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Review Article

Plasma nutrient status of patients with Alzheimer's disease: Systematic review and meta-analysis

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Abstract

Background: Alzheimer disease (AD) patients are at risk of nutritional insufficiencies because of physiological and psychological factors. Nutritional compounds are postulated to play a role in the pathophysiological processes that are affected in AD. We here provide the first systematic review and meta-analysis that compares plasma levels of micronutrients and fatty acids in AD patients to those in cognitively intact elderly controls. A secondary objective was to explore the presence of different plasma nutrient levels between AD and control populations that did not differ in measures of protein/energy nourishment.

Methods: We screened literature published after 1990 in the Cochrane Central Register of Controlled Trials, Medline, and Embase electronic databases using Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines for AD patients, controls, micronutrient, vitamins, and fatty acids, resulting in 3397 publications, of which 80 met all inclusion criteria. Status of protein/energy malnutrition was assessed by body mass index, mini nutritional assessment score, or plasma albumin. Meta-analysis, with correction for differences in mean age between AD patients and controls, was performed when more than five publications were retrieved for a specific nutrient.

Results: We identified five or more studies for folate, vitamin A, vitamin B12, vitamin C, vitamin D, vitamin E, copper, iron, and zinc but fewer than five studies for vitamins B1 and B6, long-chain omega-3 fatty acids, calcium, magnesium, manganese, and selenium (the results of the individual publications are discussed). Meta-analysis showed significantly lower plasma levels of folate and vitamin A, vitamin B12, vitamin C, and vitamin E ($P < .001$), whereas nonsignificantly lower levels of zinc ($P = .050$) and vitamin D ($P = .075$) were found in AD patients. No significant differences were observed for plasma levels of copper and iron. A meta-analysis that was limited to studies reporting no differences in protein/energy malnourishment between AD and control populations yielded similar significantly lower plasma levels of folate and vitamin B12, vitamin C, and vitamin E in AD.

Conclusions: The lower plasma nutrient levels indicate that patients with AD have impaired systemic availability of several nutrients. This difference appears to be unrelated to the classic malnourishment that is well known to be common in AD, suggesting that compromised micronutrient status may precede protein and energy malnutrition. Contributing factors might be AD-related alterations in feeding behavior and intake, nutrient absorption, alterations in metabolism, and increased utilization of nutrients for AD pathology-related processes. Given the potential role of nutrients in

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the pathophysiological processes of AD, the utility of nutrition may currently be underappreciated and offer potential in AD management.

Keywords: Vitamin A; Vitamin B1; Vitamin B6; Folate; Vitamin B12; Vitamin C; Vitamin D; Vitamin E; Omega-3 fatty acids (DHA and EPA); Calcium; Copper; Iron; Magnesium; Manganese; Selenium; Zinc

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder of unknown origin and the leading cause of dementia. Age is the primary risk factor for AD [1,2], whereas a family history of AD (in familial AD) and the presence of the ApoE4 genotype (in sporadic AD) are the most important inherited determinants known for this disease [3,4]. Other risk factors for AD include gender, education, and potentially modifiable lifestyle factors including diet and physical activity [5,6]. Diet-related disorders such as obesity, hypertension, hypercholesterolemia, and diabetes have consistently been shown to be associated with AD [7–9]. Risk factors of nutritional origin are extensively analyzed for their possible role in AD onset and progression [3,10–13]. Epidemiological studies have suggested a positive correlation between AD and the consumption of a diet rich in saturated fatty acids and alcohol and low in antioxidants and vitamins. However, adherence to a diet rich in fruits, vegetables, and unsaturated fatty acids and low in saturated fat and refined sugar seems to reduce the risk of dementia and cognitive decline [10,14–17].

The first nutrients identified to be of pivotal importance for neuronal functioning and cognition were the B vitamins [18]. They were discovered at the beginning of the 20th century as being essential nutrients able to relieve beriberi and pellagra, deficiency diseases affecting the nervous system [19]. In 1929, Frederick Gowland Hopkins [20] and Christiaan Eijkman were awarded the Nobel Prize in Physiology for this discovery. Since then, several nutrients, including antioxidants, choline, and omega-3 fatty acids, have been suggested to influence cerebral functioning (reviewed in Bourre [21] and in Smith and Blumenthal [22]).

Therefore, it is no surprise that these nutrients have been postulated to play roles in the pathophysiological processes in AD. For example, antioxidants reduce reactive-oxygen-species-induced damage and stabilize membranes; the fatty acid docosahexaenoic acid (DHA) affects abnormal protein processing (amyloid-β, tau); and DHA, choline, and uridine modulate neuronal membrane formation [23]. Recent evidence suggests that a multinutrient intervention comprising DHA, eicosapentaenoic acid (EPA), uridine monophosphate (UMP), choline, folate, vitamin B6, vitamin B12, vitamin C, vitamin E, selenium, and phospholipids (PLs) modulated functional connectivity measures (assessed by electroencephalography) in AD, indicative of preserved synaptic function [24]. Therefore, increasing specific nutrient levels may modulate synaptic function and prevent neurodegeneration and eventually neuronal loss. Despite the potential importance of nutrient availability for brain function in AD, evidence on its systemic availability in AD is not conclusive. The results of the many studies that examined plasma nutrient levels in AD are not fully consistent, and systematic reviews are lacking.

The primary objective of the current systematic review is to evaluate the presence of differences in the systemic availability of nutrients between AD patients and cognitively intact elderly controls. Because protein/energy malnutrition may be present more among patients with AD than among control subjects and can potentially be associated with differential micronutrient and fatty acid status, the secondary objective was to compare plasma nutrient levels of AD patients and controls that were reported not to differ in measures of protein/energy malnourishment. All relevant literature published after 1990 in Medline, Embase, and the Cochrane Central Register of Controlled Trials was reviewed using the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines recently published by Moher and colleagues [25].

2. Methods

2.1. Search strategy and selection criteria

The literature published from 1990 to March 26, 2012 was systematically screened in the Cochrane Central Register of Controlled Trials, Medline, and Embase electronic databases according to PRISMA guidelines [25] using the following search terms in the title, abstract, or descriptors:

(Alzheimer* and [humans or patients or inpatients or outpatients or persons or volunteers or participants or subjects] and [nutrition or nutritional or nutrient or nutrients or micronutrient or micronutrients or diet or diets or dietary or vitamin or vitamins or mineral or minerals or trace-element or trace-elements or fatty-acid or fatty-acids or pufa or pufas])

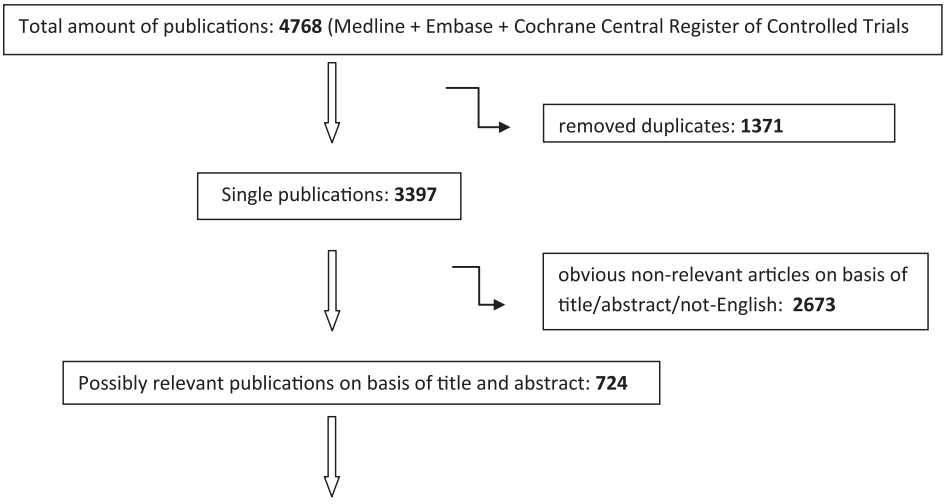
The search in the Cochrane Central Register of Controlled Trials, Embase, and Medline resulted in 4768 published studies that were imported into Endnote. Duplicate references (684) were automatically removed, followed by manual examination, which retrieved another 687 duplicate references. The title and abstract of the remaining 3397 publications were evaluated according to predefined exclusion and inclusion criteria.

