Nutrition and metabolic profiles in Alzheimer's disease

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Chapter 4.1

Associations between nutrient intake and corresponding nutritional biomarker levels in a memory clinic cohort: the NUDAD project

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Abstract

Introduction: Nutrition is a putative determinant of Alzheimer's disease (AD), but the relation between nutrient intake and nutrient status has not been investigated in clinical populations. We examined associations between self-reported nutrient intake and blood nutrient status in a memory clinic cohort.

Methods: We included 22 patients with mild cognitive impairment (MCI), 29 patients with AD and 39 controls. The 238-item HELIUS food frequency questionnaire (FFQ) was used to assess nutrient intake from food and supplements. Nutritional biomarker concentrations in blood were measured to assess nutrient status. Nutrient intake and nutrient status of vitamin A, B1, B6, B12, C, folate, zinc, total omega-3 fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) were examined. Linear regression models were used to examine associations between nutrient intake and nutrient status. If interaction terms for diagnostic group*nutrient intake were significant ($p<0.10$), analyses were stratified by diagnostic group (control, MCI, AD).

Results: In the total cohort, nutrient intake and their corresponding nutritional biomarker levels were associated for vitamin B1, vitamin B12, folate and EPA (range B(SE) 0.35-0.49(0.09-0.11), $p\leq0.001$) and a trend was found for vitamin A (0.23(0.12), $p=0.057$). Interactions with diagnosis indicated associations for vitamin B6 in all diagnostic groups (strongest in MCI), for omega-3 fatty acids and DHA in controls, and for vitamin C in AD.

Discussion: In this small study, nutrient intake and nutrient status were moderately associated, and similarly associated across diagnostic groups, particularly for B-vitamins and EPA. Our findings indicate that the HELIUS FFQ provides valid estimates of nutrient intake in a memory-clinic cohort.

Introduction

Diet is a promising intervention target to prevent or slow Alzheimer's disease (AD) [1, 2]. Clinical stages of cognitive decline offer a unique opportunity for dietary interventions [2]. Nutritional assessment methods to estimate nutrient intake have, however, not been validated in memory-clinic cohorts.

Nutrient intake is not expected to perfectly correspond to nutrient status, as many genetic and metabolic factors influence nutrient status [3, 4]. Moreover, nutritional assessment methods rely on memory and are sensitive to misreporting [5]. To identify dietary components that could affect health status, however, the relation between dietary intake and nutrient status is relevant. Several studies have compared the nutrient intake assessed by a food frequency questionnaire (FFQ) with their corresponding nutritional biomarker levels in blood in healthy populations. For nutrients such as β-carotene, vitamin C, folate and omega-3 fatty acids moderate correlations (range correlation coefficients: 0.17-0.46) have been reported [6-9]. In addition, correlations between nutrient intake and nutrient status have been shown to be much stronger when dietary supplement intake is included [10].

Far fewer studies have investigated the relation between nutrient intake and nutrient status in cognitively impaired individuals. One study, in subjects with and without mild cognitive impairment (MCI), found associations between FFQ estimates of intake and biomarker levels of omega-3 fatty acids and carotenoids, but only in cognitively normal subjects and not in subjects with MCI [11]. A second study however, showed similar associations between FFQ estimates of intake and biomarker levels of omega-3 fatty acids in cognitively healthy subjects and subjects with MCI and dementia [12]. So far, no studies have investigated associations of nutrient intake and nutrient status in memory-clinic populations. Hence, we assessed the association between nutrient intake assessed by FFQ and nutrient status measured by nutritional biomarkers in blood in a clinical sample of controls, MCI and AD patients.

Methods

Study population

NUDAD (Nutrition, the Unrecognized Determinant in Alzheimer's Disease) is a prospective cohort study investigating nutritional determinants in predementia stages and AD [13]. The NUDAD study is part of the Amsterdam Dementia Cohort [14]. All patients underwent standardized cognitive screening including neuropsychological and neurological examination, blood sampling, a lumbar puncture, and magnetic resonance imaging. Diagnoses were made in

a multidisciplinary consensus meeting. As controls, we used individuals with subjective cognitive decline (SCD), who presented with memory complaints at our memory clinic, but performed normal on all clinical examinations, i.e. criteria for MCI, dementia, neurological or psychiatric disorders were not fulfilled. The diagnosis MCI or probable AD was based on the core clinical criteria of the National Institute on Aging-Alzheimer's Association criteria [15, 16]. Apolipoprotein E (APOE) genotype was determined using a QIAxcel DNA Fast Analysis kit (Qiagen, Venlo, The Netherlands) after DNA isolation from 7-10ml (ethylenediaminetetraacetic acid) EDTA blood. Participants were classified as ε4 carrier (≥ 1 allele) or non-carrier [17].

Within the NUDAD project, a subgroup consented to participate in an in-depth study on nutrition [18]. In the present study we used the cross-sectional baseline data of the 91 participants from the NUDAD subgroup, who completed a food frequency questionnaire (FFQ) and had at least one nutritional biomarker measurement; 30 patients with AD, 22 patients with MCI and 39 controls. Written informed consent was obtained from all participants and the local Medical Ethical Committee approved the study.

