

The effects of bioactive compounds on Alzheimer's disease and Mild cognitive impairment

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ABSTRACT

As the prevalence and rate of Alzheimer's disease are increasing around world, identifying effective treatments to manage cognitive impairment and neurodegeneration has become a global health priority. Decades of scientific research have led to a more comprehensive understanding of Alzheimer's Disease and Mild Cognitive Impairment (MCI), a transitional stage of accelerated cognitive decline. This understanding has enabled researchers to envision and develop novel Alzheimer's Disease therapies, including the use of bioactive compounds found in plants and animals. Within the past 15 years, a significant amount of clinical research has been published documenting the effects specific bioactive compounds have on patients with Alzheimer's Disease and MCI. This article reviews the results of this research, along with relevant epidemiological studies, measures of cognition, and disease pathologies to discuss whether sufficient evidence exists to support the use of specific bioactive compounds by individuals diagnosed with Alzheimer's Disease and MCI. The results of clinical trials within the last 15 years do not conclusively prove that the studied quantities of vitamin B, Omega-3 fatty acids, or bioactive compounds within Fortasyn Connect are beneficial for use in the management of Alzheimer's Disease or MCI. Additionally, the documented effects of these bioactive compounds do not warrant development of a functional food product to help manage AD or MCI.

Keywords: Bioactive compounds, Alzheimer's Disease, Mild cognitive impairment, Dementia, Vitamin B, Omega-3 fatty acids, DHA, EPA, Fortasyn Connect, Souvenaid.

BACKGROUND

Alzheimer's disease (AD) is the most common form of dementia and the most prevalent neurodegenerative disease in the world [1]. While cognitive decline is a natural part of aging, AD patients exhibit an unnatural level of cognitive impairment [2]. This impairment is commonly thought of as a manifestation of underlying neurodegeneration, including synaptic

dysfunction and neuron loss. When the mean age of a population increases, the prevalence of AD is also expected to rise because AD is an age-associated disease [1]. Currently the mean age of the entire human population is rising, and AD is already a leading cause of death in developed countries [1, 3]. Global population aging can be seen as one of humanities greatest achievements, but longer life expectancies come with inherent medical demands. Approximately 46.8 million people in the world today have Dementia. By 2030, this number is expected to reach 74.7 million [1]. Future healthcare systems will face an unprecedented demand for AD management. Fortunately, our evolving capacity to understand diet's relationship to AD may help develop novel therapies to address this health issue.

The field of functional food science seeks to understand diet's relationship with AD on a very comprehensive level. Functional food researchers study which compounds within plants and animals are responsible for specific biological effects, in addition to what dosages of these compounds produce specific effects. These compounds are referred to as bioactive compounds. In this article, this term does not apply to compounds derived from chemical synthesis. The safety and price of many bioactive compounds, relative to pharmaceuticals, makes them very interesting candidates to help manage AD. Epidemiological studies also support an association between the risk of developing AD and the intake of specific bioactive compounds. Within recent decades these studies have helped identify three combinations of bioactive compounds as candidates for AD Management. These three bioactive compound combinations include vitamin B, Omega-3 fatty acids, and a multi-nutrient combination named Fortasyn Connect. This article focuses exclusively on the bioactive compounds in these combinations, because unlike other compounds these compounds have been studied in multiple clinical trials that recruited patients with AD and MCI.

MCI is a transitional state between normal cognitive decline and AD [4]. Not all patients with MCI progress to AD, but they are at a significantly higher risk to develop AD and experience the same protein related neurodegeneration as AD patients [4, 5]. Candidate AD therapies have been tested on both MCI and AD patients due to their shared pathomechanisms and accelerated rates of cognitive decline. This article reviews the results of recent clinical trials that utilized the three mentioned bioactive compound combinations to manage cognitive decline and neurodegeneration in patients with AD and MCI. This article also discusses whether the results of these trials supply the necessary evidence for future development of a functional food product.

Retrieval of Published Studies

Electronic searches of published studies were conducted utilizing PubMed®, and EBSCOhost. Both review and research papers were included. No language limitations were imposed. A publication date limitation was imposed when clinical trials were retrieved. All clinical trials searched were limited to following range of years 2000-2016. Keywords for the search included: Alzheimer's Disease, Mild cognitive impairment, Dementia, Clinical trials, vitamin B, Folic acid, B12, B6 Omega-3 fatty acids, DHA, EPA, Fortasyn Connect, Souvenaid, Bioactive food compounds, Epidemiological, Amyloid Beta, Tau, Pathology, Mechanisms, Neurodegeneration, Cognitive examinations, MMSE, ADAS, Neuroimaging, MRI.