Included were those papers that might contain plasma nutrient levels of AD patients, even if not explicitly mentioned in the abstract. One fifth (724) of the retrieved publications was identified to be of potential relevance. The full-text articles of these 724 publications were analyzed according to the following inclusion criteria: contained an AD patient population according to the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association criteria [26] and/or the Diagnostic and Statistical Manual for Mental Disorders [27] criteria for AD and mentioned the use of a cognitively intact elderly control group, mean plasma or serum levels of nutrients, number of AD patients and number of controls, and mean age or age range of the AD and control group.

Analysis of the retrieved documents revealed that some of these publications concerned data that were derived from the same patient population. In this case, the publication with the most complete set of data was included. The following papers were excluded: those concerning other cognitive disorders (e.g., mild cognitive impairment [MCI], vascular dementia, frontotemporal dementia, or psychogeriatric disease), those that included subjects using vitamin supplements, and those not written in English. Botanicals, resveratrol, curcumin, β-carotene, and heavy metals (aluminum and mercury) were also excluded because these are not considered micronutrients.

This systematic review resulted in the identification of 80 publications that met all inclusion criteria, some of which described plasma levels of multiple nutrients.

Flow Chart Literature Search



Nutrients found	Number of relevant publications	Studies used for meta-analysis	Studies used for meta-analysis substudy	Nutrients found	Number of relevant publications
Vitamin A	9	9	6	Vitamin B1	2
folate	31	31	14	Vitamin B6	2
Vitamin B12	33	33	16	DHA	4
Vitamin C	8	8	5	EPA	4
Vitamin D	5	5	-	calcium	4
Vitamin E	20	20	11	magnesium	3
copper	5	5	-	manganese	2
iron	5	5	-	selenium	4
zinc	5	5	-		

For meta-analysis, a minimum of five publications was required [28,29].

2.2. Statistical analysis

All reported comparisons of plasma/serum levels in AD patients and controls were integrated and summarized into a final result per nutrient, using meta-analysis (regression) methods [30], according to the PRISMA statement [25]. For each nutrient, we extracted from the articles the mean and standard deviation of plasma/serum levels in AD patients and in controls as well as the number of subjects and the average age per group. To allow comparison across studies, all plasma/serum levels were converted to the same unit value. These data were analyzed using the random-effects meta-analysis model [30] fitted by restricted maximum likelihood using the program metareg of Stata (StataCorp. 2001. Statistical Software: Release 12.1. College Station, TX, USA) [31]. For this analysis, a minimum of five studies is generally recognized to be required [28,29].

Because it is known that the plasma levels of several nutrients can vary with age [32], the meta-analyses were conducted with and without a correction by meta-regression for differences in mean age between AD patients and controls. The final result for a given nutrient was based on the result of the meta-analysis with age adjustment. Given the fact that plasma and serum levels were of the same magnitude, both were used in the same analysis. For clarity, we use the term plasma in the remainder of this systematic review.

2.3. Analysis of bias

According to the PRISMA statement [25], the quality of a systematic review depends on the quality of the individual studies and the absence of bias for their inclusion. To avoid the risk of bias, general search terms were chosen (such as nutrition and diet) to guarantee that papers reporting nutrient levels as a secondary outcome would also be included. The quality of the studies was assessed by several inclusion and exclusion criteria (listed in *Search strategy and selection criteria*). Furthermore, the results of the meta-analyses were statistically analyzed for possible bias because of small studies tending to have larger effects or smaller effects using a funnel plot accompanied by the Egger's test [31]. Meta-analyses that are based on small studies reporting larger (smaller) effects may tend to overestimate (underestimate) the actual outcome. Funnel plots, which plot the standard error against the reported mean difference for each publication, can give an indication of the overestimation or underestimation of the actual difference occurring in the meta-analysis. We used Egger's test as implemented in the Stata program meta-bias [31] to test the association between standard error and effect size in the funnel plot. For this analysis, a minimum of eight publications is generally recognized to be required [28,29]. Statistically significant results of the funnel plot analysis are indicated in the *Results* section.

2.4. Meta-analysis limited to AD patients and controls reported not to differ in measures of protein/energy malnourishment

A secondary objective was to explore the presence of different plasma nutrient levels between AD and control subjects that were reported not to differ in measures of protein/energy malnutrition. Therefore, a meta-analysis was conducted for each nutrient including only a subset of studies that reported no significant differences in body mass index (BMI), mini nutritional assessment (MNA) score, or plasma albumin levels between the AD patients and control subjects [33,34]. Publications were also included in which the authors explicitly stated that there were no differences in protein/energy malnourishment status between AD patients and controls.

3. Results

Publications were found concerning the following nutrients: vitamin A (9 studies), vitamin B1 (2 studies), vitamin B6 (2 studies), folate (31 studies), vitamin B12 (33 studies), vitamin C (8 studies), vitamin D (5 studies), vitamin E (20 studies), the omega-3 fatty acids DHA and EPA (4 studies), and 7 minerals or trace elements (calcium [4 studies], copper [5 studies], iron [5 studies], magnesium [3 studies], manganese [2 studies], selenium [4 studies], and zinc [5 studies]). Table 1 provides a summary of this systematic review, listing for each nutrient the results reported in the retrieved publications.

The primary objective was to assess whether differences in plasma nutrient levels exist between AD patients and cognitively intact elderly controls. Therefore, a meta-analysis was performed with a correction by meta-regression for differences in mean age between AD patients and controls. This meta-analysis was possible for vitamin A, folate, vitamin B12, vitamin C, vitamin D, vitamin E, copper, iron, and zinc because our literature search had yielded more than five publications for each of these nutrients (see Figures 1–9 and Table 2). Significantly lower plasma levels of vitamin A, vitamin C, vitamin E, folate, and vitamin B12 ($P < .001$) and nonsignificantly lower levels of vitamin D ($P = .075$) and zinc ($P = .050$) were found in AD patients compared with controls. No significant differences were observed for plasma levels of copper and iron. These results are based on a meta-analysis with age adjustment. For completeness, the results of the meta-analyses without age correction are also provided in the figures.

