Differences in Nutritional Status Between Very Mild Alzheimer's Disease Patients and Healthy Controls

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Abstract.
Background: Studies on the systemic availability of nutrients and nutritional status in Alzheimer's disease (AD) are widely available, but the majority included patients in a moderate stage of AD.
Objective: This study compares the nutritional status between mild AD outpatients and healthy controls.
Methods: A subgroup of Dutch drug-naïve patients with mild AD (Mini-Mental State Examination (MMSE) $\geq$20) from the Souvenir II randomized controlled study (NTR1975) and a group of Dutch healthy controls were included. Nutritional status was assessed by measuring levels of several nutrients, conducting the Mini Nutritional Assessment (MNA®) questionnaire and through anthropometric measures.
Results: In total, data of 93 healthy cognitively intact controls (MMSE 29.0 [23.0–30.0]) and 79 very mild AD patients (MMSE = 25.0 [20.0–30.0]) were included. Plasma selenium ($p < 0.001$) and uridine ($p = 0.046$) levels were significantly lower in AD patients, with a similar trend for plasma vitamin D ($p = 0.094$) levels. In addition, the fatty acid profile in erythrocyte membranes was different between groups for several fatty acids. Mean MNA screening score was significantly lower in AD patients ($p = 0.008$), but not indicative of malnutrition risk. No significant differences were observed for other micronutrient or anthropometric parameters.
Conclusion: In non-malnourished patients with very mild AD, lower levels of some micronutrients, a different fatty acid profile in erythrocyte membranes and a slightly but significantly lower MNA screening score were observed. This suggests that subtle differences in nutrient status are present already in a very early stage of AD and in the absence of protein/energy malnutrition.

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INTRODUCTION

Alzheimer's disease (AD) is a progressive, neurodegenerative disorder with unknown etiology. There are multiple risk factors for AD including age, certain genetic alleles (e.g., apolipoprotein E4), and specific nutritional characteristics [1–3]. Epidemiological studies suggest that a low intake of n-3 fatty acids, B-vitamins, and antioxidants increases the risk of AD [4, 5]. Several nutrients are hypothesized to play a role in the pathological processes of AD. For instance, the n-3 long-chain polyunsaturated fatty acid (n-3 LC-PUFA) docosahexaenoic acid (DHA) reduces abnormal amyloid-β (Aβ) and tau processing [6]. In addition, antioxidants like vitamin E have been shown to protect against Aβ-induced oxidative stress and to stabilize neuronal membranes [7]. These observations suggest that systemic availability of several nutrients is of importance in AD.

AD patients are frequently reported to have lower plasma levels of certain nutrients compared with cognitively intact controls. These lower plasma nutrient levels may be due to reduced daily consumption, increased nutrient use and/or a different metabolism in AD [8]. Recently, a systematic review and meta-analysis compared plasma levels of vitamins, minerals, trace elements, and fatty acids in AD patients with those in cognitive intact controls [9]. Significantly lower plasma levels of vitamin A, C, E, folate, and vitamin B12 were found in AD patients, while there was a trend for lower levels of vitamin D and zinc [9]. About half of the studies included in this review mentioned the stage of the disease of the included AD patients [9], reporting average Mini-Mental State Examination (MMSE) scores ranging from 8 to 21.5. This suggests that limited data are available on nutrient levels in very mild AD patients. Besides lower micronutrient levels, (risk on) protein/energy malnutrition frequently occurs in cognitively impaired elderly subjects [10], and data suggest a positive association between the presence of (risk of) protein/energy malnutrition and the stage of AD [11–14]. Protein/energy malnutrition can potentially be associated with differential blood nutrient levels [15]. Furthermore, (risk of) protein/energy malnutrition as assessed by the Mini Nutritional Assessment (MNA®) questionnaire has been shown to be associated with disease progression in very mild AD patients [16], indicating the importance of studying nutritional status in the early stage of AD.

The aim of the current study was to compare the nutritional status between mild AD patients and healthy controls. We assessed nutritional status by measuring levels of several micronutrients and fatty acids. We used the MNA questionnaire and anthropometric measures to determine (risk of) protein/energy malnutrition. The current study is a substudy of the 24-week, double-blind, randomized, controlled, multi-center Souvenir II study in which the effect of a medical food on memory performance of mild AD patients was investigated [17].