Epidemiologic Studies of Patients with Dementia and Cognitive Impairment

Much of the recent AD research involving bioactive compounds is a product of initial epidemiologic findings. In fact, several of the clinical trials we reviewed cited the same studies originally reported these findings [**Error! Bookmark not defined.-Error! Bookmark not defined.**]. These initial studies discovered differences between the plasma profiles and nutrient intakes of demented patients and healthy controls. These differences were uncovered through the utilization of two basic study designs. In the first study design, researchers recorded the characteristics of patients diagnosed with AD and MCI, and compared those characteristics to healthy controls. Differences between group characteristics were thought to be associated with AD and MCI. In studies of this nature, researchers found that AD patients exhibited higher plasma levels of homocysteine, and lower plasma levels of vitamin B and Omega-3 fatty acids when compared to controls [**Error! Bookmark not defined.-13**]. In the second study design, researchers recorded the characteristics of non-diseased individuals, and followed them for a significant amount of time to allow for a portion of the sample to receive an AD or dementia diagnosis. Similarities in characteristics of individuals later diagnosed were investigated as potential risk factors, while similarities in characteristics of individuals who remained healthy were investigated as potential protective factors. In studies of this nature, researchers discovered that participants with lower vitamin B and Omega-3 fatty acid status and higher baseline plasma homocysteine levels were at a significantly higher risk of developing Dementia [**Error! Bookmark not defined., Error! Bookmark not defined.**]. Individuals who consumed fish more frequently or had a higher intake of Omega-3 fatty acids were at a significantly lower risk of developing Dementia [**Error! Bookmark not defined.-Error! Bookmark not defined.**]. Investigators theorized that the high Omega-3 Fatty acid content of fish was responsible for the apparent protective effects of frequent fish consumption. The results of the epidemiological studies listed above have not been reproduced throughout all of the literature, making the epidemiological evidence for the use of bioactive compounds by AD and MCI patients controversial [6-8].

Possible neurodegenerative mechanisms in Alzheimer's disease and Mild cognitive impairment

All cognitive ability reflects the brain's functionality on a physical level, as this physical level is where neurodegeneration from AD and MCI pathology occurs. Our current understanding of many neurodegenerative mechanisms can be traced back to the psychiatrist Alois Alzheimer. In 1906, Alzheimer identified two types of insoluble protein aggregates, now known as Amyloid Beta ($A\beta$) plaques and Neurofibrillary tangles (NFT), in the brain of a cognitively impaired patient [9]. Since Alzheimer's initial discovery, over a hundred years of research has further developed our understanding of these disease related proteins. Amyloid Beta ($A\beta$) plaques predominantly consist of $A\beta$ protein, and NFT's predominantly consist of tau protein, today both $A\beta$ and tau proteins are considered central to AD and MCI pathology. Healthy neurons produce $A\beta$ and tau proteins, but abnormal events render these proteins neurotoxic [20, 21]. Each of these proteins have a monomeric, oligomeric and fibrillar form which behave in distinctly different

ways. It is easier to explain the roles these proteins play in disease related neurodegeneration separately as both proteins undergo separate aggregation processes.

The three forms of A β protein relevant to AD are A β monomers, A β oligomers and A β plaques. All three of these forms contain A β protein, which was initially created from the proteolysis of Amyloid precursor protein (APP) [Error! Bookmark not defined., 22]. A β monomers are proteolytically cleaved off APP into the extracellular space of a neuron [Error! Bookmark not defined., Error! Bookmark not defined.]. The creation and subsequent clearance of A β monomers occurs in healthy brain tissue, however, in diseased brain tissue; an imbalance between A β monomer production and clearance leads to an excess amount of A β monomers. Un-cleared or overproduced A β monomers may then aggregate into toxic A β oligomers [Error! Bookmark not defined., 13]. The oligomeric form of A β protein is considered by many to be the most neurotoxic form of A β protein. A β oligomers travel to the intercellular space of neurons and disrupt cellular processes, which leads to cell death [Error! Bookmark not defined., Error! Bookmark not defined.]. A β oligomers that remain in the extracellular space and aggregate form large masses of A β protein called A β plaques [Error! Bookmark not defined.-Error! Bookmark not defined.]. A β plaques are insoluble forms of the A β protein that cannot move into the intercellular space of a neuron. [Error! Bookmark not defined., Error! Bookmark not defined.].