Assessment of dietary and supplement intake

Usual dietary intake was measured using the semi-quantitative 238-item HELIUS FFQ with a reference period of the prior four weeks [19]. We selected 10 nutrients that have been associated with pathological mechanisms of AD [20, 21]. The HELIUS FFQ was specifically developed to measure nutrient intake of mono-, di- and polysaccharides, fiber, animal and vegetable protein, total fat, fatty acids clusters, alcohol, calcium, iron, vitamin A, vitamin B2, folate, vitamin B12, vitamin C, and vitamin D [19]. From these prioritized nutrients, we included vitamin A, vitamin B12, vitamin C, folate, total omega-3 fatty acids, docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA). In addition, we included three other nutrients that have been associated with AD; vitamin B1, vitamin B6 and zinc. Daily nutrient intake was obtained by multiplying the nutrient intake of each food item (from the Dutch Food Composition Table 2011) by reported frequency of intake, and subsequent summing over all food items [22]. One women with an implausible energy intake >3500 kcal was excluded, leaving 90 participants for data-analysis [4]. We additionally asked participants to report their nutritional supplement use. For each nutritional supplement, the frequency and amount of intake was recorded. If not specified by the participant, nutrient dosage was obtained from the (online) instructions leaflet of a supplement. If the nutrient dosage was unavailable (n=11), it was imputed with the nutrient dosage of the most frequently used comparable supplement in this study. To account for differences in bioavailability of natural and synthetic folate, folate intake was expressed as folate equivalents. Total folate intake was

defined as the sum of dietary folate intake µg/day + 1.7* supplement folic acid (µg/day) [23]. Vitamin A and β-carotene (provitamin A) were expressed as retinol activity equivalents, using the following conversion factors for supplements: 1 µg retinol = 2 µg β-carotene [24]. Nutrient intake was defined as the sum of dietary and supplement intake.

Nutritional biomarker measurements

Most blood samples (68-83%) were obtained within three months from the FFQ assessment. Both folic acid and vitamin B12 which were measured in non-fasting samples on a routine basis at patients' baseline visit. Vitamin A levels was measured in both fasting (89%) and non-fasting (11%) samples. All other nutritional biomarker were measured in fasting plasma, serum or whole blood samples. Serum and EDTA plasma samples were collected in 6 mL tubes and whole blood was collected in 2mL heparin tubes. For vitamin C measurements EDTA plasma was stabilized using metaphopshoric acid (Merck, Kenilworth, USA). Within 2 hours, blood was centrifuged at 1800x *g* for 10 minutes at room temperature, aliquoted in polypropylene vials of 0.5 ml (Sarstedt, Numbrecht, Germany) and stored at -80^{0}C until further analysis. **Supplementary text 1** contains detailed information on measurement methods of the nutritional biomarkers.

Statistical analyses

Statistical analyses were performed using R version 3.5.3 (2019-03-11). Nutrient intake (diet + supplement) and nutritional biomarkers were log transformed and subsequently converted to z-scores to enable comparison of effect sizes. Diagnostic group differences were tested using analysis of variance with post-hoc Bonferroni adjusted t-tests, χ^2 and Kruskal-Wallis tests. The difference in nutritional biomarker levels and dietary intake of nutrients between nutritional supplements users and non-supplement users was assessed using linear regression analyses adjusted for sex, age and clinical diagnosis. To investigate associations of nutrient intake (diet + supplement) with nutritional biomarker levels we used linear regression analyses. We present an unadjusted model (model 1) and a model adjusted for sex, age, clinical diagnosis, and total energy intake (model 2). To assess if these associations differed per diagnostic group (MCI, AD, controls), we added interaction terms (dummy diagnosis * nutrient intake) to the model. If there was an interaction between diagnosis and nutrient intake ($p<0.10$), we stratified the results by diagnostic group and showed the effect sizes for each diagnostic group separately. If there was no significant interaction, the interaction term was removed and the model without interaction term is shown. Lastly, we performed four sensitivity analyses: 1) excluding supplement users for each nutrient separately (e.g. excluding vitamin B1 supplement users for the association between vitamin B1 nutrient intake and blood levels) , 2) excluding

imputed supplement dosages of 11 participants, 3) excluding blood samples taken more than 3 months from FFQ administration 4) excluding those who filled in the FFQ by, or with the help of, a relative/interviewer. In the sensitivity analyses, we used the same stratifications by diagnostic group as indicated by the interaction terms (dummy diagnosis * nutrient intake) in the main analyses. A p-value < 0.05 was considered significant.

Results

Controls were younger than patients with MCI or AD (**Table 1**). AD patients had fewer years of education and a lower MMSE score than patients with MCI and controls. AD patients were more often APOE ε4 carriers than controls. In AD, the FFQ was more often filled in by, or with the help of, a proxy than in controls. Vitamin and mineral supplement use was observed in 49% of the controls, 41% of the MCI patients and 41% of the AD patients (p>0.05). Intake (diet + supplement) of omega-3 fatty acids was higher in controls than AD patients. Levels of zinc were higher in controls and MCI than AD, levels of total omega-3 fatty acids and EPA were higher in MCI than in AD (**Table 1**). All nutritional biomarker levels were higher in supplement users than in non-supplement users, except for levels of vitamin A and zinc (**Supplementary table 1**). Dietary intake of vitamin B1 and zinc was lower in supplement users than in non-supplement users.