MATERIALS AND METHODS

Study population

The study population of this exploratory Souvenir II substudy consisted of a subgroup of patients with mild AD from the double-blind, randomized, controlled, multi-center, multi-country Souvenir II study and a group of healthy controls, all recruited in the Netherlands. The Dutch Trial Registration Number for the Souvenir II study is NTR1975.

Patients

A total of 84 AD patients across nine study centers agreed to participate. The methodology of the Souvenir II study has been described in detail previously [17]. Briefly, major eligibility criteria included 1) diagnosis of probable AD according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria [18], 2) mild AD as defined by a MMSE score ≥20, 3) age ≥50 years, 4) being drug-naïve for AD medication (cholinesterase inhibitor or N-methyl-D-aspartate receptor antagonist, 5) the presence of a responsible caregiver, and 6) the provision of written informed consent from both the patient and caregiver.

Healthy controls

A total of 98 healthy controls were included across seven (out of nine) Dutch study centers. Healthy controls could be either caregivers, other contacts of the patients or other volunteers and met the following eligibility criteria: 1) age ≥50 years, 2) no current diagnosis of AD, 3) no other condition that might interfere with the definition 'healthy person' according to the investigator's judgment, 4) no participation in another interventional clinical study, and 5) the provision of written informed consent.

Nutritional supplement use

In addition to the eligibility criteria described above, the following exclusion criteria regarding the use of nutritional supplements or dietary habits were applied to both the inclusion of AD patients and healthy controls: 1) use within two months prior to study participation of a) n-3 fatty acid containing supplements or b) oily fish (when consumed more than twice a week) and 2) use within one month prior to study participation of a) vitamins B, C and/or E > 200% of recommended daily intake or b) high energy and/or high protein nutritional supplements and/or medical foods.

Study procedures

For the current analysis, the authors used data on nutritional status collected at the baseline visit of the Souvenir II study for the AD patients and data collected at a single time-point for the healthy controls. In addition, information on demographic data and the use of nutritional supplements was collected from all subjects. At all centers, trained research nurses performed all assessments.

Outcome measures
Plasma micronutrients and fatty acids in plasma phospholipids and erythrocyte membrane: Non-fasting blood samples were taken to determine plasma folate, vitamins B6 and B12, choline, homocysteine (Hcy), uridine, vitamins A, D, and E, selenium, albumin, and plasma phospholipid fatty acids levels. In addition, erythrocytes were collected from the same blood sample to determine fatty acid levels in the erythrocyte membrane.

Anthropometry: Measures of anthropometry included calf circumference and body weight and height to calculate body mass index (BMI) (=body weight [kg]/body height [m]^2). Calf circumference was assessed to the nearest 0.1 cm of the largest part of the left lower leg using calibrated equipment, with the subject in a sitting position, knee and ankle at a 90° angle and with the muscles relaxed. Body height was measured, without shoes, to the nearest cm. Body weight was measured, without shoes and heavy clothing, to the nearest 0.1 kg.

Mini Nutritional Assessment: The MNA® questionnaire was used as a measure of nutritional status [19–21]. The questionnaire contains a 6-item screening part and a 12-item assessment part. All subjects completed the MNA screening part. When subjects scored ≤11 out of 14 points on the screening part (indicative of increased risk for protein/energy malnutrition), the 12-item assessment part was completed as well and a total score was calculated (MNA total = sum of scores on screening part and assessment part; maximum score = 30, a score between 17 and 23.5 is indicative of risk for protein/energy malnutrition, a score lower than 17 is indicative for malnutrition). BMI and calf circumference are part of the MNA screening and total score, respectively.