The second protein central to AD and MCI pathology is the tau protein. Three AD-related forms of tau protein include hyper-phosphorylated tau monomers, tau oligomers, and neurofibrillary tangles (NFT). Tau protein is often referred to as a microtubule associated protein, because tau protein stabilizes the structure of microtubules in the axon of a neuron [24-17]. In the axon of diseased neurons, tau protein becomes abnormally phosphorylated and dislocates from the microtubules, generating untethered tau monomers [Error! Bookmark not defined.-18]. Current theories associate this dislocation with microtubule dysfunction, leading to mitochondrial dysfunction, synaptic dysfunction, and ultimately cell death [Error! Bookmark not defined.-17]. Untethered tau monomers circulating in the intercellular space may aggregate to form tau oligomers [Error! Bookmark not defined., Error! Bookmark not defined.]. Researchers believe the oligomeric form of tau protein can travel to other neurons and propagate the destabilization of tau in foreign axons. The potential for tau oligomers to spread tau pathology is why many consider the oligomeric form of tau to be the most toxic [17]. Tau oligomers that remain inside a neuron's intracellular space may aggregate together to form filaments, which can further aggregate into the large insoluble masses of tau protein known as NFT's [11, 19]. There is not much evidence supporting that NFTs or A β plaques directly contribute to neurodegeneration the way lower order aggregates of tau and A β protein do; nonetheless, they remain some of the most visually pronounced results of AD and MCI pathology [13, 17].

Possible Effects of Bioactive Compounds on Alzheimer's disease and Mild cognitive impairment

There are many theories describing how vitamin B, Omega-3 fatty acids, and the bioactive compounds within Fortasyn Connect, mitigate the neurotoxic effects of both A β and tau protein.

The theory most cited throughout the trials reviewed states that specific bioactive compounds may positively affect the health of neuronal membranes, increasing a neuron's resistance to disease-related neurodegeneration [20, 21]. Neuronal membranes are continuously formed and maintained, requiring an adequate stock of neuronal membrane precursors and cofactors [20]. Hypothetically, a suboptimal stock of compounds could lead to suboptimal neuronal membrane health. Furthermore, an individual with poor neuronal membrane health may sustain more damage from the AD related neurotoxic agents. The bioactive compounds within the vitamin B, Omega-3 fatty acid, and the Fortasyn Connect combinations, are supplemented to increase a deficient individuals stock of neuronal membrane precursors or cofactors and restore adequate neuronal membrane health [20, 21]. Theoretically the restoration of adequate neuronal membrane health will improve the individual's resistance to disease-related neurodegeneration. This theory regarding improved neuronal membrane health and resistance to neurodegeneration has also provided the basis for several bioactive compounds proposed effects on cognition.

Measures of Cognition and Neurodegeneration

The cognitive outcomes of the participants in the reviewed clinical trials were assessed by the use of cognitive examinations. There are two main types of cognitive examination. The first type examines overall cognitive ability by testing a subjects performance in a large set of mental functions such as memory, perception, attention, language, visual and spatial processing, and executive functions [22]. The results of these examinations are used as diagnostic criteria for AD and MCI. One of the most widely used overall cognitive examinations in clinical research is the Mini-Mental State Examination (MMSE). The MMSE assesses a subject's attention, memory, language and several other mental abilities through a 30 point questionnaire to determine an overall evaluation of mental state [22]. Many of the studies we reviewed utilized MMSE scores as inclusion/exclusion criteria and/or a measurable cognitive outcome. Another commonly used overall cognitive examinations is the Alzheimer's Disease Assessment Scale–Cognitive subscale (ADAS-cog). The ADAS-cog is a 11 item evaluation originally designed to assess cognitive impairment in AD patients [23]. Since its inception the function of the ADAS-cog has expanded, assessing cognitive impairment at levels insufficient to bring about a AD diagnosis. ADAS-cog scores are currently being utilized to determine Mild Cognitive Impairment [23]. The second type of cognitive examination tests a subject's performance on one or few specific cognitive functions. Studies often use this type of examination, in addition to those of overall cognitive ability, to more comprehensively assess a subject's cognitive abilities. Many of the specific cognitive function examinations have non-intuitive abbreviated names. Throughout the text the specific cognitive function being examined is displayed adjacent to these abbreviations in parenthesis. Neither type of cognitive examination quantifies physical neurodegeneration, but instead are believed to record the cognitive ramifications of neuronal damage.