Table 1 Characteristics of the study population according to diagnostic group

Characteristics	Categories	Controls	MCI	AD
General				
N		39	22	29
Age (years), mean±SD		62.8 ± 6.9	70.1 ± 7.1 [a]	69.9 ± 9.4 [a]
Female, n(%)		21 (54)	6 (27)	15 (52)
Living situation, n(%)	Independent, with partner	33 (85)	20 (91)	21 (72)
	Independent, alone	6 (15)	2 (9)	8 (28)
Education (Verhage scale), median (IQR)		6 (5 - 7)	6 (5 - 7)	5 (4 - 6) [ab]
MMSE, median (IQR)		29 (27 - 30)	26 (25 - 28) [a]	24 (21 - 26) [ab]
APOE ε4 carrier, n (%)		14 (38)	13 (68)	18 (69) [a]
BMI, mean±SD		25.7 ± 4.0	25.1 ± 3.3	25.9 ± 4.5
Vitamin/Mineral supplement users, n(%)		19 (49)	9 (41)	12 (41)
FFQ filled in by, n(%)	Participant	32 (84)	13 (62)	12 (41) [a]
	Partner/Child/Interviewer	2 (5)	3 (14)	7 (24) [a]
	Participant with partner	4 (11)	5 (24)	10 (34) [a]
Meals prepared mostly by, n(%)	Participant	20 (57)	6 (32)	13 (48)
	Partner	15 (43)	13 (68)	14 (52)
	Ready-made meals	0 (0)	0 (0)	0 (0)
Nutrient (diet + supplement) intake assessed by FFQ				
Retinol activity equivalents, mcg/d		1028.4 (700.8 - 1526.0)	997.9 (702.5 - 1144.8)	749.9 (601.5 - 1105.0)
Vitamin B1[#], mg/d		1.0 (0.9 - 1.5)	1.0 (0.9 - 1.2)	0.9 (0.8 - 1.3)
Vitamin B6[#], mg/d		1.8 (1.5 - 2.6)	1.7 (1.4 - 2.1)	1.6 (1.4 - 2.1)

Table 1 Continued.

Characteristics	Categories	Controls	MCI	AD
Vitamin B12, mcg/d		5.5 (3.9 - 7.8)	6.6 (4.6 - 8.0)	4.6 (4.2 - 7.4)
Vitamin C, mg/d		144.1 (112.0 - 234.5)	146.8 (124.9 - 207.5)	131.2 (91.9 - 195.3)
Folate equivalents, mcg/d		313.9 (273.2 - 487.2)	318.4 (268.2 - 410.3)	307.7 (245.1 - 372.3)
Zinc[#], mg/d		10.9 (8.9 - 13.1)	11.8 (9.2 - 13.5)	10.5 (9.4 - 12.9)
Omega-3 fatty acids, % of total fatty acids intake		3.0 (2.7 - 3.8)	3.0 (2.3 - 3.9)	2.7 (2.0 - 3.2) [a]
DHA, % of total fatty acids intake		0.2 (0.1 - 0.4)	0.2 (0.0 - 0.3)	0.1 (0.0 - 0.3)
EPA, % of total fatty acids intake		0.2 (0.1 - 0.3)	0.2 (0.1 - 0.3)	0.1 (0.0 - 0.2)
Nutrient status assessed by nutritional biomarkers in blood				
Vitamin A, µmol/L		2.4 (2.0 - 2.5)	2.2 (2.0 - 2.5)	2.1 (1.7 - 2.5)
Vitamin B1, nmol/L		128.0 (108.0 - 142.0)	123.0 (108.0 - 130.0)	124.0 (110.0 - 151.0)
Vitamin B6, nmol/L		92.3 (72.6 - 116.0)	92.3 (78.4 - 123.0)	83.7 (65.6 - 136.0)
Vitamin B12, pmol/L		280.0 (223.0 - 318.0)	254.0 (224.5 - 314.5)	284.0 (174.0 - 499.0)
Vitamin C, µmol/L		79.9 (56.0 - 98.8)	65.4 (43.5 - 75.9)	70.0 (43.7 - 84.7)
Folate, nmol/L		17.7 (13.2 - 23.7)	15.3 (13.6 - 25.8)	16.3 (11.6 - 23.4)
Zinc, µmol/L		14.4 (13.6 - 15.3)	13.8 (13.1 - 14.6)	12.9 (11.3 - 14.0) [ab]
Omega-3 fatty acids, % of plasma total fatty acids		3.9 (3.2 - 4.6)	4.3 (3.7 - 5.0)	3.2 (2.8 - 4.0) [b]
DHA, % of plasma total fatty acids		1.7 (1.2 - 2.1)	2.0 (1.4 - 2.3)	1.4 (1.2 - 1.9)
EPA, % of plasma total fatty acids		1.0 (0.6 - 1.3)	1.4 (1.1 - 1.7)	0.8 (0.7 - 1.0) [b]

Abbreviations: AD, Alzheimer's disease; APOE, apolipoprotein E; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FFQ, food frequency questionnaire; IQR, interquartile range; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; SD, standard deviation

Different $p<0.05$ from [a] controls, [b] MCI. [#] the HELIUS FFQ was not designed to measure these nutrients. Education was rated using Verhage's scale ranging from 1 (low) to 7 (high) [25]. Group differences were tested with one-way analysis of variance (ANOVA) with post-hoc Bonferroni adjusted t-tests, χ^2 or Kruskal-Wallis test when appropriate. Measures of nutrient intake and nutrient status were log-transformed and converted to z-scores prior to analysis.