Subject characteristics and nutritional supplement use

Subject characteristics that were collected for the study included age, gender, education (years of formal education after finishing primary school), and the MMSE score (a short cognitive test with a score ranging from 0 [severe cognitive deficit] to 30 [no cognitive deficit]). Furthermore, the health status of the healthy controls was assessed using the Cumulative Illness Rating Scale for Geriatrics (CIRS-G), which measures chronic medical conditions while considering the severity of chronic diseases in elderly [22]. Diseases are scored by organ systems and grouped into 14 categories. Each category is scored on severity, ranging from 0 (no problem) to 4 (extremely severe). For this study, the CIRS-G total score was calculated (i.e., the sum of scores on the 14 categories, maximum score = 56). If applicable, information was collected on the use of nutritional supplements.

Ethics

Written informed consent was obtained from patients and their caregivers and the healthy controls prior to study participation. The Ethics Committee of each participating study center reviewed and approved the protocol. The study was conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonization Guidelines for Good Clinical Practice as appropriate for nutritional products and the local laws and regulations of the Netherlands.

Biochemical analyses

Blood was collected in tubes containing ethylenediaminetetraacetic acid. All samples were centrifuged (1300 g, 15 min, 4°C), and plasma and erythrocyte aliquots were stored at −80°C until analysis at a central laboratory. Plasma folate and vitamin B12 levels were determined using a competitive protein binding ligand assay, plasma B6 levels were measured by high-performance liquid chromatography

(HPLC). HPLC-electrochemical detection of plasma-free choline was performed according to a method adapted from Fossati et al. [23] as described previously [24]. Plasma albumin was determined using a colorimetric kit, and plasma selenium levels were measured using graphite furnace atomic absorption spectrometry. A chemiluminescent microparticle immuno assay (ARCHITECT assay) was used to determine plasma vitamin D (total 25-hydroxyvitamin D) levels. Plasma vitamin A (retinol) and vitamin E (α-tocopherol) levels were determined simultaneously by HPLC, using ultraviolet-absorbance for detection of retinol and fluorometric properties for detection of α-tocopherol, by comparing with standard solutions [25]. For the determination of plasma Hcy levels, thiol amino acids (free and protein-bound) were reduced with tri-n-butylphosphine. After precipitation with trichloroacetic acid, thiol groups were derivatized with ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate, followed by separation using HPLC with a fluorescence detector [26, 27]. In order to determine plasma uridine levels, perchloric acid was added to the sample. Uridine was extracted by vortexing the solution, followed by separation from other nucleotides/nucleosides using reversed-phase HPLC [28]. Uridine was quantified by measuring its absorbance compared with a standard. Fatty acid compositions of the erythrocyte lipid fraction and the plasma phospholipid fraction were analyzed qualitatively on a gas chromatograph after extraction of the lipids from the erythrocytes/plasma (plasma phospholipids were separated from the other lipid classes in plasma by solid phase extraction) and a methylation step [29–32].

The analysis of plasma Hcy levels was repeated due to relatively high interassay variations in the analyses. Results from the second analysis were used for statistical comparisons due to lower interassay variations in the second compared with the first analysis. Also, the analysis of plasma uridine levels was performed twice, because the assay is sensitive to variation. Pooled data from these analyses were used for statistical comparisons as there were no clear indications to use either one of the analyses.

Statistical analyses

Statistical analyses were performed using SAS® software (SAS Enterprise Guide 4.3 for Windows, SAS Institute Inc., Cary, NC, USA). Data are presented as means $\pm$ standard deviation (SD) unless stated otherwise. Statistical significance was set at $p < 0.05$.

Given the exploratory nature of the study, no sample size calculations were performed. Despite the attempt to select a group of healthy controls with a mean age comparable to the AD patients, there was an age difference at baseline between the healthy controls ($n = 98$) and AD patients ($n = 84$) (respectively 70.4 ± 10.1 versus 74.2 ± 7.6 years, $p = 0.005$). To overcome this imbalance at baseline, it was decided to exclude the five oldest AD patients and the five youngest healthy controls from the dataset prior to the main statistical analyses. Indeed, this resulted in a better balance between groups with respect to age (Table 1), while other subject characteristics at baseline did not change. Therefore, data from 93 healthy controls and 79 AD patients were used for the analyses presented here.