Throughout the clinical trials reviewed, the only assessment used to quantify physical neurodegeneration was Magnetic Resonance Imaging (MRI). MRI's create a detailed structural image of the brain providing valuable information regarding the effects of AD and MCI pathology on specific structures [24]. Although MRI's cannot detect the presence of A β plaques and NFTs, they can be used to both precisely measure reductions in brain matter volume, and calculate rates of brain atrophy. The volumetric reductions of brain matter seen in patients with

AD and MCI is thought to predominantly reflect the loss of neurons and dendrites [24]. The ability for MRI's to show physical degeneration has been exceedingly valuable in clinical research [25].

Vitamin B in Clinical Trials

The vitamin B nutrient combination consists of three bioactive compounds: B6, B12, and folic acid. In the past 15 years, five research papers were published that documented the effects of B vitamin supplementation patients diagnosed with AD and MCI. The study designs and results of all reviewed vitamin B clinical trials are indexed in Table 1. These investigations measured B vitamin supplementation's, safety and tolerability, capacity to decrease plasma levels of homocysteine, and effect on disease related cognitive decline and neurodegeneration. It must be mentioned that three of the five research papers investigated the same sample of MCI patents, denoted in table one by the term VITACOG trial [26-28].

Vitamin B supplementation was not associated with serious adverse effects throughout the majority of the literature. However, one trial reported a higher number of depression related adverse effects in the vitamin B group [30]. Two separate clinical trials successfully reduced the levels of plasma homocysteine with vitamin B supplementation. The VITACOG trial's invention consisting of 0.5 mg/d of B12, 20 mg/d of B6, and 0.8 mg/d of folic acid reduced plasma homocysteine (tHcy) levels by 23% [26]. The Aisen et al RCT's invention consisting of 1 mg/d of B12, 25 mg/d of B6, and 5 mg/d of folic acid reduced plasma tHcy levels by 27% [29]. The VITACOG trial produced the only positive effects on cognitive performance of the trials reviewed. Independent of baseline plasma tHcy levels the VITACOG trial's intervention reportedly stabilized CLOX test (executive function) performance, but did not improve performance in measures of global cognitive function, memory, or clinical status [27]. The intervention group of the VITACOG trial also demonstrated reductions in the rate of whole brain atrophy and total 2-year gray matter loss independent of baseline plasma tHcy levels [26,28]. VITACOG trial participants taking vitamin B with higher than median baseline plasma tHcy level exhibited greater cognitive improvements and greater reductions in brain atrophy. Despite the positive cognitive effects seen within the VITACOG trial's vitamin B group, no positive effects were seen on measures of cognition in the other two clinical trial samples [Error! Bookmark not defined.,30]. No other trial reviewed besides the VITACOG trial measured brain atrophy outcomes.

Table 1. The effects of vitamin B on AD and MCI

Study	Participants	Intervention	Duration	Outcome
Smith AD, 2010 [26] Randomized controlled trial	271 individuals aged 70 years old or older diagnosed with MCI	0.5 mg of B12, 20 mg of B6, and 0.8 mg of Folic acid was taken daily.	24 months	A subsample of 168 individuals completed the MRI portion of the trial. Independent of baseline tHcy levels the rate of whole brain atrophy in the vitamin B group was 27.1% less per year compared to control group. The rate of whole brain atrophy in the vitamin B group was 53.3% less per year compared to control group for individuals with baseline tHcy levels of >13 μmol/L After 24 months the vitamin group B experienced a 22.5 % reduction in plasma tHcy levels, and the placebo group