Table 2 shows the associations between nutrient (diet + supplement) intake and their corresponding nutritional biomarker levels in blood. In the total cohort, intake and blood levels were associated for vitamin B1, vitamin B12, folate and EPA (B(SE) 0.48 (0.09), 0.35 (0.10), 0.49 (0.09), 0.41 (0.11), p≤0.001), and a trend was observed for retinol activity equivalents (B(SE) 0.23 (0.12), p=0.057). In addition, interactions were found between diagnostic group and nutrient intake for vitamin B6, vitamin C, total omega-3 fatty acids and DHA. Stratification by diagnostic group showed that intake and blood levels of vitamin B6 were associated in all diagnostic groups, but associations were strongest in MCI (B(SE) controls; 0.78 (0.06), p<0.001, MCI; 1.05 (0.20), p<0.001, AD; 0.45 (0.14), p=0.006). Intake and blood levels of total omega-3 fatty acids and DHA were only significantly associated in controls (B(SE) controls; 0.50 (0.20), p=0.021, 0.38 (0.13), p=0.009, MCI; -0.38 (0.30), p=0.230, -0.13 (0.27), p=0.646, AD; 0.25 (0.17), p=0.166, -0.27 (0.25), p=0.285) and vitamin C was only associated in AD (B(SE) controls; 0.22 (0.15), p=0.152, MCI; 0.03 (0.27), p=0.927, AD; 0.75 (0.11), p<0.001). No associations were found for zinc.

Next, we performed a set of sensitivity analyses. First, we re-analyzed the data excluding supplement users for each nutrient separately (**Supplementary table 2**). Associations became stronger for folate and remained comparable in effect size for EPA (B (SE) total cohort; 0.82 (0.26), p=0.003, 0.27 (0.17) p=0.118)), for total omega-3 fatty acids and DHA (B (SE) controls; 0.41 (0.20), p=0.048; 0.31 (0.23), p=0.189) and for vitamin C (B (SE) AD; 1.03 (0.30), p=0.004). Effect sizes for vitamin B6 remained comparable in controls only (B (SE) controls; 0.60(0.30), p=0.061, MCI; 0.48 (0.41), p=0.266, AD; -0.04 (0.60), p=0.944), associations for vitamin B1 and B12 attenuated and lost significance (B(SE) total cohort; - 0.41(0.60), p=0.493; -0.22 (0.47), p=0.640).

In a second sensitivity analysis, we excluded imputed nutrient intake values (n=11). All associations remained comparable, indicating that our findings were not driven by the imputed data (**Supplementary table 3**). Third, we excluded blood samples taken longer than 3 months from FFQ administration. Associations remained similar for all nutrients, indicating that long time intervals between blood sampling and FFQ administration did not drive our results (**Supplementary table 4**).

Last, to assess the effect of FFQ's filled in by, or with the help of, a proxy, we repeated the analysis including only participants with a self-administered FFQ (32 controls, 13 MCI, 12 AD). Here, the significant association between intake and blood levels of vitamin B6 in AD was lost (B(SE) -0.07 (0.38), p=0.860), but all other findings remained essentially unchanged (**Supplementary table 5**).

Table 2 Associations between nutrient intake and nutritional biomarker levels in blood.

Determinant	Outcome	Model	All			Controls			MCI			AD		
Nutrient intake	Nutritional biomarker		B(SE)	p	n	B(SE)	p	n	B(SE)	p	n	B(SE)	p	n
Retinol activity equivalents, mcg/d	Vitamin A, µmol/L	Model 1	0.24 (0.10)	0.025	87									
		Model 2	0.23 (0.12)	0.057	87									
Vitamin B1[#], mg/d	Vitamin B1, nmol/L	Model 1	0.47 (0.09)	<0.001	79									
		Model 2	0.48 (0.09)	<0.001	79									
Vitamin B6[#], mg/d	Vitamin B6, nmol/L	Model 1				0.74 (0.08)	<0.001	33	0.97 (0.22)	<0.001	21	0.53 (0.15)	0.002	25
		Model 2				0.78 (0.06)	<0.001	33	1.05 (0.20)	<0.001	21	0.45 (0.14)	0.006	25
Vitamin B12, mcg/d	Vitamin B12, pmol/L	Model 1	0.38 (0.10)	<0.001	77									
		Model 2	0.35 (0.10)	0.001	77									
Vitamin C, mg/d	Vitamin C, µmol/L	Model 1				0.25 (0.15)	0.102	33	0.07 (0.26)	0.781	20	0.78 (0.11)	<0.001	25
		Model 2				0.22 (0.15)	0.152	33	0.03 (0.27)	0.927	20	0.75 (0.11)	<0.001	25
Folate equivalents, mcg/d	Folate, nmol/L	Model 1	0.51 (0.09)	<0.001	77									
		Model 2	0.49 (0.09)	<0.001	77									
Zinc, mg/d	Zinc, µmol/L	Model 1	-0.03 (0.11)	0.768	79									
		Model 2	-0.10 (0.11)	0.373	79									
Omega-3 fatty acids, % of total fatty acids intake	Omega-3 fatty acids, % of plasma total fatty acids	Model 1				0.50 (0.17)	0.005	33	-0.20 (0.26)	0.440	21	0.10 (0.16)	0.527	25
		Model 2				0.50 (0.20)	0.021	33	-0.38 (0.30)	0.230	21	0.25 (0.17)	0.166	25
DHA, % of total fatty acids intake	DHA, % of plasma total fatty acids	Model 1				0.40 (0.11)	0.002	33	-0.18 (0.25)	0.494	21	-0.15 (0.27)	0.576	25
		Model 2				0.38 (0.13)	0.009	33	-0.13 (0.27)	0.646	21	-0.27 (0.25)	0.285	25
EPA, % of total fatty acids intake	EPA, % of plasma total fatty acids	Model 1	0.40 (0.10)	<0.001	79									
		Model 2	0.41 (0.11)	<0.001	79									

Abbreviations: AD, Alzheimer's disease; DHA, docosahexaenoic acid ; EPA, eicosapentaenoic acid; MCI, mild cognitive impairment; SD, standard deviation. [#] the HELIUS FFQ was not designed to measure these nutrients.