Basun and colleagues [35] reported comparisons of plasma vitamin B12 levels between AD patients and controls for five different age groups. Because these concerned five independent observations, all are shown in Figure 3. Figure 3 further shows that the results reported by Dominguez and colleagues [36] and by Postiglione and colleagues [37] deviated from those reported in the other publications. The reason for this discrepancy may be attributed to the higher vitamin B12 levels that were measured in the control group (713 pmol/L in Dominguez et al. [36] and 780 pmol/L in Postiglione et al. [37]) as

Table 1
Results of the systematic review

Nutrient	Total number of publications	Total number of AD patients	Total number of control subjects	Studies reporting significantly lower levels in AD patients than in controls	Studies reporting significantly higher levels in AD patients than in controls	Studies reporting no significant differences between AD patients and controls
Vitamin A	9 studies [44,65,72,74,75,78,100–102]	310	674	4 studies $P < .005$ [74] $P < .05$ [65,72,78]		5 studies [44,75,100–102]
Folate	31 studies [36,37,46,84,85,103–128]	2108	2447	14 studies $P < .005$ [37,46,84,85,104,106, 108,114,123,124,127] $P < .05$ [36,109,126]		17 studies [103,105,107,110–113, 115–122,125,128]
Vitamin B12	33 studies [35–37,44,46,49,84,85,103, 105,107–112,114–123,125–131]	2264	2784	9 studies $P < .005$ [37,84,114,123,127] $P < .05$. [44,46,118,129]		24 studies [35,36,49,85,103,105, 107–112,115–117,119–122, 125,126,128,130,131]
Vitamin C	8 studies [44,74,75,77,79,132–134]	223	211	4 studies $P < .005$ [44,74,75,132]		4 studies [77,79,133,134]
Vitamin D	5 studies [47,98,99,135,136]	394	471	2 studies $P < .005$ [98,99]		3 studies [47,135,136]
Vitamin E	20 studies [44,65,69,71–78,100,101, 132,133,137–141]	830	1349	9 studies $P < .005$ [44,69,72,74,75,78,141] $P < .01$ [65] $P < .05$ [100]		11 studies [71,73,76,77,101,132, 133,137–140]
Copper	5 studies [39,40,51,53,142]	178	575	1 study $P < .01$ [51]	1 study $P = .001$ [40]	3 studies [39,53,142]
Iron	5 studies [39,51,53,129,142]	153	545	2 studies $P < .05$ [39,51]		3 studies [53,129,142]
Zinc	5 studies [39,51,53,142,143]	157	554	2 studies $P < .05$ [51,143]		3 studies [39,53,142]
DHA in PL	3 studies [38,41,42]	69	80	2 studies $P < .05$ [41] $P < .001$ [38]		1 study [42]
EPA in PL	3 studies [38,41,42]	69	80	3 studies $P < .05$ [41,42] $P < .001$ [38]		
DHA in CE	3 studies [38,42,43]	78	106	1 study $P < .001$ [43]	1 study $P < .01$ [38]	1 study [42]
EPA in CE	3 studies [38,42,43]	78	106	2 studies $P < .05$ [42] $P < .001$ [43]		1 study [38]
Vitamin B1	2 studies [44,45]	53	50	2 studies $P < .05$ [44,45]		
Vitamin B6	2 studies [44,46]	48	48	1 study $P < .005$ [44]		1 study [46]
Calcium	4 studies [39,47–49]	76	90	1 study $P < .05$ [47]		3 studies [39,48,49]

(*Continued*)

Table 1
Results of the systematic review (*Continued*)

Nutrient	Total number of publications	Total number of AD patients	Total number of control subjects	Studies reporting significantly lower levels in AD patients than in controls	Studies reporting significantly higher levels in AD patients than in controls	Studies reporting no significant differences between AD patients and controls
Magnesium	3 studies [39,50,51]	76	83	2 studies $P < .001$ [51] $P < .01$ [50]		1 study [39]
Manganese	2 studies [52,53]	54	58			2 studies [52,53]
Selenium	4 studies [39,51,54,55]	129	141	2 studies $P < .001$ [51,54]	1 study $P < .05$ [39]	1 study [55]

Abbreviations: AD, Alzheimer's disease; DHA, docosahexaenoic acid; PL, phospholipids; EPA, eicosapentaenoic acid; CE, cholesteryl ester.

NOTE. Columns 1–4: publications retrieved per nutrient and numbers of included AD patients and controls. Columns 5–7: studies reporting significantly lower levels (Column 5), significantly higher levels (Column 6), and no significant differences (Column 7) in AD patients vs controls.

compared with the values reported for the control subjects in the other studies. Omission of these two studies from the meta-analysis (and from the funnel plot analysis, see next paragraph) did not change the results.

Subsequent funnel plot analysis was conducted to identify underestimations or overestimations of the actual difference [31]. This analysis required a minimum of eight publications and was possible for vitamin A, folate, vitamin B12, vitamin C, and vitamin E. All funnel plot analyses were not significant except for vitamin B12. The funnel plot for vitamin B12 ($P = .008$) indicated a statistically significant underestimate of the actual difference. The nonsignificant results of the funnel plot analyses for vitamin A, folate, vitamin C, and vitamin E indicated that the results of the meta-analyses were not biased because of the inclusion of small studies reporting large effects.

A secondary objective was to assess the presence of different plasma nutrient levels between AD and control subjects that were explicitly reported not to differ in measures of protein and energy nourishment. Therefore, a meta-analysis with age correction was conducted on a subset of publications reporting to have included only AD patient and control populations that did not differ in the status of protein and energy nourishment (as reflected by BMI, MNA, or albumin). For vitamin A, folate, vitamin B12, vitamin C, and vitamin E, sufficient publications were available to allow for the meta-analysis substudy. The meta-analysis substudies showed statistically significant lower plasma levels of folate, vitamin B12, vitamin C, and vitamin E ($P \leq .001$) in AD patients compared with controls, whereas a trend toward lower levels of vitamin A ($P = .052$) were found (see Table 2). Thus, these findings indicate that even when only AD and control subjects are taken that are reported not to differ in signs of protein and energy malnutrition, similar statistically significant lower micronutrient levels were found in AD patients compared with cognitively intact elderly controls.

For several nutrients, insufficient publications were retrieved to perform the meta-analysis. The results of individual studies are discussed in the following subsections. None of these publications reported significantly higher plasma nutrient levels in AD patients compared with controls, except for one study reporting higher levels of DHA in the cholesteryl ester (CE) moiety [38], one study reporting higher levels of selenium [39], and one study reporting higher levels of copper [40] (indicated in Column 6 of Table 1).

3.1. Fatty acids

The electronic database search with our search string retrieved three publications that investigated DHA and EPA levels in PLs [38,41,42] and three that investigated these levels in the CE lipid moiety [38,42,43] in the plasma of AD patients and of cognitively intact elderly controls. Levels were expressed as weight percentage of total fatty acids in the particular lipid moiety. The publications by Corrigan and colleagues [38,42] concerned two different

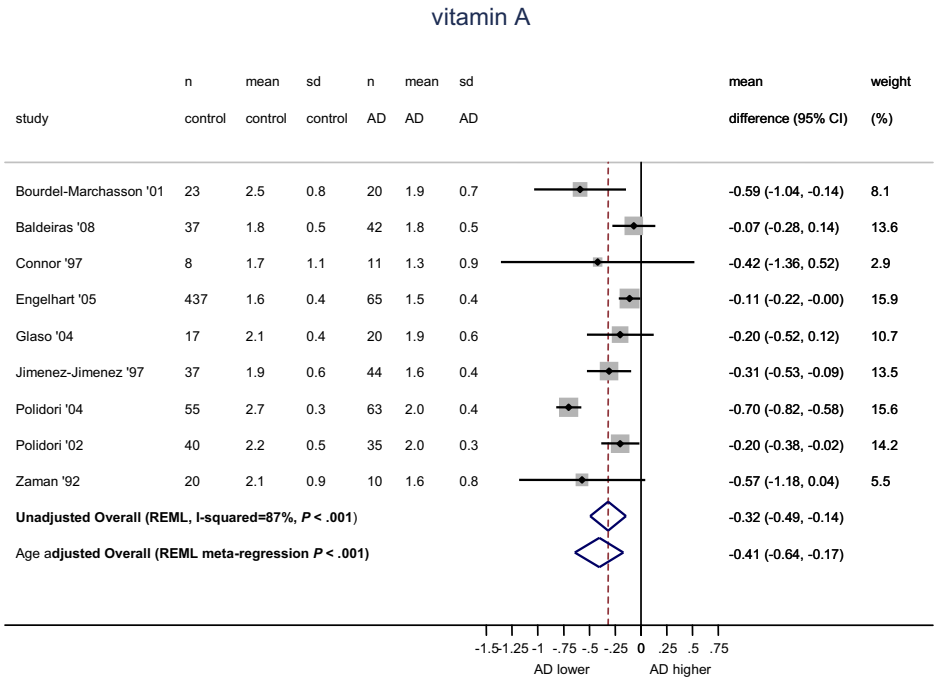


Fig. 1. The age-adjusted overall mean difference in plasma vitamin A (retinol) levels (μmol/L) between AD patients and controls was significant ($P < .001$) and approximately 20% of the absolute amount in the control subjects. Abbreviations: AD, Alzheimer's disease; CI, confidence interval; REML, restricted maximum likelihood.