Study	Participants	Intervention	Duration	Outcome
				experienced a increase in plasma tHcy levels of 7.7%.
de Jager CA, 2012 [27] Randomized controlled trial	VITACOG 266 individuals aged 70 years old or older diagnosed with MCI	VITACOG 0.5 mg of B12, 20 mg of B6, and 0.8 mg of Folic acid was taken daily.	24 months	Independent of baseline tHcy, vitamin B stabilized CLOX Test performance, but did not improve performance in measures of global cognitive function, episodic memory, semantic memory, or clinical status. In subjects with a baseline plasma tHcy level of $\geq 11.3 \mu\text{mol/L}$, vitamin B supplementation had a beneficial effect on measures of episodic memory, semantic memory, and global cognitive function. In subjects with a baseline plasma tHcy level of $\geq 13.1 \mu\text{mol/L}$, vitamin B supplementation improved clinical status (CDR and IQCODE) compared to controls.
Douaud G, 2013 [28] Randomized controlled trial	VITACOG 156 individuals aged 70 years old or older diagnosed with MCI	VITACOG 0.5 mg of B12, 20 mg of B6, and 0.8 mg of Folic acid was taken daily.	24 months	Subjects with a baseline plasma tHcy level of $\leq 11.6 \mu\text{mol/L}$, received no benefit from vitamin B supplementation on the volume of total grey matter lost. Vitamin B supplementation significantly reduced the amount of grey matter atrophy compared to placebo in subjects with a baseline plasma tHcy level of $> 11.6 \mu\text{mol/L}$. Within this higher baseline plasma tHcy subsample, subjects taking the placebo sustained an average total gray matter volume loss of 5.2% and subjects taking vitamin B showed an average total gray matter volume loss of 0.6%.
Aisen PS, 2008 [29] Randomized controlled trial	409 individuals diagnosed with mild to moderate AD.	1 mg of B12, 25 mg of B6, and 5 mg of Folic acid was taken daily.	18 months	Vitamin B supplementation had no beneficial effect on subjects ADAS-cog, MMSE, and CDR-SOB scores. After 18 months the vitamin B group experienced a 27 % reduction in plasma tHcy levels, and the placebo group experienced a 7 % reduction in plasma tHcy levels.
van Uffelen J, 2008 [30] Randomized controlled trial	152 individuals aged between 70 and 80 years diagnosed with MCI	0.4 mg of B12, 50 mg of B6, and 5 mg of Folic acid was taken daily.	12 months	Vitamin B supplementation had no beneficial effect on measures of memory, attention, executive function, and information processing. Vitamin B's effect on plasma tHcy levels was not analyzed.

Omega-3 Fatty Acids in Clinical Trials

The Omega-3 Fatty Acid combination that was utilized the most to manage AD consisted of two bioactive compounds, docosahexanoic acid (DHA) and eicosapentaenoic acid (EPA). In the past 15 years, there have been six comparable clinical trials that document the effects that Omega-3 Fatty Acid supplementation has on patients diagnosed with AD and MCI. The study designs and results of all reviewed Omega-3 fatty acid clinical trials are indexed in Table 2. These investigations measured Omega-3 Fatty Acid supplementation's, safety and tolerability, capacity to increase DHA and EPA status, and effect on disease related cognitive decline and neurodegeneration. It should be noted that the Quinn et al 2010 randomized controlled trial (RCT) was supplemented with only DHA. All other trials utilized both DHA and EPA in their investigations [31].

Throughout the majority of studies reviewed, Omega-3 fatty acid supplementation was described as well tolerated and safe. The few adverse effects reported across these studies were

described as mild and gastrointestinal by nature [33, 35]. Omega-3 fatty acid supplementation increased plasma, serum, or erythrocyte levels of DHA and EPA in several different dosages. Additionally, the Quinn et al 2010 RCT reported that a 2000 mg supplement 45-55% DHA by weight, significantly increased the levels DHA within the cerebrospinal fluid [31]. The amount of DHA and EPA given in these trials varied; daily doses of DHA ranged between 650 mg-1700 mg, and daily doses of EPA ranged between 450 mg-1080 mg [31-36]. Although Omega-3 fatty acid supplementation had positive effects on subject’s DHA and EPA status, it failed to produce positive effect on the scores of overall cognition, independent of baseline cognitive impairment [31-36]. This lack of effect on cognition was especially significant, because it was reproduced in several different trials utilizing the ADAS-cog and MMSE examinations [31-36]. Only subsamples with a lower level of baseline cognitive impairment showed improvements in overall cognitive ability [34, 35]. Independent of baseline cognitive impairment, Omega-3 fatty acid did have a beneficial effect on measures of specific cognitive function [33-35]. Unfortunately, none of these specific cognitive function improvements were reproduced utilizing the same test in more than one study. Finally the Quinn et al 2010 RCT tested DHA supplementation’s effect on the rates of brain atrophy, but no difference was seen between the DHA intervention group and the placebo group [31].