Linear regression analyses were used, using separate models for each nutritional biomarker. We first entered syndrome diagnosis and interaction terms (dummy diagnosis x nutrient intake) to test if associations between nutrient intake and nutritional biomarker level differed according to diagnostic group. When we found an interaction between syndrome diagnosis and nutrient intake ($p < 0.10$), we stratified the results for syndrome diagnosis. When no significant interaction was found, only the effect size for the total group effect size was reported. Data were log-transformed and converted to z-scores before data-analysis. We present an unadjusted model (model 1) and a model adjusted for sex, age, diagnosis (total group model) and total energy intake. Effect size is change of SD nutritional biomarker blood level per 1 SD increase in nutrient intake.

Discussion

The main finding of this study is that intake of nutrients, as assessed by the HELIUS FFQ and self-reported supplement use, are moderately associated with nutritional biomarker levels of omega-3 fatty acids, B- and C vitamins in a memory-clinic cohort of controls, patients with MCI and AD. Associations were largely similar across diagnostic groups, except for total omega-3 fatty acids and DHA that were only associated in controls, and vitamin C that was only associated in AD. Almost half of the participants used nutritional supplements. Associations between nutrient intake and nutritional biomarker levels remained, however, largely comparable in non-supplement users. Our findings together suggest that the HELIUS FFQ (with or without supplement intake) is a valid method to estimate nutrient intake in a memory-clinic population.

Our findings of moderate associations between nutrient intake and nutritional biomarker levels of omega-3 fatty acids, B- and C vitamins are in line with previous population-based studies [6-9]. This suggests that the nutritional assessment methods for these nutrients perform similar in our memory-clinic cohort population as in a cognitively healthy population. Moreover, the associations found in our study were largely comparable in cognitively impaired and cognitively healthy individuals. Previously, the NAME study included 273 elderly home-care clients with normal cognition, MCI and dementia and found no group differences for the associations between intake and plasma-based estimates of EPA and DHA [12]. These results are in line with our findings for EPA, but in contrast to our findings for DHA and total omega-3 fatty acids that were only associated in controls, but not in MCI and AD in our study. In addition, a study in 19 cognitively normal older adults and 19 patients with MCI found that DHA intake and blood levels were associated in cognitively normal individuals, but not in MCI [11]. One possible explanation for these conflicting findings could be that the last study used self-administered FFQs [11], while the NAME study used only interviewer-administered FFQs [12]. In our study we used both self-administered and proxy-administered FFQs. Our sensitivity analysis excluding FFQs filled in by proxies showed similar associations as observed in the total cohort, suggesting that both self-reported and proxy-reported FFQs can be reliably used in MCI and AD. Another explanation for the conflicting findings on omega-3 fatty acids in cognitively impaired individuals could be related to power. It is possible that we did not find associations for DHA and omega-3 fatty acids in MCI and AD due to our smaller group sizes (n=90) in comparison to the NAME study (n=273). We found that intake and status of vitamin C was only associated in AD. This is difficult to interpret as the current knowledge about the relation between vitamin C intake and vitamin C status in AD is limited. Previous studies in healthy individuals that report on the association

between vitamin C intake and plasma levels have shown conflicting results [26, 27]. This might be a consequence of the instability of vitamin C in plasma and its saturation effect, meaning that absorption of vitamin C attenuates with higher intake levels [26, 28]. We did stabilize vitamin C in our plasma samples by immediately adding metaphosphoric acid after spinning. In summary, we found that associations between nutrient intake and nutritional biomarker levels in blood were comparable across the diagnostic groups for most nutrients.

We found that for vitamin B1, B6 and B12 nutrient intake from diet and supplement use combined was more strongly associated with nutritional biomarker levels in the total cohort than in non-supplement users alone. This finding is in line with previous studies and is a logical consequence of the supraphysiological nutrient dosages in supplements in comparison to the nutrient intake from food [10, 29]. In addition, the HELIUS FFQ was not designed to measure vitamin B1 and B6 [19]. As some food items relevant for the intake of vitamin B1 and B6 might not have been included in the HELIUS FFQ, actual intake might be higher than estimated from the HELIUS FFQ. Nonetheless, most associations between nutrient intake and nutrient status remained comparable after exclusion of supplement users, indicating that the HELIUS FFQ also provides valid estimates of nutrient intake from diet alone.

A valid and reliable method to measure nutrient intake in clinical populations will contribute to the development of dietary interventions for AD. For example, stratification of participants based on their nutrient or food group intake will help to define the optimum range of intake that can provide protective benefits for AD. Subsequently, these findings from observational studies will be of help in selecting patients for specific nutritional interventions.

Strengths of this study were that we were able to study associations of nutrient intake and nutrient status of multiple nutrients in different stages of cognitive decline. As all our participants underwent extensive, uniform cognitive screening, our population was well characterized. Moreover, the HELIUS FFQ has been validated in the Dutch population [19]. There are however some limitations to consider. The sample size of our study is relatively small, and as a consequence we were unable to evaluate non-linear relations or effect modification other than by diagnosis. Lastly, most plasma and serum biomarkers reflect relatively short-term intake [3, 30]. Future studies, should, therefore, include larger populations and more nutritional biomarkers that provide information on longer-term intake, such as nutritional biomarkers measured in adipose tissue or erythrocytes [3].