populations of patients and controls; therefore, they were both included in our systematic analysis. The second study of Corrigan and colleagues [42] only concerned the fatty acid profiles of the isolated plasma high-density lipoprotein fraction. With regard to plasma PLs, the individual papers reported significantly lower levels of DHA (Conquer et al. [41] $P < .05$; Corrigan et al. [38] $P < .001$) and EPA (Conquer et al. [41] $P < .05$; Corrigan et al. [42] $P < .05$; Corrigan et al. [38] $P < .001$) in AD patients compared with their cognitively intact contemporaries (see Table 1). These studies comprised 80 control subjects and 69 AD patients.

With regard to the CE moiety, one of the three publications (Corrigan et al. [38] $P < .01$) reported a significantly higher weight percentage of DHA in AD patients versus controls. However, Tully et al. [43] reported a significantly lower weight percentage of DHA in AD patients ($P < .001$), whereas in the second study by Corrigan and colleagues [42] no significant differences were observed between AD patients and controls (see Table 1). The weight percentage of EPA in the CE moiety was found to be significantly lower in AD patients (vs controls) in the publications of Corrigan et al. [42] ($P < .05$) and in the publication of Tully et al. [43] ($P < .001$). In the other publication no statistical difference was reported (see Table 1). These studies comprised 106 control subjects and 78 AD patients.

Vitamin B1 [44,45] and vitamin B6 [44,46] were each only mentioned in two publications. The individual papers reported significantly lower levels of vitamin B1 (Glasö et al. [44] $P < .05$; Molina et al. [45] $P < .05$) and vitamin B6 (Glasö et al. [44] $P < .005$) in AD patients compared with controls (see Table 1).

3.2. Calcium

Four papers were retrieved that measured calcium levels [39,47–49], including in total 90 control subjects and 76 AD patients. Of these four studies, one study [47] reported significantly ($P < .05$) lower levels of calcium in AD patients versus controls (see Table 1). In the other studies, no significant differences were reported.

3.3. Magnesium

Three publications were found to report on plasma magnesium levels in AD and controls [39,50,51], including in total of 83 control subjects and 76 AD patients. Two [50,51] of them measured significantly lower plasma levels of magnesium in AD patients compared with controls (Vural et al. [51] $P < .001$; Kurup et al. [50] $P < .01$) (see Table 1).

3.4. Manganese

Only two publications, including in total 58 controls and 54 AD patients, reported manganese plasma levels [52,53]. In both publications, differences between AD patients and controls were not significant (see Table 1).

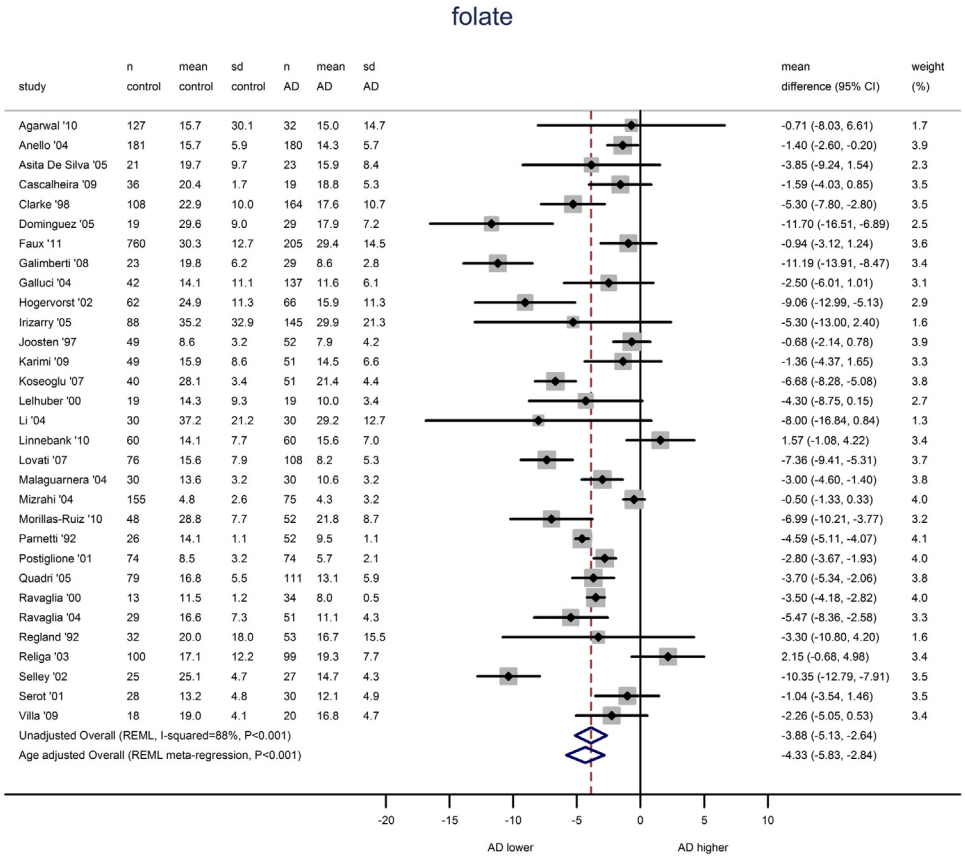


Fig. 2. The age-adjusted overall mean difference in plasma folate levels (nmol/L) between AD patients and controls was significant ($P < .001$) and approximately 20% of the absolute amount in the control subjects. Abbreviations: AD, Alzheimer's disease; CI, confidence interval; REML, restricted maximum likelihood.

3.5. Selenium

Four studies were found that measured plasma selenium levels in AD patients compared with controls, including in total 141 control subjects and 129 AD patients [39,51,54,55]. Two of the studies [51,54] reported significantly lower levels in AD patients ($P < .001$), whereas one study [39] reported significantly higher ($P < .05$) levels of selenium in AD patients (see Table 1).

4. Discussion

To our knowledge, this is the first systematic comparison of plasma nutrient levels in AD patients to those in cognitively intact elderly controls. Conclusive evidence that plasma nutrient levels in AD patients are lower than in controls had thus far been lacking. The magnitude of the effect—15–30% lower concentration for several nutrients—was surprising. Integrating the results of all published studies in the current meta-analysis provides an unequivocal answer to our research objective for vitamin A, folate, vitamin B12, vitamin C, and vitamin E ($P < .001$). Funnel plotting of these results further showed that the meta-analyses were not biased because of the inclusion of small studies reporting large effects. In fact, the funnel plot for vitamin B12 indicated a statistically significant underestimate of the actual difference. It is important to note that in the meta-analysis that included only studies with no difference in measures of protein/energy malnourishment between AD patients and controls, the same statistically significant differences were observed, except for vitamin A, for which a trend was found toward lower levels ($P = .052$). This indicates that the lower nutrient levels in AD patients may not be a mere consequence of protein/energy malnourishment.