Table 2. The effects of Omega-3 fatty acids on AD and MCI

Study	Participants	Intervention	Duration	Outcomes
Quinn JF, 2010 [31] Randomized, double-blind, placebo-controlled trial	402 individuals diagnosed with mild to moderate AD	2000 g of Algal DHA, (45%-55% DHA by weight) was taken daily.	18 months	DHA supplementation had no beneficial effect on ADAS-cog or CDR sum of boxes scores. In a subsample of 102 participant’s MRIs revealed no difference in the rate of brain atrophy between DHA group and placebo group.
Phillips M, 2015 [32] Randomized, double-blind, placebo-controlled trial	76 individuals diagnosed with either Alzheimer’s disease, or CIND (Cognitive Impairment No Dementia).	625 mg of DHA and 600 mg of EPA was taken daily.	4 months	The Omega-3 supplementation had no beneficial effect on cognitive function based on MMSES7, MMSEWB scores, and three separate verbal memory examinations.
Lee LK, 2012 [33] Randomized, double-blind, placebo-controlled trial	36 individuals aged 60 years or older diagnosed with MCI and low socioeconomic status.	1300 mg of DHA and 450 mg of EPA, was taken daily.	12 months	No difference was seen between omega-3 and placebo group scores, on MMSE, CDT, GDS, visual reproduction II, RAVLT total immediate recall, block design, digit symbol substitution, and matrix reasoning. Omega-3 supplementation had a beneficial effect the following specific cognitive function examinations compared to placebo, digit span, visual reproduction I, and RAVLT delayed recall.
Freund-Levi Y,	204 individuals	1700 mg of DHA and	12 months	At 6 months there was no difference

Study	Participants	Intervention	Duration	Outcomes
2006 [34] Randomized, double-blind, placebo-controlled clinical trial.	diagnosed with mild to moderate AD	600 mg of EPA acid was taken daily. 4 mg of vitamin E taken daily by all groups. At month 6 placebo group began taking Omega-3 supplement, and Omega-3 group continued to take Omega-3 supplement.		between the MMSE or ADAS-cog scores of the Omega-3 and placebo group. At 12 months there was no difference seen in MMSE or ADAS-cog scores between the Omega-3/Omega-3 group and Placebo/Omega-3 group. At 6 months in subgroups with very mild cognitive impairment, Omega-3 supplementation had a beneficial effect on MMSE scores, compared to the placebo.
Chiu C-C, 2008 [35] Randomized double-blind placebo-controlled study	46 individuals diagnosed with either MCI or mild to moderate AD between the ages of 55 and 90 years old.	720 mg of DHA and 1080 mg of EPA was taken daily	24 weeks	There was no difference observed in ADAS-cog, MMSE, and HDRS scores between groups. Omega-3 supplementation had a beneficial effect on CIBIC-plus scores compared to placebo. In subgroups with MCI, Omega-3 supplementation had a beneficial effect on ADAS-cog scores compared to placebo.
Shinto L, 2014 [36] Randomized Placebo-Controlled Pilot Trial	39 individuals diagnosed with probable AD aged 55 years or older.	Omega-3 group was given 675 mg of DHA and 975 mg of EPA daily Linoleic group was given 675 mg of DHA, 975 mg of EPA, and 600 mg of LA daily	12 months	There was no difference observed in ADAS-cog and ADL scores between groups. Both Omega-3 group and Linoleic group showed a lower amount of decline in IADL scores. Linoleic group showed a lower amount of decline in MMSE scores compared to both Omega-3 group and placebo.