In conclusion, we found that nutrient intake as assessed by the HELIUS FFQ and self-reported supplement use are moderately associated with their nutrient status

in blood for B- and C vitamins, and omega-3 fatty acids. The associations were largely comparable in controls, MCI and AD. These results combined indicate that the HELIUS FFQ provides reliable estimates of nutrient intake in a memory clinic cohort, regardless of a clinical diagnosis of MCI or AD.

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Competing interests

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Supplementary text 1

Analyses were performed at the department of Clinical Chemistry of the Amsterdam UMC, Amsterdam, the Netherlands. Exceptions were zinc, which was measured at Medlon (Enschede, the Netherlands,) and vitamin C which was measured at the Reinier de Graaf Groep (Delft, the Netherlands). Missing values ranged from 3-14% per nutritional biomarker and were due to a lack of volume. Vitamin A was measured in plasma using high performance liquid chromatography (HPLC) with UV detection [1]. The lower limit of quantitation (LLOQ) was 0.1 µmol/L for vitamin A and inter-assay variation were determined at two different concentrations and ranged from 0.7-1.0%. Vitamin B1 and B6 were measured in whole blood using HPLC combined with mass spectrometry (MS). The LLOQ was 3 nmol/L for vitamin B1 and B6, inter-assay CV's ranged from 2.2-4.5%. Serum folate and serum vitamin B12 were measured by competitive luminescence immunoassay on an Architect analyzer (Abbott Diagnostics, Abbott Laboratories, Abbott Park, USA). The LLOQ was 2 nmol/L for folate and 44 pmol/L for vitamin B12 and inter-assay CV's were 9% for folate and 6.3% for vitamin B12. Vitamin C was measured in stabilized plasma using HPLC-UV. The LLOQ was 2.8 µmol/L and the inter-assay CV determined at two different concentrations ranged from 8.8-11.5%. Plasma zinc was detected by electron multiplier using inductively coupled mass spectrometry (ICP-MS) (NexION 300D, Perkin Elmer, Waltham, Massachusetts, USA). The LLOQ was 0.3 µmol/L and inter-assay CV was 2.9%. Omega-3 fatty acids, including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) were measured in plasma using a Hewlett Packard GC 5890 equipped with an Agilent J&W HP-FFAP, 25m, 0.20mm, 0.33µm GC Column and flame ionization detection. The LLOQ was 3.0 µmol/L, inter-assay CV's ranged from 3.9-18.7%.

Supplementary table 1 Nutritional biomarker concentrations and dietary intake of supplement and non-supplement users.

	Supplement users		Non-supplement users	
Nutritional biomarkers				
	Median (IQR)	n	Median (IQR)	n
Vitamin A, µmol/L	2.55 (2.23 - 2.75)	14	2.19 (1.93 - 2.46)	73
Vitamin B1, nmol/L	147.00 (129.12 - 178.00)	18	120.00 (104.00 - 131.00) ***	61
Vitamin B6, nmol/L	236.00 (121.00 - 311.50)	18	81.40 (67.60 - 98.10) ***	61
Vitamin B12, pmol/L	296.00 (246.50 - 526.75)	22	266.00 (193.00 - 318.50) *	55
Vitamin C, µmol/L	87.17 (78.02 - 98.37)	20	67.92 (42.47 - 81.16) **	58
Folate, nmol/L	28.35 (22.93 - 34.08)	22	14.70 (11.65 - 17.80) ***	55
Zinc, µmol/L	13.60 (12.53 - 14.07)	18	14.00 (12.70 - 14.60)	61
Omega-3 fatty acids, % of plasma total fatty	4.22 (4.02 - 7.65)	9	3.65 (3.00 - 4.47) **	70
DHA, % of plasma total fatty	2.15 (1.73 - 2.84)	9	1.58 (1.20 - 2.02) **	70
EPA, % of plasma total fatty	1.40 (1.12 - 3.04)	9	0.96 (0.67 - 1.32) ***	70
Dietary intake				
Retinol activity equivalents, mcg/d	564.60 (454.61 - 1021.63)	15	888.47 (601.69 - 1152.54)	75
Vitamin B1[#], mg/d	0.71 (0.61 - 0.97)	19	0.91 (0.83 - 1.06) *	71
Vitamin B6[#], mg/d	1.42 (1.10 - 1.73)	19	1.60 (1.36 - 1.89)	71
Vitamin B12, mcg/d	4.28 (3.34 - 5.89)	22	4.65 (3.66 - 6.63)	68
Vitamin C mg/d	125.37 (91.82 - 176.22)	22	125.98 (92.95 - 164.77)	68
Folate equivalents, mcg/d	282.74 (230.03 - 354.96)	22	284.69 (242.77 - 336.74)	68
Zinc[#], mg/d	8.37 (7.26 - 10.39)	19	10.35 (8.76 - 12.51) *	71
Omega-3 fatty acids, % of total fatty acids intake	2.29 (2.09 - 3.19)	10	2.91 (2.32 - 3.62)	80
DHA, % of total fatty acids intake	0.17 (0.07 - 0.33)	10	0.13 (0.04 - 0.28)	80
EPA, % of total fatty acids intake	0.16 (0.09 - 0.22)	10	0.11 (0.05 - 0.20)	80

Abbreviations: DHA, docosahexaenoic acid ; EPA, eicosapentaenoic acid; IQR, interquartile range.

Different from supplement users: * $p<0.05$, ** $p<0.01$, *** $p<0.001$ [#] the HELIUS FFQ was not designed to measure these nutrients. Pairwise comparisons, using log-transformed z-scores of nutritional biomarkers, compared with linear regression analysis adjusted for age, sex and diagnosis.