Because weight loss and malnutrition are common problems in AD as well as a predictive factor of mortality, recommendations on the nutritional care in AD have focused on protein/energy malnutrition, particularly for the end stages of the disease when this risk increases [56–58]. For example, in mild-moderate AD (Mini-Mental State Examination [MMSE] score = 20), only 3% of the patients were shown to be malnourished (MNA score < 17.5) [59], whereas in severe AD (MMSE score < 10), 50% of AD patients had protein and energy malnutrition [60]. The present systematic review is the first to focus on the nutritional status of micronutrients and fatty acids, several of which with suggested potential to modulate pathophysiological processes in AD but have thus far not been part of recommendations for

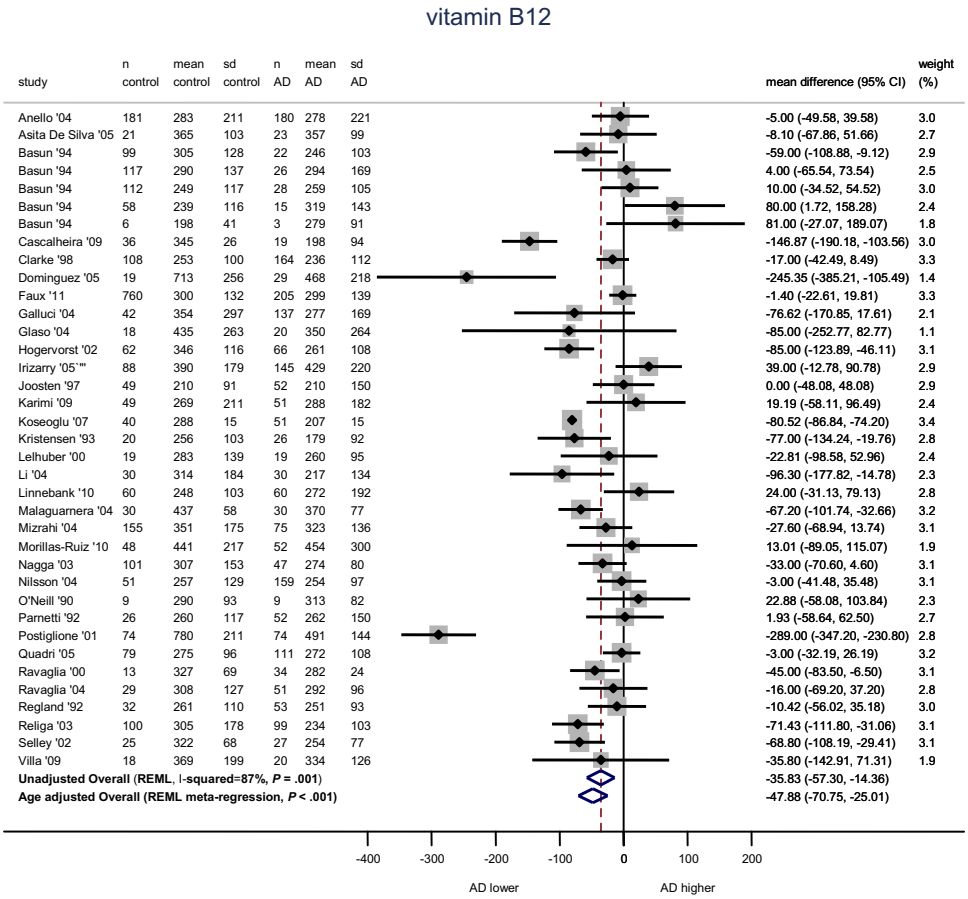


Fig. 3. The age-adjusted overall mean difference in plasma vitamin B12 (cobalamin) levels (pmol/L) between AD patients and controls was significant ($P < .001$) and approximately 15% of the absolute amount in the control subjects. Abbreviations: AD, Alzheimer's disease; CI, confidence interval; REML, restricted maximum likelihood.

nutritional and clinical care. The reported MMSE values of the AD patient populations in the studies that we retrieved were in the moderate MMSE range of 20 to 10, and the AD patients were not reported to have protein and energy malnutrition. This is in agreement with a previous study that investigated the protein and energy status of a group of 93 AD patients with an average MMSE of 14.8 ± 5.7 [61]. Thus, we believe that the lower levels of plasma nutrients found in the meta-analyses are not due to, and probably precede, protein and energy malnutrition. The lower plasma nutrient levels may be caused by altered feeding behavior [62,63], nutrient absorption, utilization, and metabolism. In particular, the last two processes have been implicated in the pathophysiology of AD [64–66].

It is a widely held view that oxidative damage plays an important role in the neurodegenerative process of AD [67]. Oxidative stress may lead to an increased use of antioxidant molecules and therefore cause a disease-specific decrease in their circulating levels [65]. Neuronal membranes are a potential target for the damaging effects of oxidative stress because of their high content of polyunsaturated fatty acids, which are vulnerable to oxidation. Lipid peroxidation can alter membrane composition [68], which is particularly evident in AD. Vitamin A, vitamin C, vitamin E, and selenium serve as antioxidants to protect the lipid precursors and the resulting membrane components from lipid peroxidation. The primary objective of many publications that reported plasma levels of antioxidants (e.g., vitamins A, C, and E) was to investigate whether AD patients have alterations in their capacity to combat oxidative stress [65,69–79]. In addition to anthropometric measures and nutritional examination of vitamin intakes, Bourdel-Marchasson and colleagues [65] conducted a measurement of actual levels of lipid peroxidation in AD patients and controls. This study indicated that the lower plasma antioxidant levels were due to enhanced brain consumption secondary to an excessive production of free radicals. The lower amounts of vitamins A, C, and E found in our meta-analysis may be caused by such increased utilization. This is also expected for selenium; however, insufficient publications were retrieved to assess this hypothesis. Because a proper antioxidant capacity is of pivotal importance to protect neuronal membranes, a process that is particularly affected in AD, AD patients may have an increased need for these vitamins compared with cognitively intact elderly people to compensate for increased utilization.

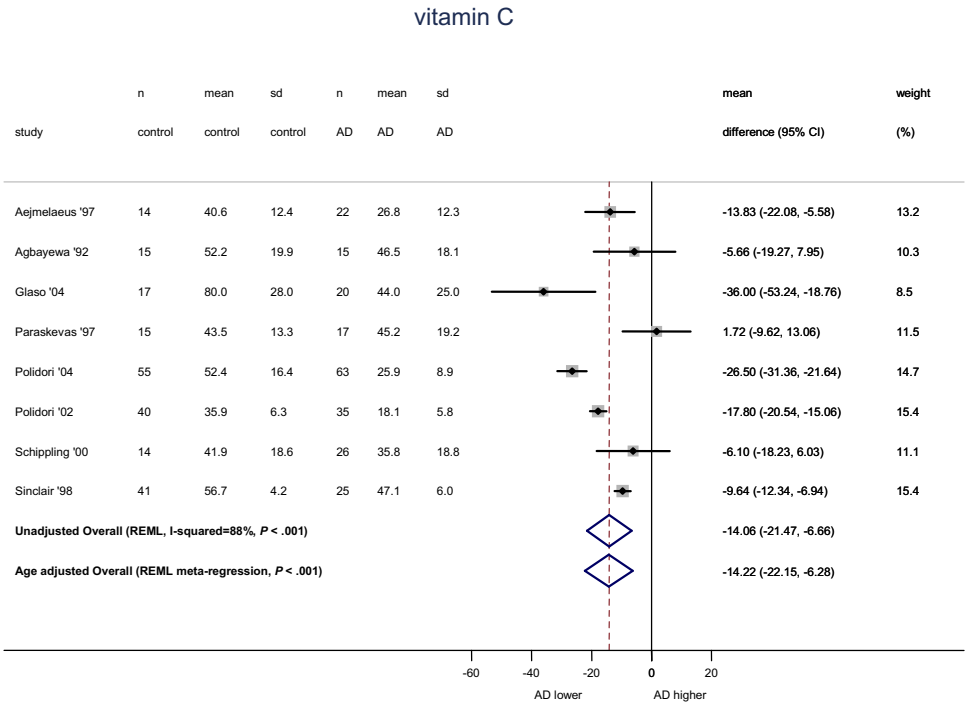


Fig. 4. The age-adjusted overall mean difference in vitamin C (ascorbate) levels (μmol/L) between AD patients and controls was significant ($P < .001$) and approximately 30% of the absolute amount in the control subjects. Abbreviations: AD, Alzheimer's disease; CI, confidence interval; REML, restricted maximum likelihood.