Fortasyn Connect Nutrient Combination in Clinical Trials

The Fortasyn Connect nutrient combination consists of several bioactive compounds: Omega-3 fatty acids, vitamin B, Phospholipids, Choline, Uridine, Selenium, Vitamin E, and Vitamin C. In the past 15 years, there have been three comparable clinical trials that document the effects Fortasyn Connect has on patients diagnosed with AD and MCI. The study designs and results of all reviewed Fortasyn Connect clinical trials are indexed in Table 3. The trademarked Fortasyn Connect nutrient combination was administered in the form of the medical food Souvenaid, and the specific quantities of bioactive compounds within Souvenaid are listed directly below Table 3. The three investigations documented Souvenaid supplementation’s, safety and tolerability, capacity to influence plasma levels of targeted nutrients, and effect on disease related cognitive decline. The daily amount of Souvenaid given to participants was the same throughout all of the reviewed trials.

All trials concluded that Souvenaid containing the Fortasyn Connect nutrient profile had a high level of safety and tolerability [37-40]. Souvenaid supplementation also consistently influenced erythrocyte and plasma levels of target nutrients. In all three trials, Souvenaid increased omega3-fatty acids and vitamin E status, and decreased plasma tHcy levels [38-40]. Souvenaid’s effect on AD-related cognitive decline was assessed by a variety of measures, including standardized cognitive examinations, a Neuropsychiatric inventory, a

Neuropsychological Test Battery (NTB), and Electroencephalography (EEG). In the Souvenir I trial, Souvenaid supplementation had no beneficial effects on examinations of overall cognitive ability or a 12 point Neuropsychiatric inventory, but was associated with improved performance in delayed verbal recall testing [38]. In the Souvenir II study, the Souvenaid group’s NTB memory domain Z-scores improved compared to control, but there was no difference reported in NTB executive function domain Z-scores between groups [39]. The Souvenir II study also utilized EEG data to measure participant’s functional brain connectivity. EEG analysis revealed a positive difference in the Souvenaid group’s delta frequency band trajectory over the 24-week treatment period compared to the control group [39]. No such difference was observed in any other frequency bands. Lastly, the Shah et al 2013 RCT concluded that there were no differences in ADAS-cog scores between the experimental group and the placebo group [40].

Table 3. The effects of the Fortasyn Connect nutrient combination on AD and MCI

Study	Participants	Intervention	Duration	Outcome
Scheltens P, 2010 [38] Souvenir I Randomized controlled trial	225 drug-naïve individuals mild AD aged 50 years or older.	125 ml of Souvenaid was taken daily	12 weeks	Souvenaid supplementation had a beneficial effect on delayed verbal recall testing. No difference was observed between group scores on modified ADAS-cog, CIBIC-plus, ADCS-ADL, and 12 item Neuropsychiatric inventory.
Scheltens P, 2012 [39] Souvenir II Randomized controlled trial	259 drug-naïve individuals with mild AD aged 50 years or older.	125 ml of Souvenaid was taken daily	24 weeks	Souvenaid supplementation had a beneficial effect on subjects NTB memory domain Z-scores, and one of four measures of functional brain connectivity based on EEG analysis. No difference was seen between groups in three of four measures of functional brain connectivity, or NTB executive function domain Z-scores.
Shah RC, 2013 [40] Randomized controlled trial	527 individuals diagnosed with mild to moderate AD, taking AD medications.	125 ml of Souvenaid was taken daily	24 weeks	ADAS-cog scores determined Souvenaid treatment had no beneficial effect on cognition compared to placebo.

Souvenaid ingredients: EPA (300mg), DHA (1200mg), Phospholipids (106mg), Choline (400mg), Uridine (625mg), Vitamin E (40mg), Vitamin C (80mg), Vitamin B6 (1mg), Vitamin B12 (3µg), Selenium (60µg), and Folic acid (400µg) [41].

DISCUSSION

Throughout the reviewed vitamin B trials, the most beneficial effects on measures of cognition were seen in a subgroup within the VITACOG sample that studied participants with higher than median baseline plasma tHcy levels [27]. Within this subgroup, vitamin B supplementation improved memory, global cognitive function and clinical status. The other vitamin B trials reviewed did not define baseline plasma tHcy subgroups, and vitamin B had no beneficial effects on measures of overall cognitive ability independent of baseline plasma tHcy levels [29, 30]. B