Differences between AD patients and healthy controls were primarily analyzed using an independent samples *t*-test. The non-parametric Mann-Whitney test was used for parameters with a non-normal distribution. As a sensitivity analysis, additional between-group analyses were performed using analysis of covariance (ANCOVA) to correct for the potential confounders 'age' (continuous; years), 'nutritional supplement use' (dummy variable; yes/no), 'use of lipid-modifying agents' (dummy variable; yes/no), and 'years of education' (continuous; years). As some subjects used nutritional supplements, a specific dummy variable for nutritional supplement use was defined for each outcome parameter. Results of the ANCOVA analyses were compared with those of the *t*-test. No correction for multiple testing was performed, and, in line with the exploratory character of the study, differences in levels between groups were tested for each parameter separately using an alpha level of 0.05.

Two AD patients and one healthy control were excluded from the analysis of vitamin B6, vitamin B12, folate, choline, and Hcy because of the recent use of vitamin B12 injections, which might interfere with plasma levels of these parameters.

RESULTS

Subject characteristics of both the AD patients and the healthy controls are shown in Table 1. There were no significant differences between groups for age (*t*-test, $p = 0.125$) and gender (Fisher's Exact test, $p = 0.760$). Years of education was significantly higher in healthy controls compared with AD patients (median [range], 5.0 [0.0–25.0] versus 4.0 [0.0–16.0], Mann-Whitney test, $p = 0.005$). The total MMSE score was significantly higher in healthy controls compared with

Table 1
Subject characteristics

	Healthy controls ($n = 93$)	Mild AD ($n = 79$)	*p*-value
Age, years [range]	71.5 (9.3) [53 90]	73.4 (7.2) [54–84]	0.125*
Male, *n* (%)	43 (46.2%)	39 (49.4%)	0.760[†]
Years of education beyond primary school	5.0 [0.0–25.0]	4.0 [0.0–16.0]	0.005[‡]
MMSE, total score	29.0 [23.0–30.0]	25.0 [20.0–30.0]	<0.001[‡]
CIRS-G, total score	3.76	–	n/a
Nutritional supplement use, *n* (%)	13 (14.0%)	12 (15.2%)	0.832[†]
Multivitamins	4 (4.3%)	1 (1.3%)	0.376[†]
Vitamin E	0	1 (1.3%)	0.459[†]
Vitamin D and analogues	4 (4.3%)	2 (2.5%)	0.688[†]
Vitamin B complex, plain	0	1 (1.3%)	0.459[†]
Hydroxocobalamin	1 (1.1%)	2 (2.5%)	0.594[†]
Folic acid	0	1 (1.3%)	0.459[†]
Calcium	4 (4.3%)	5 (6.3%)	0.734[†]
Other	4 (4.3%)	0	0.126[†]
Lipid-modifying agents use, *n* (%)	20 (21.5%)	31 (39.2%)	0.013[†]

AD, Alzheimer's disease; CIRS-G, Cumulative Illness Rating Scale for Geriatrics; MMSE, Mini-Mental State Examination; –, not measured; n/a, not applicable. Data are mean (standard deviation) or median [range]. *T-test; [†]Fisher's exact test; [‡]Mann-Whitney test.

AD patients (median [range], 29.0 [23.0–30.0] versus 25.0 [20.0–30.0], Mann-Whitney test, $p < 0.001$), suggestive of a cognitively intact, healthy control group versus a very early AD patient group. Moreover, the CIRS-G score in the healthy controls (3.76) was indicative of low co-morbidity. The number of subjects using one or more nutritional supplements was comparable between healthy controls and AD patients (13 (14.0%) versus 12 (15.2%), Fisher's Exact test, $p = 0.832$), while the number of subjects using one or more lipid-modifying agents was higher in the group of AD patients compared with healthy controls (31 (39.2%) versus 20 (21.5%), Fisher's Exact test, $p = 0.013$).

Plasma micronutrients

Results for the comparison of plasma micronutrient levels between the AD patients and the healthy controls are shown in Table 2. Plasma selenium and uridine levels were significantly lower in AD patients compared with healthy controls (*t*-test, respectively $p < 0.001$ and $p = 0.046$). There was a trend for lower plasma vitamin D levels in AD patients compared with healthy controls (*t*-test, $p = 0.084$). No significant differences were observed between the healthy controls and AD patients for plasma levels of folate, choline, vitamin B6, vitamin B12, Hcy, vitamin A, vitamin E, and albumin.