In addition to alterations in the utilization of nutrients, AD pathology has been shown to be accompanied by alterations in the metabolism of nutrients by the liver. One of the enzymes, peroxisomal D-bifunctional protein, which mediates the conversion of dietary α-linolenic acid (ALA) to DHA in the liver, was found to be less active in AD patients, potentially because of oxidative stress [64]. It is interesting to note that the enzymes acting more upstream in the

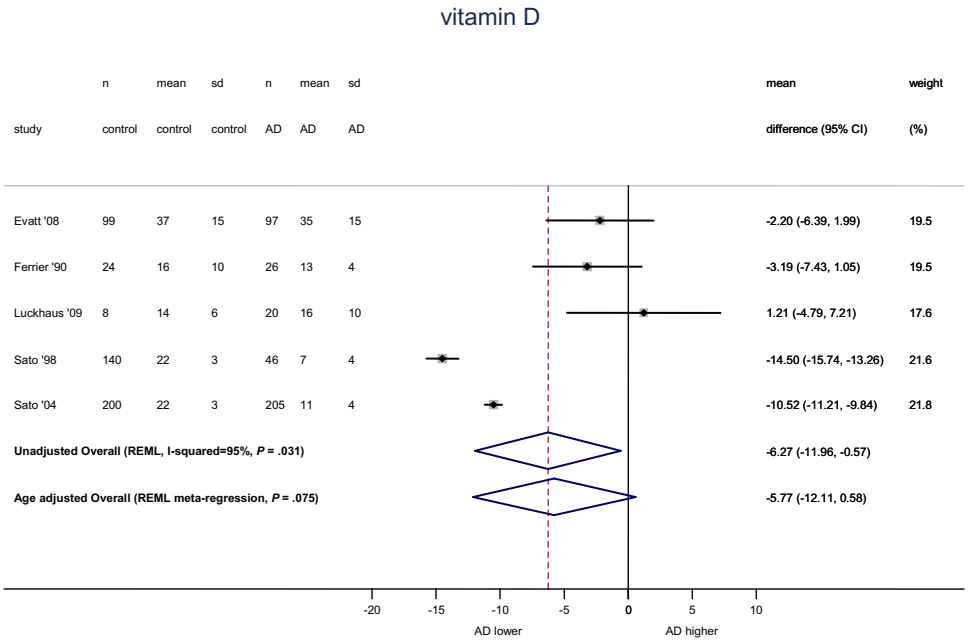


Fig. 5. A trend ($P = .075$) toward lower age-adjusted vitamin D (calcidiol) levels (ng/mL) in AD patients vs controls was observed. We verified that the two studies of Sato et al. [98,99] concerned different AD and control populations. Abbreviations: AD, Alzheimer's disease; CI, confidence interval; REML, restricted maximum likelihood.

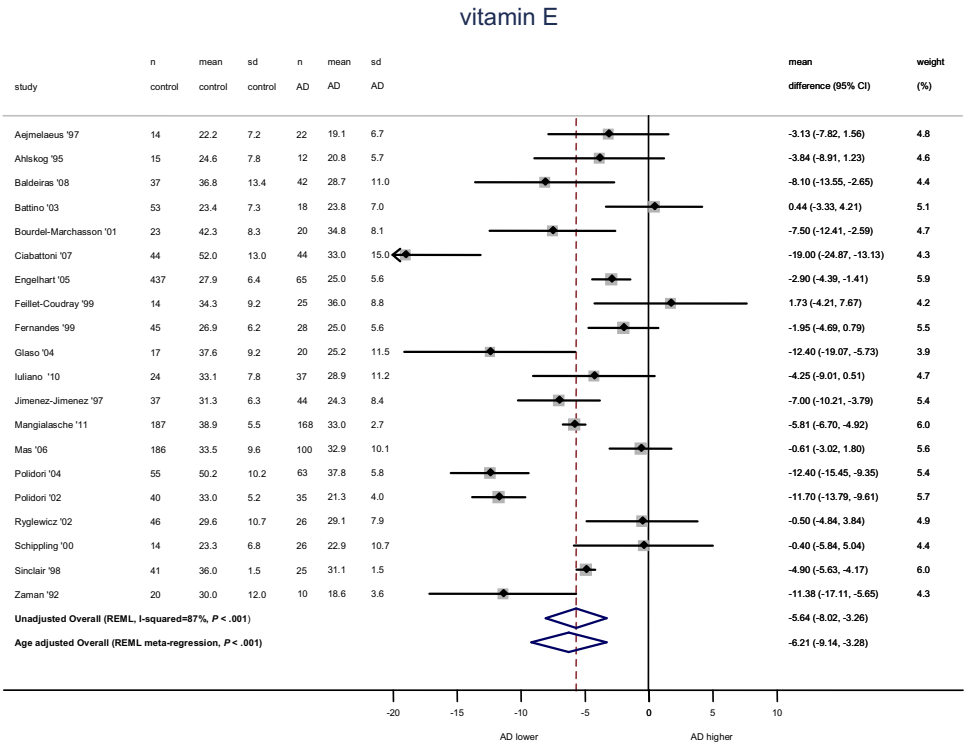


Fig. 6. The age-adjusted overall mean difference in α-tocopherol/vitamin E levels (μmol/L) between AD patients and controls was significant ($P < .001$) and approximately 20% of the absolute amount in the control subjects. Vitamin E levels were used uncorrected for cholesterol amounts. Abbreviations: AD, Alzheimer's disease; CI, confidence interval; REML, restricted maximum likelihood.

ALA to DHA metabolic pathway were not affected [64]. The livers of AD patients were shown to contain significantly lower levels of DHA-containing phosphatidylethanolamine ($P = .003$) compared with control subjects. The reduced liver and brain DHA concentrations correlated with the decline in MMSE scores experienced by the patients [64].