vitamin supplementation had a beneficial effect on measures of neurodegeneration via rates of brain atrophy in the VITACOG trial [26, 28]. Future studies will need to replicate the VITACOG trial results in additional sample populations, before vitamin B's effect on brain atrophy can be considered conclusive. Throughout the Omega-3 fatty acid clinical trials, supplementation had a beneficial effect on measures of both overall cognitive ability and specific cognitive function in participants with lower levels of baseline cognitive impairment [34, 35]. Omega-3 fatty acid supplementation did not elicit any beneficial effects on measures of overall cognitive ability, independent of baseline cognitive impairment [31-36]. There were reports of improvements in specific cognitive function examinations from Omega-3 supplementation independent of baseline cognitive impairment, but no single report was replicated by another study [31-36]. The clinical trials that utilized the Fortasyn Connect nutrient combination via the medical food Souvenaid, showed that supplementation provided a beneficial effect on one of four measures of functional brain connectivity, and two separate memory examinations [38, 39]. Souvenaid was not associated with similar positive effects on scores of overall cognition and or other measures of functional brain connectivity [38, 40].

Supplementation with vitamin B, Omega-3 fatty acids, or bioactive compounds in Fortasyn Connect was not conclusively proven to have a beneficial effect on AD or MCI patient cognitive outcomes, and there is not enough data to conclude an effect on measures of neurodegeneration. Despite this determination, several positive effects from bioactive compound supplementation have been seen within individual studies, justifying further investigation. Potential topics of further investigation include, the effect of bioactive compounds on AD and MCI patients with higher baseline plasma tHcy levels, and lower baseline levels of cognitive impairment, and the effects of vitamin B supplementation on rates brain atrophy in patients with MCI.

Considerations for Functional Food Development

The results of these bioactive compound clinical trials are of primary importance to the field of functional food science because a core objective of functional food research is to substantiate the effects of bioactive compounds. The Functional Food Center, based in the United States, has comprehensively defined a functional food as, "Natural or processed foods that contains known or unknown biologically-active compounds; which, in defined, effective non-toxic amounts, provide a clinically proven and documented health benefit for the prevention, management, or treatment of chronic disease" [42]. Although previously specified quantities of vitamin B, Omega-3 fatty acids, and bioactive compounds in Fortasyn Connect have proven to be non-toxic throughout the reviewed studies, their clinical efficacy to manage AD or MCI remains unproven. For this reason, we do not recommend that these clinically tested quantities of bioactive compounds be considered for development of a functional food.

CONCLUSION

The results of clinical trials within the last 15 years do not conclusively prove that the specified quantities of vitamin B, Omega-3 fatty acids, or bioactive compounds within Fortasyn Connect, are beneficial for use in the management of AD or MCI. Additionally, the documented effects of these bioactive compounds do not warrant development of a functional food product utilizing the previously specified quantities to help manage AD or MCI. The effects bioactive compound

supplementation had on measures of neurodegeneration, and cognition in individuals with higher levels of baseline plasma tHcy and lower levels of cognitive impairment warrants further investigation.

List of Abbreviations: Alzheimer's Disease, AD; Mild Cognitive Impairment, MCI; Homocysteine, tHcy; Amyloid Beta, A β ; Neurofibrillary tangles, NFT; Mini-Mental State Examination, MMSE; Mini-Mental State Examination Serial Sevens, MMSES7; Mini-Mental State Examination World Backwards, MMSEWB; Alzheimer's Disease Assessment Scale–Cognitive subscale, ADAS-cog; Magnetic Resonance Imaging, MRI; Docosahexanoic acid, DHA; Eicosapentaenoic acid, EPA; Cognitive Impairment No Dementia, CIND; Omega-3 fatty acids, Omega-3; Randomized Controlled Trial, RCT; Neuropsychological Test Battery, NTB; Electroencephalography, EEG; Clinical Dementia Rating, CDR; Informant Questionnaire on Cognitive Decline in the Elderly, IQCODE; Clinicians Global Impression of Change, CIBIC-plus; Activities of Daily Living, ADL; Alzheimer's Disease Cooperative Study Activities of Daily Living, ADCS-ADL; Instrumental Activities of Daily Living, IADL; Hamilton Depression Rating Scale, HDRS; Clock-Drawing Test, CDT; Global Deterioration Scale, GDS; Rey Auditory Verbal Learning Test, RAVLT.

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Authors' Contributions: All authors contributed to this review

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