Correction for the potential confounders did not affect any of the observed differences, except for the difference in plasma vitamin D levels, which was not significant after correction for the confounders (ANCOVA, F(1, 166) = 2.34, $p = 0.128$).

Table 2
Descriptive statistics for plasma micronutrients, anthropometrics, and malnutrition scoring for mild stage AD patients and healthy controls

	Healthy controls ($n = 93$)	Mild AD ($n = 79$)	*p*-value*
Plasma micronutrients			
Homocysteine (μM)	13.59 (5.27)	13.84 (5.65)	0.771
Folate (nM)	17.53 (8.59)	16.07 (10.12)	0.309
Choline (μM)	8.45 (2.31)	8.79 (2.19)	0.345
Vitamin B12 (pM)	314.5 (98.4)	348.4 (188.8)	0.158
Vitamin B6 (nM)	61.28 (44.73)	57.85 (47.27)	0.631
Uridine (μM)	3.38 (1.29)	3.03 (0.95)	0.046
Selenium (μM)	1.18 (0.26)	1.04 (0.24)	<0.001
Vitamin A (μM)	3.30 (0.69)	3.46 (0.73)	0.151
Vitamin D (nM)	52.92 (22.23)	47.30 (19.69)	0.084
Vitamin E (μM)	32.89 (7.23)	31.89 (9.07)	0.432
Anthropometrics			
Body weight (kg)	73.79 (11.96)	74.89 (12.79)	0.560
Body height (m)	1.70 (0.08)	1.69 (0.08)	0.361
BMI (kg/m^2)	25.4 (3.6)	26.2 (4.1)	0.212
Calf circumference (cm)	35.5 (3.1)	35.9 (3.2)	0.390
Malnutrition scoring			
MNA screening score	13.2 (1.2)	12.6 (1.5)	0.008
MNA total score[†]	24.8 (1.6)	23.4 (2.7)	0.171
Albumin (g/L)	40.51 (2.83)	41.14 (2.76)	0.141

AD, Alzheimer's disease; BMI, body mass index; MNA, Mini Nutritional Assessment. Data are mean (standard deviation). *Independent samples *t*-test, except for MNA total score (see [†]) [†]MNA total score: Healthy controls ($n = 10$), AD ($n = 10$). Fisher's Exact test.

Fatty acids in erythrocyte membrane and plasma phospholipids

Table 3 shows the results of the between-group comparisons of the erythrocyte and plasma fatty acid

Table 3
Descriptive statistics for fatty acids in erythrocyte membranes and plasma phospholipids

	Erythrocyte fatty acids			Plasma phospholipid fatty acids		
	Healthy controls ($n = 93$)	Mild AD ($n = 79$)	p-value*	Healthy controls ($n = 93$)	Mild AD ($n = 79$)	p-value*
EPA (%)	0.98 (0.40)	0.97 (0.44)	0.849	1.21 (0.62)	1.15 (0.72)	0.523
DHA (%)	3.44 (0.99)	2.99 (1.14)	0.006	3.63 (0.93)	3.55 (0.96)	0.572
DPA (%)	1.78 (0.36)	1.64 (0.54)	0.046	0.94 (0.17)	0.96 (0.17)	0.509
DHA+EPA (%)	4.42 (1.30)	3.95 (1.48)	0.028	4.84 (1.38)	4.69 (1.54)	0.511
n-3 LC-PUFA (%)	6.20 (1.54)	5.59 (1.92)	0.024	5.78 (1.44)	5.65 (1.60)	0.577
Palmitic acid (%)	24.82 (1.40)	26.64 (2.76)	<0.001	27.90 (1.49)	28.52 (1.28)	0.005
Stearic acid (%)	9.00 (0.72)	9.12 (0.84)	0.309	13.19 (1.24)	12.87 (1.09)	0.077
Elaidic acid (%)	0.18 (0.11)	0.23 (0.11)	0.006	0.13 (0.05)	0.12 (0.06)	0.119
Oleic acid (%)	15.80 (1.72)	15.59 (1.54)	0.387	9.12 (1.20)	9.18 (1.47)	0.788
Cis-vaccenic acid (%)	1.14 (0.16)	1.14 (0.17)	0.981	1.34 (0.20)	1.36 (0.22)	0.515
Linoleic acid (%)	15.01 (2.53)	13.06 (2.41)	<0.001	21.10 (2.86)	20.61 (2.71)	0.255
Gamma linolenic acid (%)	0.15 (0.10)	0.09 (0.09)	<0.001	0.12 (0.06)	0.13 (0.07)	0.227
Alpha linolenic acid (%)	0.28 (0.10)	0.16 (0.09)	<0.001	0.22 (0.09)	0.18 (0.06)	<0.001
Stearidonic acid (%)	0.09 (0.04)	0.11 (0.13)	0.231	0.05 (0.01)	0.05 (0.01)	0.632
Dihomo gamma linolenic acid (%)	1.49 (0.30)	1.30 (0.33)	<0.001	2.97 (0.77)	2.91 (0.68)	0.586
Arachidonic acid (%)	10.94 (1.43)	9.76 (2.69)	<0.001	9.47 (1.84)	9.91 (2.38)	0.194
Cis docosapentaenoic acid (%)	0.63 (0.14)	0.70 (0.25)	0.026	0.21 (0.06)	0.21 (0.08)	0.790