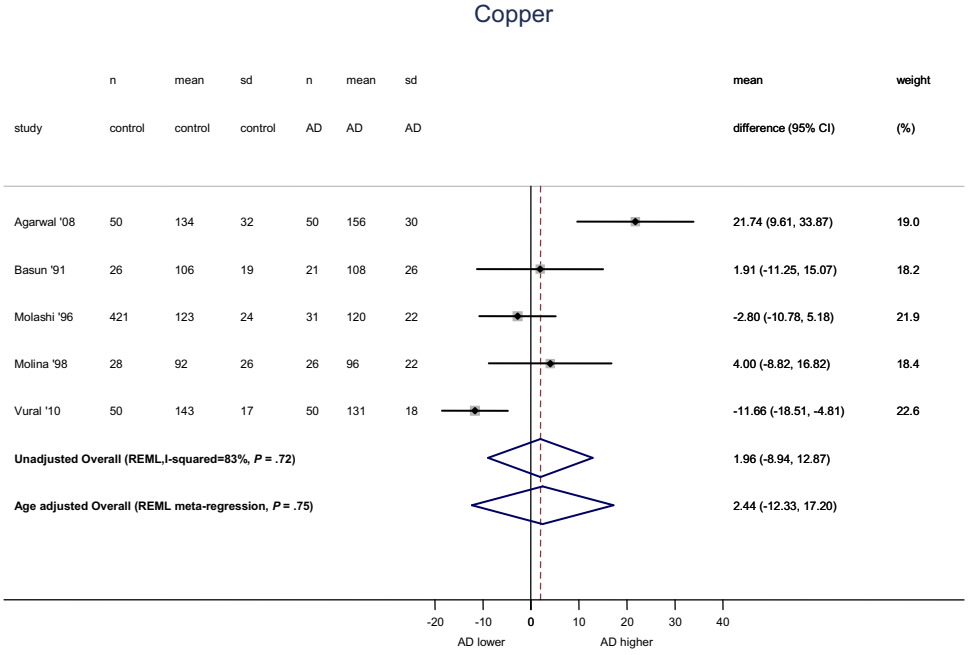


Fig. 7. The age-adjusted overall mean difference in copper levels (μg/dL) between AD patients and controls was not significant. Abbreviations: AD, Alzheimer's disease; CI, confidence interval; REML, restricted maximum likelihood.

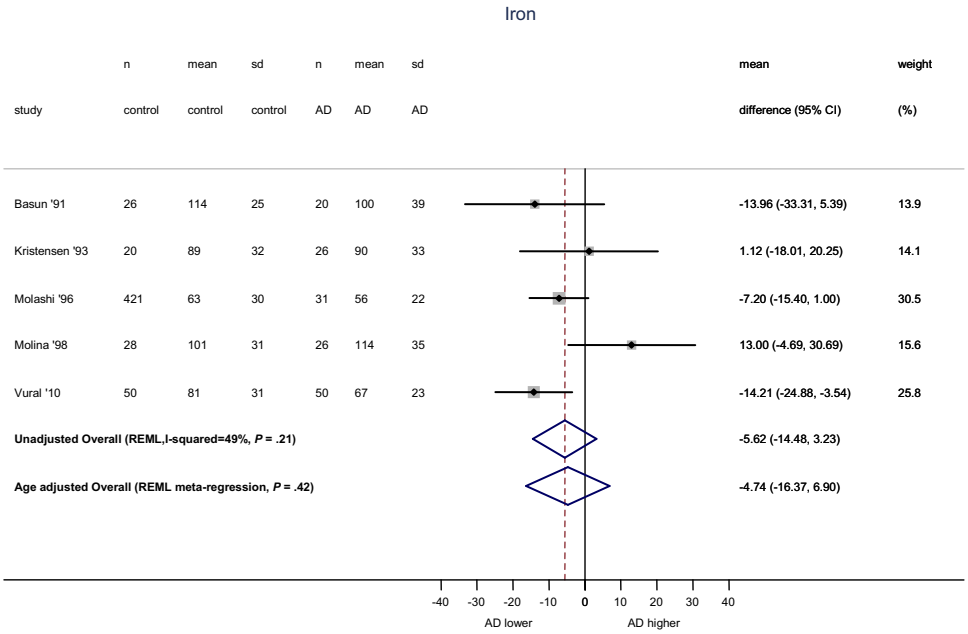


Fig. 8. The age-adjusted overall mean difference in iron levels (μg/dL) between AD patients and controls was not significant. Abbreviations: AD, Alzheimer's disease; CI, confidence interval; REML, restricted maximum likelihood.

The publications that were retrieved in our systematic search also indicated lower levels of DHA in plasma PLs [38,41]. The metabolic change in the de novo DHA synthesis in the liver may contribute to the lower levels of DHA in plasma PLs found in AD patients. Circulating DHA can be obtained from DHA present in some foods and from the conversion of ALA to DHA in the liver. Hence, because of the hepatic metabolic alterations, AD patients may need higher dietary intake of DHA.

Impaired digestion and absorption of nutrients has also been reported in AD. For example, absorption of calcium and zinc depends on active transport by ion channels. Impairments in channel function may lead to impaired dietary uptake (observed by Ferrier et al. [47]) and lower plasma levels. Zinc and calcium share many transport and signaling pathways [80] and have been implicated in the disease process [81]. We found a trend toward lower plasma levels of zinc ($P = .050$) in AD patients. Insufficient publications

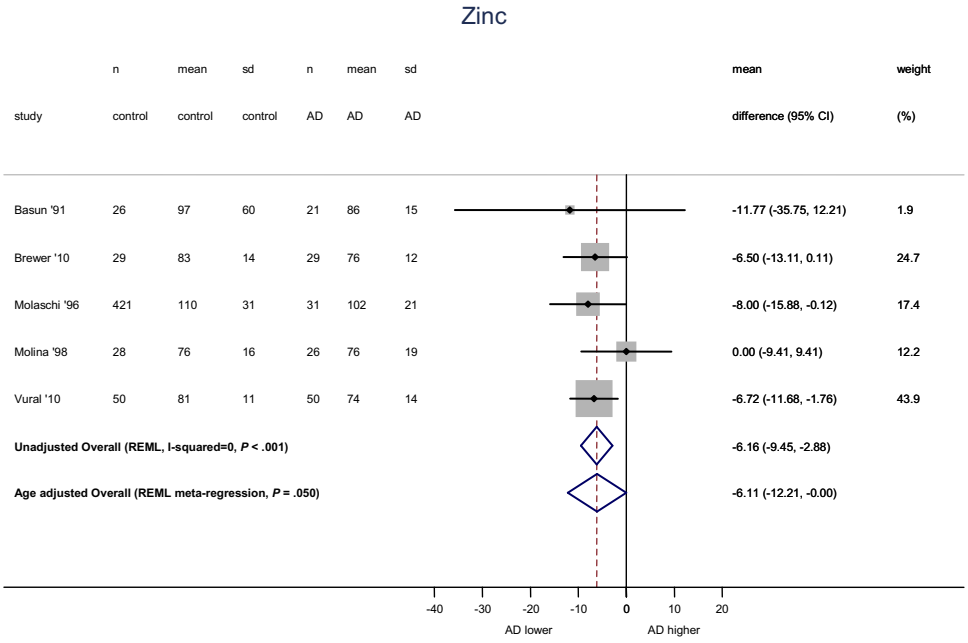


Fig. 9. A trend ($P = .050$) toward lower age-adjusted zinc levels (μg/dL) in AD patients vs controls was observed. Abbreviations: AD, Alzheimer's disease; CI, confidence interval; REML, restricted maximum likelihood.

Table 2
Results of meta-analyses

Nutrient	Number of publications	Meta-analysis In all cases levels were lower in AD patients than in controls	Substudy on AD and control populations reported not to differ in protein and energy nourishment	
			Number of publications	Meta-analysis In all cases levels were lower in AD vs controls
Vitamin A	9 studies [44,65,72,74,75,78,100–102]	$P < .001$	6 studies [44,65,74,75,78,100]	$P = .052$
Folate	31 studies [36,37,46,84,85,103–128]	$P < .001$	14 studies [46,84,105,107,109–112,114, 116,120,122,123,125]	$P \leq .001$
Vitamin B12	33 studies [35–37,44,46,49,84,85,103,105, 107–112,114–123,125–131]	$P < .001$	16 studies [44,46,49,84,105,107,109–112, 114,116,120,122,123,125]	$P \leq .001$
Vitamin C	8 studies [44,74,75,77,79,132–134]	$P < .001$	5 studies [44,74,75,79,133]	$P \leq .001$
Vitamin D	5 studies [47,98,99,135,136]	$P = .075$	NA	NA
Vitamin E	20 studies [44,65,69,71–78,100,101,132, 133,137–141]	$P < .001$	11 studies [44,65,69,74,75,78,100,133,137, 139,141]	$P \leq .001$
Copper	5 studies [39,40,51,53,142]	NS	NA	NA
Iron	5 studies [39,51,53,129,142]	NS	NA	NA
Zinc	5 studies [39,51,53,142,143]	$P = .05$	NA	NA

Abbreviations: AD, Alzheimer's disease; NS, not significant; NA, not applicable.

