (hazard ratio [HR] 1.92; 95% confidence intervals [CI] 1.05–3.52), and with lower levels of uridine in subjects with AD (HR

0.78; 95% CI 0.62–0.99). Lower levels of uridine were also associated with clinical progression in subjects with a positive amyloid test. Based on these findings, the authors recommended targeting uridine and cholesterol levels in individuals with cognitive decline [94].

In summary, the evidence suggests that metabolic derangements associated with ageing and disease pathology can affect the ability of the body to utilize nutrients and generate brain synapses [80, 87, 88]. This is reflected in lower levels of the nutrients as measured in the blood and brains of individuals with MCI and very mild AD, and progressive loss of cognitive performance.

## INCREASING URIDINE AND CHOLINE AVAILABILITY PROMOTES NEUROREGENERATION AND IS NEUROPROTECTIVE

The metabolic pathways involved in the conversion of uridine to UTP and subsequently to CTP for use in the PC pathway depend on low-affinity enzymes; consequently, providing the brain with uridine will increase the formation of PC [17]. Preclinical experiments have shown that administration of uridine with other key substrates (choline and DHA) stimulates neuroregeneration (reviewed in [95]), increasing the production of synaptic proteins [96–98], the formation of neurites and synapses [98–102], and the levels of neurotransmission [96, 103–105], which in turn may lead to improvements in memory performance [103, 106–109]. Preclinical experiments have also shown that uridine administration may provide neuroprotection [95], evidenced by reducing Aβ production and plaque formation [103, 110], and diminishing neurodegeneration [103, 106, 107, 110]. It is important to note that these neuroprotective effects were observed by administering uridine with other nutrients including choline and DHA. For example, administering a multinutrient containing uridine, choline, and DHA was shown to protect the cholinergic system against

Aβ<sub>42</sub>-induced toxicity in rats [103] and to reduce AD-like pathology in Aβ<sub>42</sub>/PS1 mice [110].

There is evidence from the clinical setting showing that uridine administration may have positive effects on cognitive functions. A controlled study in 17 healthy volunteers showed that administration of uridine increases brain membrane phospholipid precursors (measured using 31-phosphorus magnetic resonance spectroscopy [MRS]) [111]. Another MRS study in healthy volunteers ( $n = 16$ ) showed that administration of cytidine diphosphate-choline (CDP-choline) also affects phospholipid membrane turnover and may increase the availability of phospholipid membrane components needed to synthesize and maintain cell membranes [112].

There is only limited evidence from clinical studies to show that administration of uridine or choline improves cognitive performance. A small clinical trial ( $n = 12$ ) showed that administration of CDP-choline (which increases uridine levels in the brain [113]) improved performance in individuals with relatively inefficient memory [114]. A population-based study in 1391 subjects (aged 36–83 years) free from dementia showed that concurrent choline intake was positively correlated with cognitive function tests and inversely correlated with white-matter hyperintensity volume [115]. Another population study ( $n = 2497$  dementia-free men aged 42–60 years) showed that higher intake of PC was associated with lower risk of incident dementia and better cognitive performance [116]. In the dementia setting, a randomized controlled trial showed that choline alfoscerate decreased cognitive impairment due to mild to moderate AD [117].

## THE NUTRITIONAL NEED FOR URIDINE AND CHOLINE IN MCI CANNOT BE MET WITH A NORMAL DIET OR SINGLE SUPPLEMENTS

Long-term adherence to a healthy diet appears to support cognitive function in ageing

individuals at risk for dementia [20, 118, 119]. Recent research suggests that preventive strategies including diet, exercise, cognitive training, and vascular risk monitoring may be more effective if started early, before pronounced structural brain changes develop [120].

McGrattan and colleagues performed a systematic review of randomized controlled trials of dietary interventions (dietary pattern or supplements) in subjects with any form of MCI diagnosed by a physician according to internationally accepted criteria [121]. The literature search done in June 2016 identified 16 trials, including one using a multinutrient intervention containing uridine, choline, and DHA [122]. The authors reported inconsistent findings among the heterogeneous studies, which overall did not provide clear evidence to support any particular dietary intervention to improve cognitive function in MCI, or evidence of a significant effect on progression from MCI to dementia [123]. Our literature search did not identify any more recent clinical studies of uridine or choline supplementation in subjects diagnosed with MCI due to AD.

The apparent nutritional need in MCI due to AD cannot be addressed simply by modifying the normal diet or administering multivitamin/mineral supplements, as these may unnecessarily increase the intake of other nutrients associated with increased risk of dementia (e.g. cholesterol, trans fatty acids, saturated fat, and vitamin A) [124, 125]. Dietary modifications to address shortages of uridine and choline in individuals with MCI due to AD appear to be particularly challenging. Uridine obtained from dietary sources is unavailable to the adult brain (due to degradation by the liver) [126], while food substances purported to increase uridine levels, such as beer [127], are impractical and potentially harmful. As an essential nutrient, choline must be obtained from the diet. Although available from many dietary sources, it is estimated that up to 90% of Americans consume below the adequate intake for choline [128].