AD, Alzheimer's disease; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; n-3 LC-PUFA, n-3 long-chain polyunsaturated fatty acids. Data are mean (standard deviation). *Independent samples t-test.

profiles. The percentage of DHA, docosapentaenoic (DPA), DHA + eicosapentaenoic acid (EPA), linoleic acid, alpha, gamma and dihomo gamma linolenic acid, arachidonic acid, and n-3 LC-PUFA of total fatty acids in the erythrocyte membrane was significantly lower in the AD patients versus healthy controls, whereas the percentage of palmitic acid, elaidic acid, and cis-docosapentaenoic acid was higher in AD patients. The proportion of the other analyzed fatty acids in the erythrocyte membrane, including EPA, was not significantly different between groups. In plasma phospholipids, the percentage of alpha linolenic acid of total fatty acids was significantly lower and the percentage of palmitic acid significantly higher in AD patients versus healthy controls. The other fatty acids in plasma phospholipids were not significantly different between groups.

Correction for the potential confounders did not affect the results of the fatty acid analyses, except for the percentage of elaidic acid in plasma phospholipids, which was significantly lower in AD patients versus healthy controls after correction for the confounders (ANCOVA, F(1, 159) = 3.96, p = 0.048).

Anthropometry

Calf circumference, body weight, height, and BMI were not significantly different between AD patients and healthy controls (Table 2). Also after correction for potential confounders, no differences between groups were observed.

Mini nutritional assessment

Results from the MNA questionnaire are shown in Table 2. The MNA screening score was significantly lower in the AD patients compared with the healthy controls (12.6 ± 1.5 versus 13.2 ± 1.2, t-test, p = 0.008). The MNA total score (applied to the subset of subjects with an initial low MNA screening score of ≤ 11, n = 10 in each group) was not significantly different between groups (t-test, p = 0.171). The mean MNA total score, however, was below the cut-off point for normal nutritional status (i.e., <24.0) in the AD patients ($23.4 \pm 2.7/n = 5$ scored <24.0), but not in the healthy controls ($24.8 \pm 1.6/n = 1$ scored <24.0), suggesting increased risk for protein/energy malnutrition in only a small subgroup of AD patients.

Correction for potential confounders did not affect any of the observed differences.