Supplementary table 2 Associations between nutrient intake and nutritional biomarker levels in blood in non-supplement users.

Determinant	Outcome	All			Controls			MCI			AD		
Nutrient intake	Nutritional biomarker	B (SE)	p	n	B (SE)	p	n	B (SE)	p	n	B (SE)	p	n
Retinol acitivty equivalents, mcg/d	Vitamin A, µmol/L	0.30 (0.16)	0.056	73									
Vitamin B1[#], mg/d	Vitamin B1, nmol/L	-0.41 (0.60)	0.493	61									
Vitamin B6[#], mg/d	Vitamin B6, nmol/L				0.60 (0.30)	0.061	25	0.48 (0.41)	0.266	17	-0.04 (0.60)	0.944	19
Vitamin B12, mcg/d	Vitamin B12, pmol/L	-0.22 (0.47)	0.640	55									
Vitamin C, mg/d	Vitamin C, µmol/L				0.19 (0.40)	0.642	22	-0.06 (0.44)	0.892	17	1.03 (0.30)	0.004	19
Folate equivalents, mcg/d	Folate, nmol/L	0.82 (0.26)	0.003	55									
Zinc[#], mg/d	Zinc, µmol/L	-0.08 (0.29)	0.789	61									
Omega-3 fatty acids, % of total fatty acids intake	Omega-3 fatty acids, % of plasma total fatty acids				0.41 (0.20)	0.048	28	-0.43 (0.27)	0.137	18	0.26 (0.18)	0.155	24
DHA, % of total fatty acids intake	DHA, % of plasma total fatty acids				0.31 (0.23)	0.189	28	-0.13 (0.27)	0.646	18	-0.32 (0.25)	0.219	24
EPA, % of total fatty acids intake	EPA, % of plasma total fatty acids	0.27 (0.17)	0.118	70									

Abbreviations: AD, Alzheimer's disease; DHA, docosahexaenoic acid ; EPA, eicosapentaenoic acid; MCI, mild cognitive impairment; SD, standard deviation

[#] the HELIUS FFQ was not designed to measure these nutrients. Linear regression analyses adjusted for sex, age, diagnosis and total energy intake were used, using separate models for each nutritional biomarker. We first entered syndrome diagnosis and interaction terms (dummy diagnosis x dietary intake) to test if associations between dietary intake and nutritional biomarker level differed according to diagnostic group in the total cohort. When we found an interaction between syndrome diagnosis and nutrient intake ($p < 0.10$), we stratified the results for syndrome diagnosis. When no significant interaction was found, only the effect size for the total group effect size was reported. Data were log-transformed and converted to z-scores before data-analysis. Effect size is change of SD nutritional biomarker blood level per 1 SD increase in nutrient intake.

Supplementary table 3 Associations between nutrient intake and nutritional biomarker levels in blood excluding supplement users with imputed values.

Determinant	Outcome	All			Controls			MCI			AD		
Nutrient intake	Nutritional biomarker	B(SE)	p	n	B(SE)	p	n	B(SE)	p	n	B(SE)	p	n
Retinol activity equivalents, mcg/d	Vitamin A, µmol/L	0.26 (0.12)	0.040	83									
Vitamin B1[#], mg/d	Vitamin B1, nmol/L	0.51 (0.10)	<0.001	75									
Vitamin B6[#], mg/d	Vitamin B6, nmol/L				0.75 (0.07)	<0.001	32	1.11 (0.20)	<0.001	20	0.52 (0.15)	0.003	23
Vitamin B12, mcg/d	Vitamin B12, pmol/L	0.30 (0.10)	0.006	72									
Vitamin C, mg/d	Vitamin C, µmol/L				0.20 (0.20)	0.318	30	0.02 (0.29)	0.951	18	0.75 (0.11)	<0.001	25
Folate equivalents, mcg/d	Folate, nmol/L	0.52 (0.10)	<0.001	72									
Zinc[#], mg/d	Zinc, µmol/L	0.05 (0.12)	0.703	76									
Omega-3 fatty acids, % of total fatty acids intake	Omega-3 fatty acids, % of plasma total fatty acids				0.50 (0.20)	0.018	32	-0.36 (0.31)	0.267	20	0.25 (0.17)	0.167	25
DHA, % of total fatty acids intake	DHA, % of plasma total fatty acids				0.36 (0.14)	0.016	32	-0.12 (0.28)	0.672	20	-0.27 (0.25)	0.285	25
EPA, % of total fatty acids intake	EPA, % of plasma total fatty acids	0.41 (0.11)	<0.001	77									

Abbreviations: AD, Alzheimer's disease; DHA, docosahexaenoic acid ; EPA, eicosapentaenoic acid; MCI, mild cognitive impairment; SD, standard deviation.

[#] the HELIUS FFQ was not designed to measure these nutrients. We performed a sensitivity analyses excluding supplement users where we imputed supplement dosages. Linear regression analyses adjusted for sex, age, diagnosis and total energy intake were used, using separate models for each nutritional biomarker. We included interaction terms (dummy diagnosis x nutrient intake) to test if associations between nutrient intake and nutritional biomarker level differed according to diagnostic group. When we found an interaction between syndrome diagnosis and nutrient intake ($p < 0.10$), we stratified the results for syndrome diagnosis, and the results are displayed for each diagnostic group separately. When no significant interaction was found, the interaction term was removed from the model and the overall effect size was reported. Data were log-transformed and converted to z-scores before data-analysis. Effect size is change of SD nutritional biomarker blood level per 1 SD increase in nutrient intake.