Dietary supplements have been suggested to increase levels of specific nutrients in individuals with MCI and AD [28, 46, 113, 129, 130]; however, to date, nutrient intervention studies

have shown that while single-agent supplements are effective in elevating plasma levels, they generally fail to demonstrate clinical benefits [131–134]. We found only limited evidence from randomized controlled clinical trials to support single-agent supplementation with uridine, choline (or CDP-choline), or DHA in MCI due to AD or probable AD [121, 135–138]. As uridine and choline work synergistically with DHA to increase PC formation, there is a compelling rationale for combining these nutrients [139].

## CLINICAL EVIDENCE FOR URIDINE- AND CHOLINE-ENRICHED MULTINUTRITIONAL INTERVENTION IN MCI DUE TO AD

A specific uridine-, choline-, and DHA-enriched multinutrient (Souvenaid; Nutricia) has been developed to support synapse formation in patients with AD and MCI due to AD (Table 1). The first randomized controlled clinical trials of this multinutrient were conducted in patients with mild–moderate AD because of the high medical and nutritional needs in this population [140–143]. An early trial of the product in 527 patients with mild–moderate AD dementia (MMSE 19.5, receiving drug therapy for AD) showed no significant cognitive improvements over a 24-week intervention period [143]. The authors speculated that patients with moderate AD may have progressed to such an extent that neuronal damage and synaptic dysfunction was irreversible and not responsive to either pharmacologic or non-pharmacologic interventions. They suggested that the potential to benefit from multinutritional interventions to increase synaptogenesis may be limited in moderate AD compared with mild AD because of the higher levels of neurodegeneration [143]. Two further clinical trials showed that the multinutrient was associated with a statistically significant improvement in memory in patients with mild and very mild AD dementia (MMSE 23.9 [141] and MMSE 25 [142]) over 12–48 weeks, respectively [140–142]. Since the data implied effects were most likely to be achieved at the early end

**Table 1** Randomized controlled studies of uridine- and choline-enriched nutrient intervention in subjects with a diagnosis of dementia

References	Subjects	Intervention	Cognitive outcomes	Other outcomes
Prodrormal AD LipidiDiet [122, 144, 146]	<i>n</i> = 311 (average MMSE 26.6)	Active* vs control for 24 (+ 12 months)	NTB (5-item): no difference at 24 months; significant reduction in decline at 36 months  CDR-SOB: significant reduction in decline at 24 and 36 months  ADCOMS: significant reduction in decline at 24 months	Significant reduction of MRI- assessed hippocampal atrophy and less expansion of ventricular volume
Mild AD Souvenir II [140, 142]	<i>n</i> = 259 (average MMSE 25)	Active* vs control for 24 (+ 24 weeks)	NTB: Z-score significantly increased over 24-week intervention period	EEG measures of brain functional connectivity improved  Significant increase in erythrocyte DHA and EPA, plasma vitamin E, and decrease in plasma Hcy
Souvenir I [141]	<i>n</i> = 225 (average MMSE 23.9)	Active* vs control for 12 (+ 12 weeks)	WMS-r delayed verbal recall: significant improvement at 12 weeks  ADAS-Cog (13-item): no change	Significant increase in erythrocyte DHA and EPA, plasma vitamin E, and decrease in plasma Hcy
Mild–moderate AD S-Connect [143]	<i>n</i> = 527 (average MMSE 19.5)	Active* vs control for 24 weeks	ADAS-Cog (11-item), ADCS-ADL, and CDR-SOB: no significant differences between study groups	Significant increase in erythrocyte DHA and EPA, plasma vitamin E, and decrease in plasma Hcy

*ADAS-cog* Alzheimer's Disease Assessment Scale—cognitive subscale, *ADCOMS* Alzheimer Disease Composite Score, *ADCS-ADL* Alzheimer's Disease Co-operative Study—Activities of Daily Living, *CDR-SOB* Clinical Dementia Rating Sum of Boxes, *DHA* docosahexaenoic acid, *EEG* electroencephalography, *EPA* eicosapentaenoic acid, *Hcy* homocysteine, *MMSE* Mini-Mental State Examination, *MRI* magnetic resonance imaging, *NTB* Neuropsychological Test Battery, *WMS-r* Wechsler Memory Scale—revised

\*Active group took a once-daily multinutrient drink containing uridine monophosphate (625 mg), choline (400 mg), docosahexaenoic acid (1200 mg), eicosapentaenoic acid (300 mg), vitamin C (80 mg), vitamin E (40 mg), folic acid (400 mcg), vitamin B6 (1 mg), vitamin B12 (3mcg) selenium (60mcg), and phospholipids (106mcg)



of the AD spectrum, the LipiDiDiet study was designed to test multinutrient intervention in patients with MCI due to AD (prodromal AD) [122].