NOTE. Columns 1–3: meta-analysis per nutrient with included publications. Columns 4–5: meta-analysis substudies using AD and control populations reported not to differ in protein/energy nourishment. In all cases, levels were lower in AD vs controls.

were retrieved to draw definite conclusions on plasma calcium levels, and more research is warranted.

The reason for the significantly lower B-vitamin levels in AD patients remains to be elucidated. The meta-analysis substudies indicate that these lower levels cannot merely be attributed to differences in nutritional status compared with controls. The B vitamins vitamin B6, vitamin B12, and folate are components of the one-carbon metabolism and are essential to maintain the methylation capacity of the cell. Folate and vitamin B12 act as cofactors in the remethylation of homocysteine (HCy) to methionine, and vitamin B6 is the cofactor in the conversion of HCy to cysteine. Hyperhomocysteinemia is considered to be a marker for compromised B-vitamin status. In AD, increases in plasma HCy have been observed compared with control subjects [82–87], and hyperhomocysteinemia can be resolved by dietary addition of B vitamins [66]. It is currently unclear whether Hcy is high because of low B-vitamin intake or if high Hcy and low B-vitamin levels are the result of alterations in liver function and metabolism. Recent research indicates that intake of B vitamins not only decreases plasma HCy, but it also increases plasma DHA and choline levels in a dose-dependent manner [88,89]. DHA and choline are both, as is uridine, rate-limiting precursors in the formation of synaptic membranes (reviewed by Wurtman et al. [90]). Low plasma B-vitamin levels in AD may contribute to compromised release of DHA and choline into the circulation. An increase in the intake of B vitamins may not only resolve effects directly mediated by B vitamins and hyperhomocysteinemia, but it may also affect neuronal functioning by correcting the compromised metabolism of other nutritional substrates.

The lower plasma nutrient levels indicate that patients with AD have impaired systemic availability of several nutrients. Contributing factors might be AD-related alterations in eating behavior, nutrient absorption, digestion, and metabolism and increased utilization of nutrients due to AD pathophysiology (e.g., to combat the oxidative stress). At the same time, the nutrient needs might be increased in AD (e.g., due to a higher requirement for the formation of neuronal membranes and synapses) [91]. This implies that patients with AD may benefit from higher nutrient intake to address the disease-specific requirement and to compensate for their lower nutrient availability. Thus far, several intervention studies have investigated the effects of higher intake of vitamin B, vitamin C, vitamin E, and long-chain omega-3 fatty acids. Some of these studies that were performed in mild AD patients or in early-stage AD, such as MCI, have shown promise [92–95], although others have reported inconclusive or little improvement in neuropsychological function and other outcomes [96]. Two multicountry randomized-control trials investigated the effects of a specific multiple nutrient enrichment (DHA, EPA, UMP, choline, folate, vitamin B6, vitamin B12, vitamin C, vitamin E, selenium, and PLs) through a medical food aiming to provide the nutritional needs for the formation and functioning of synaptic membranes in AD. These studies showed improved memory performance of drug-naïve patients with mild AD and preserved functional connectivity, a derivative of synaptic function [24,97]. Thus, these studies suggest that addressing the increased nutritional requirement of the patient results in improvements in neurophysiological and neuropsychological alterations that are characteristic of AD.

A systematic review and meta-analysis is limited by the quality, quantity, and contents of the available publications. If more studies had been available on omega-3 fatty acids, vitamin B1, vitamin B6, calcium, magnesium, manganese, and selenium, we would also have been able to perform

meta-analysis on these nutrients and would have been able to draw stronger conclusions on differences in their levels between AD patients and controls. Exploration of the contribution of differences in dietary intake between AD and control subjects to plasma nutrient levels was limited by the low availability of habitual intake data. In all cases, we excluded the publications that had used subjects on vitamin supplements. With regard to the assessment of the nutritional status, we were limited by the various nutritional assessment procedures that were reported (e.g., plasma albumin levels, BMI, and MNA). Therefore, it was not possible to use a numerical value for the nutritional status as a confounding variable in the meta-analysis. Hence, we decided to repeat the meta-analysis as a whole, including only the publications reporting that the AD and control populations did not differ in nutritional status. Because malnutrition appears to be more prevalent in more advanced stages of AD, it would have been interesting to investigate whether the differences in plasma nutrient levels were dependent on the stage in the AD spectrum. However, only half of the number of publications reported the mean MMSE score of the included subjects, and those MMSE scores were in the moderate range of 10 to 20. Therefore, it was not possible to perform the meta-analysis with disease stage as a confounding variable or by using only data of mild AD patients.

In conclusion, the current investigation indicates that patients with AD have impaired systemic availability of several nutrients, even in the absence of signs of malnutrition. Provided the postulated role of nutrients in AD, the utility of nutrition may currently be underappreciated and offer potential in AD management. Nutrition-based approaches to reduce the risk of AD and for the management of diagnosed AD are potentially attractive because of the relatively low risk of side effects. The advances in diagnostic methodologies provide opportunity for earlier intervention in the prodromal and preclinical stages of AD. Therefore, an interesting future consideration is to investigate nutrient levels and metabolism in presymptomatic AD, when the damaging subclinical changes may not have yet accumulated to an irreversible degree, to further explore the potential of nutritional approaches to reduce the risk of AD.

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Contributors: S.L.S., M.G., and J.S. designed the study. S.L.S. and S.E. obtained and analyzed the data. T.S. performed the statistical analyses and produced the figures. S.L.S. prepared the first and revised drafts of this manuscript according to the suggestions from co-authors. S.L.S., J.S., M.G., K.Y., J.L., P.K., B.V., and T.S. interpreted the data and revised the manuscript. All authors approved the final version.

S.L.S., S.E., M.G., J.S., and P.K. are employees of Nutricia Advanced Medical Nutrition, Nutricia Research, Danone's specialized health-care unit, which markets the medical food Souvenaid. Nutricia paid consultancy fees to the Department of Medical Statistics, Leiden University Medical Center, Leiden, The Netherlands, for the statistical analysis performed by T.S. in the current report. B.V., K.Y., and J.L. have received consultancy and speakers' fees from Nutricia unrelated to the present study. The authors declare no other conflicts of interest. The corresponding author had full access to the study data and had final responsibility for the decision to submit for publication.

RESEARCH IN CONTEXT

1. Systematic review: To evaluate possible different systemic availability of micronutrients and fatty acids between AD patients and cognitively intact elderly controls, we searched in literature databases. We found many publications reporting various results but no comprehensive review of this area. Hence, we performed a systematic review and meta-analysis to generate an overview of this literature.
2. Interpretation: The highly significant effects across a range of nutrients provide conclusive evidence that plasma nutrients levels are lower in AD, and the lower levels may occur in the absence of, or precede, signs of protein and energy malnutrition. Provided the postulated roles for these nutrients in pathological processes of AD, the current observations provide a lead for nutritional strategies in AD.
3. Future directions: Additional research is needed to investigate the changes in AD-specific eating behavior, nutrient metabolism, and pathophysiology causing the lower nutrient levels in AD and to determine when in the disease spectrum the lower levels start to manifest.

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