DISCUSSION

We found that plasma levels of selenium and uridine were lower and plasma levels of vitamin D tended to be lower in very mild AD patients (MMSE = 25.3) compared with healthy controls. The fatty acid profile in erythrocyte membranes also showed differences between groups, including a lower proportion of docosahexaenoic and linoleic acid and total n-3 LC-PUFA and a higher proportion of palmitic and elaidic acid in AD patients. The studied AD population was not at nutritional risk: their MNA screening score was

≥12 (<12 indicates risk on protein/energy malnutrition) [10], their BMI was >23 kg/m² [33], their calf circumference >31 cm [10], and their albumin >35 g/L. Therefore, the observed differences in nutrient levels seem unlikely to have originated from protein/energy malnutrition. However, based on this study we cannot exclude that the nutritional differences measured are caused by differences in intake secondary to the cognitive decline. Other studies have proven compensatory neuronal activity in AD, which make an intrinsic shortage of certain micronutrients linked to increased needs more likely [34]. Moreover, specific nutrient intake deficiencies and weight loss may occur in AD despite adequate or even increased caloric intake, possibly due to defects in systemic nutrient homeostasis such as malabsorption and decreased interest in specific nutritional components [35]. Although unintended weight loss frequently occurs in AD (approximately 40% of patients) and has been reported during all stages of the disease [36, 37], the mild AD patients in this study did not suffer from energy malnutrition and had a relatively high mean BMI. This suggests that caloric intake was adequate in the currently studied mild AD population, but this was not specifically measured. It has recently been shown that BMI might even increase in prevalent AD and that changes in BMI during disease may depend on the patient's initial BMI [38]. Our results from the MNA questionnaire are in agreement with a recent study of the MNA in Dutch AD patients that showed that outpatients of a memory clinic showed no protein-energy malnutrition, while a subgroup (14%) showed higher risk on such malnutrition [39]. In addition, the findings on nourishment status in the currently studied AD patients are in line with literature data, which suggest a positive association between prevalence of (risk of) protein/energy malnutrition and stage of AD [11–14, 39].

Many studies have compared levels of nutrients between AD patients and controls. In a recent meta-analysis of such trials, a similar trend as in this study was found for lower vitamin D levels in AD patients [9]. Furthermore, lower levels of vitamin A, B12, E, and folate in AD patients compared with cognitive intact elderly controls were observed. In the present study, no differences between groups in the levels of these vitamins were observed, which may be explained by several factors. Firstly, in the current study very mild AD patients were included, whereas studies in the meta-analysis included patients with a more advanced stage of the disease: differences in certain nutrients might only become apparent in more advanced stages of the disease. Secondly, differences in dietary intake or

habits between studies performed in different countries may contribute to these findings and the current study was performed in one country only. Finally, the sample size may have been too small to detect differences between groups in certain nutrients.

Lower selenium levels as currently found are also described in other [40, 41], but not in all studies [42, 43]. Selenium is a micronutrient which is involved in several molecular pathways that are thought to be important in the progression of AD [44]. It is, for instance, part of the antioxidant enzyme glutathion-peroxidase which helps to protect lipid precursors and the resulting membrane components from lipid peroxidation. Lower plasma selenium levels in AD patients compared with healthy controls may have an association with the process of oxidative stress known to be present in AD. To compensate for a possible increased use of selenium in AD, a higher intake might be necessary.

Several differences in fatty acid levels in erythrocyte membranes were observed between groups in the present study, including lower proportions of n-6 and n-3 polyunsaturated fatty acids in mild AD patients. These differences are in line with reported lower dietary intake of linoleic and alpha linolenic acid and DHA in early AD compared with age-matched controls [45]. The observed lower circulating levels of n-6 fatty acids are generally believed not to be of concern due to their high availability in foods through plant oils and subsequent high and probably abundant tissue levels [46]. In addition, we found higher levels of the *trans* fatty acid elaidic acid in erythrocyte membranes of the AD patients. *Trans* fatty acids have been suggested to enhance amyloidogenic processing of the amyloid precursor protein and increase the production and aggregation of Aβ *in vitro* [47]. However, the suggested link between *trans* fatty acid intake and AD risk or cognitive decline is not consistently supported by cohort studies showing either a positive association [48] or no association [49, 50].

In the current study, no significant differences in plasma DHA, EPA, and n-3 LC-PUFA levels were found between groups. Levels of n-3 LC-PUFA in plasma, however, are a poorer marker of long term dietary intake than levels in erythrocyte membranes [51]. The observed lower erythrocyte DHA and total n-3 LC-PUFA in the mild AD patients are consistent with previous observations [52]. Recently, reduced activity of the last step enzyme in DHA synthesis pathway, peroxisomal D-bifunctional protein was found in the liver of AD patients and this coincided with lower DHA levels in the brain and liver tissue [53]. This metabolic

change may contribute to the lower DHA and not EPA erythrocyte membrane levels as currently found and is consistent with a recent meta-analytic review reporting lower plasma levels of DHA and total n-3 LC-PUFA in dementia patients [54].