Supplementary table 4 Associations between nutrient intake and nutritional biomarker levels in blood excluding blood samples taking longer than 3 months from FFQ administration.

Determinant	Outcome	All			Controls			MCI			AD		
Nutrient intake	Nutritional biomarker	B(SE)	p	n	B(SE)	p	n	B(SE)	p	n	B(SE)	p	n
Retinol activity equivalents, mcg/d	Vitamin A, µmol/L	0.21 (0.14)	0.131	71									
Vitamin B1#, mg/d	Vitamin B1, nmol/L	0.61 (0.12)	<0.001	67									
Vitamin B6#, mg/d	Vitamin B6, nmol/L				0.86 (0.09)	<0.001	26	0.67 (0.20)	0.006	18	0.46 (0.16)	0.009	23
Vitamin B12, mcg/d	Vitamin B12, pmol/L	0.46 (0.15)	0.003	52									
Vitamin C, mg/d	Vitamin C, µmol/L				0.23 (0.16)	0.165	26	0.05 (0.41)	0.900	17	0.75 (0.12)	<0.001	23
Folate equivalents, mcg/d	Folate, nmol/L	0.55 (0.11)	<0.001	52									
Zinc#, mg/d	Zinc, µmol/L	-0.13 (0.11)	0.237	67									
Omega-3 fatty acids, % of total fatty acids intake	Omega-3 fatty acids, % of plasma total fatty acids				0.47 (0.23)	0.053	26	-0.37 (0.34)	0.303	18	0.24 (0.17)	0.178	23
DHA, % of total fatty acids intake	DHA, % of plasma total fatty acids				0.39 (0.16)	0.024	26	-0.11 (0.29)	0.716	18	-0.26 (0.26)	0.334	23
EPA, % of total fatty acids intake	EPA, % of plasma total fatty acids	0.42 (0.12)	0.001	67									

Abbreviations: AD, Alzheimer's disease; DHA, docosahexaenoic acid ; EPA, eicosapentaenoic acid; MCI, mild cognitive impairment; SD, standard deviation.
the HELIUS FFQ was not designed to measure these nutrients. We performed a sensitivity analyses excluding supplement users where we imputed supplement dosages. Linear regression analyses adjusted for sex, age, diagnosis and total energy intake were used, using separate models for each nutritional biomarker.
We first entered syndrome diagnosis and interaction terms (dummy diagnosis x nutrient intake) to test if associations between nutrient intake and nutritional biomarker level differed according to diagnostic group. When we found an interaction between syndrome diagnosis and nutrient intake ($p < 0.10$), we stratified the results for syndrome diagnosis. When no significant interaction was found, only the effect size for the total group effect size was reported. Data were log-transformed and converted to z-scores before data-analysis. Effect size is change of SD nutritional biomarker blood level per 1 SD increase in nutrient intake.

Supplementary table 5 Associations between nutrient intake and nutritional biomarker levels in blood in participants with a self-administered FFQ.

Determinant	Outcome	All			Controls			MCI			AD		
Nutrient intake	Nutritional biomarker	B(SE)	p	n	B(SE)	p	n	B(SE)	p	n	B(SE)	p	n
Retinol activity equivalents, mcg/d	Vitamin A, µmol/L	0.27 (0.14)	0.049	55									
Vitamin B1[#], mg/d	Vitamin B1, nmol/L	0.31 (0.16)	0.060	48									
Vitamin B6[#], mg/d	Vitamin B6, nmol/L				0.84 (0.11)	<0.001	26	1.12 (0.29)	0.006	12	-0.07 (0.38)	0.860	10
Vitamin B12, mcg/d	Vitamin B12, pmol/L	0.42 (0.10)	<0.001	49									
Vitamin C, mg/d	Vitamin C, µmol/L				-0.01 (0.21)	0.959	26	0.23 (0.37)	0.549	11	0.64 (0.18)	0.018	10
Folate equivalents, mcg/d	Folate, nmol/L	0.31 (0.12)	0.014	49									
Zinc[#], mg/d	Zinc, µmol/L	-0.21 (0.14)	0.134	48									
Omega-3 fatty acids, % of total fatty acids intake	Omega-3 fatty acids, % of plasma total fatty acids				0.64 (0.21)	0.007	26	-0.54 (0.53)	0.342	12	-0.29 (0.36)	0.451	10
DHA, % of total fatty acids intake	DHA, % of plasma total fatty acids				0.41 (0.15)	0.011	26	-0.39 (0.37)	0.327	12	0.64 (1.06)	0.570	10
EPA, % of total fatty acids intake	EPA, % of plasma total fatty acids	0.39 (0.13)	0.005	48									

*Note :*Abbreviations: AD, Alzheimer's disease; DHA, docosahexaenoic acid ; EPA, eicosapentaenoic acid; MCI, mild cognitive impairment; SD, standard deviation

[#] the HELIUS FFQ was not designed to measure these nutrients. Linear regression analyses adjusted for sex, age, diagnosis and total energy intake were used, using separate models for each nutritional biomarker. We first entered syndrome diagnosis and interaction terms (dummy diagnosis x nutrient intake) to test if associations between nutrient intake and nutritional biomarker level differed according to diagnostic group. When we found an interaction between syndrome diagnosis and nutrient intake ($p < 0.10$), we stratified the results for syndrome diagnosis. When no significant interaction was found, only the effect size for the total group effect size was reported. Data were log-transformed and converted to z-scores before data-analysis. Effect size is change of SD nutritional biomarker blood level per 1 SD increase in nutrient intake.

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