The LipiDiDiet study was a randomized, controlled, double-blind, parallel-group multicentre trial in 311 subjects with MCI due to AD (MMSE 26.6) [122], as defined by episodic memory disorder and evidence for underlying AD pathology [3]. Subjects were randomly assigned (1:1) to receive Souvenaid or a matched control product, taken every day for 24 months [122], with the option to enter an extension study period [144]. The primary endpoint was a change in a neuropsychological test battery (NTB; composite z-score based on Consortium to Establish a Registry for Alzheimer's disease [CERAD] 10-word list learning immediate recall, CERAD 10-word delayed recall, CERAD 10-word recognition, category fluency, and letter-digit substitution test). The authors noted that cognitive decline in the LipiDiDiet study population was much lower than expected in both groups, so the primary endpoint was inadequately powered; no significant effect on the primary endpoint was found after 24 months. Interestingly, significant effects were observed for secondary endpoints, including Clinical Dementia Rating scale–Sum of Boxes (CDR-SOB) and Alzheimer Disease Composite Score (ADCOMS). The ADCOMS scale provides a composite clinical outcome measure and was designed for use in trials in MCI due to AD and mild AD dementia [145]. A post hoc analysis of data from the LipiDiDiet study showed that during the 24-month intervention period, worsening on ADCOMS was 36% less in the multinutrient group than in the control group; estimated mean treatment difference  $-0.048$  (95% CI  $-0.090$  to  $-0.007$ ;  $P = 0.023$ ) [146]. Magnetic resonance imaging (MRI) analyses also showed a significant reduction of hippocampal atrophy and less expansion of ventricular volume in subjects receiving the multinutrient intervention [122]. In line with previous trials in AD, the LipiDiDiet showed that administration of Souvenaid was well tolerated and had a high rate of adherence [122, 144].

Changes from baseline in the levels of uridine, choline, and other nutrients were not reported in the LipiDiDiet study, so it is not possible to correlate effects on clinical and brain imaging endpoints with an improvement in nutritional status. Previous randomized controlled trials in subjects with mild AD showed that Souvenaid increases levels of uridine, choline, DHA, and other key nutrients involved in PC formation [147, 148] and increases markers of phospholipid synthesis in the brain in subjects with mild AD [147]. These findings support the putative mode of action of the product on synapse formation.

At 24 months, there was no difference between groups in progression to dementia; however, preliminary data with long-term (3-year) intervention suggests a possible effect favoring multinutrient intervention [144]. Longer-term follow-up of the LipiDiDiet study will provide additional insights into the sustainability of effects observed with multinutrient intervention and hopefully elucidate additional information on which patients are most likely to benefit. It will be interesting to see whether expression of the apolipoprotein E4 (APOE4) gene modifies the effects of multinutrient intervention in MCI due to AD. APOE4, a major genetic driver of AD, is associated with decreased transport of DHA to the CSF [149] and appears to influence the effects of DHA supplementation in subjects with AD and MCI [138]. In subjects with early-stage AD, the effect of Souvenaid was assessed in predefined subgroups, including expression of the APOE4 genotype; however, no significant effect was observed [141].

Overall, clinical evidence suggests that a specific uridine- and choline-enriched multinutritional intervention may produce meaningful clinical benefits in MCI due to AD, possibly by addressing a conditional shortage in levels of uridine and other key nutrients essential for neuronal membrane formation. Additional studies are needed to extend the findings of the LipiDiDiet study to the presymptomatic stage of AD, and to correlate improvements in nutrient levels with cognitive benefits.

## CONCLUSIONS

There is strong evidence from systematic reviews and meta-analyses showing a pathologic shortage of uridine and choline in AD, including MCI due to AD, particularly in the levels of these nutrients in the brain or CSF. While the shortfall appears relatively modest, compared with healthy age-matched controls, the impact on the metabolic pathways leading to synapse formation could be significant considering the ongoing loss of synapses that characterizes progression of MCI and AD. The uridine shortage cannot be corrected simply by modifying a normal diet, making uridine a conditionally essential nutrient in affected individuals. As an essential nutrient, choline must be obtained from the diet; however, correcting the shortfall in individuals with MCI through diet alone is challenging, because brain uptake from the plasma significantly decreases with ageing. Dietary supplements have been used to improve outcomes in subjects with MCI, but there is limited evidence of effectiveness for single-agent supplements and a lack of studies specifically in subjects with MCI due to AD. Preclinical research provides a strong rationale for multinutrient intervention providing supplemental uridine and choline alongside other substrates used in the metabolic pathways for PC formation. Administration of these nutrients at the same time has been shown to increase synapse formation and provide neuroprotection in models of dementia. Clinical trials of a specific multinutrient product containing uridine, choline, and DHA have shown that the benefits are most likely to be achieved at the very early stages of the AD spectrum, when there is still a possibility to influence the processes affecting synapse formation and loss. To date, there is no evidence that multinutrient intervention can prevent progression of MCI to AD; however, preliminary brain imaging data does suggest an observable slowing of neurodegeneration.

Based on this review, we recommend that nutritional intervention be considered as a part of the personalized approach to the management of individuals with MCI due to AD,

including adherence to a healthy, balanced diet and consideration of evidence-based multinutrient supplements, as indicated. The selection of a multinutritional intervention should be based on strong evidence in a clearly defined population of subjects with MCI.

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Massachusetts Institute of Technology and licensed to Danone.

**Compliance with Ethics Guidelines.** This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors. All clinical trials cited in this review were done in compliance with the Declaration of Helsinki.

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