The plasma levels of uridine were lower in AD patients in the present study. This finding confirms the results of a very recent study reporting lower plasma uridine levels in AD patients versus healthy controls [55]. Although the cause of these lower levels of uridine is unclear, it has been speculated that _de novo_ synthesis of uridine in the liver may be reduced in AD [8]. The lower uridine observed in the plasma of AD patients is likely to result in less transport of uridine into the brain as uridine readily enters the brain via the blood-brain barrier high affinity transporter of nucleosides CNT2 [56]. Uridine is, via the Kennedy pathway, a precursor in the synthesis of phospholipids in neuronal membranes, which are depleted in AD [57, 58]. Combined dietary enrichment of uridine and DHA has been reported to promote the synthesis of brain phospholipids, hippocampal dendritic spines, and synaptic proteins, all prerequisites for synaptogenesis [59]. In AD, cerebrospinal fluid uridine levels [60] and hippocampal levels of DHA [61, 62] were found to be lower than in controls, which together may reflect a reduced capacity for neuronal membrane synthesis and may result in inability to compensate for the synaptic loss associated with AD and be associated with lower levels in the circulation. Although tissue target levels of uridine and DHA are not well defined for the general population, they should probably be higher for people with AD provided their role in compensational neuronal membrane synthesis. Further research is needed to elucidate the cause and possible clinical implications of lower circulating uridine and DHA levels in AD.

Decreased levels of certain nutrients as observed in AD patients suggest a potential role for the supplementation of specific nutrients in the management of AD. Until now, several intervention studies investigated the effects of supplementation with single nutrients, for instance vitamin B12, vitamin E, or n-3 LC-PUFA in dementia patients, showing no or little improvements of cognitive function and other outcomes [63–65]. The outcomes of single nutrient studies may be explained by the fact that nutrients most likely act in a synergistic way [66]. The findings of the current study are in line with this, since several nutrients, rather than a single nutrient, were observed to be present in lower levels. An intervention with a specific combination of nutrients in the form of a medical food has been shown to improve memory performance of drug-naïve patients

with mild AD and has been suggested to preserve functional connectivity in AD [17, 67]. Together, these data may suggest that combinations of nutrients can be more powerful than single nutrients, as there may be a positive interaction between them. A similar synergistic effect of a complex combination of nutrients has recently been described for the Mediterranean diet enriched with walnut oil for cardiovascular endpoints [68].

The present study has some limitations: 1) Control subjects are not precisely matched for age and gender. The average age of AD patients is almost 2 years higher and there are slightly more men in the AD group (49.4% versus 46.2%). These differences may have influenced the results. However, analyses on data of 66 pairs of age- and gender-matched subjects and excluding subjects using nutritional supplements yielded similar results (data not shown). 2) No data were collected on dietary intake of the study subjects, so the possible contribution of (differences in) dietary intake (specific (micro)nutrients and/or caloric intake) on the results cannot be determined. 3) Due to the exploratory character of the study, no correction for multiple testing was performed. To confirm current study findings, further research is warranted. 4) To avoid the influence of regional differences in nutrient levels via for instance different dietary habits or food fortification, this substudy was performed in one small country with relatively homogenous food habits, the Netherlands. This may, however, limit its representation for other regions.

In conclusion, the study results showed lower levels of some micronutrients, a different fatty acid profile in erythrocyte membranes, and a slightly, but significantly lower MNA screening score in very mild AD patients compared with healthy controls, whereas the AD patients were neither malnourished nor at risk of malnutrition. These data suggest that in a very early stage of AD and in the absence of protein/energy malnutrition, subtle differences in nutrient status are present as compared to healthy controls. Given the proposed role of nutrients and nourishment status in AD pathology and progress, these data need to be corroborated in an independent sample, possibly also including preclinical and prodromal stages of AD to study when these changes occur in the disease progress.

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