Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring 8 (2017) 139-146

Blood Based Biomarkers

Nutrients required for phospholipid synthesis are lower in blood and cerebrospinal fluid in mild cognitive impairment and Alzheimer's disease dementia

Nick van Wijk[a,*], Rosalinde E. R. Slot[b], Flora H. Duits[b], Marieke Strik[c], Egbert Biesheuvel[a], John W. C. Sijben[a], Marinus A. Blankenstein[c], Jörgen Bierau[d], Wiesje M. van der Flier[b], Philip Scheltens[b], Charlotte E. Teunissen[c]

[a]*Nutricia Advanced Medical Nutrition, Nutricia Research, Utrecht, The Netherlands*
[b]*Alzheimer Center and Department of Neurology, VU University Medical Center, Amsterdam, The Netherlands*
[c]*Department of Clinical Chemistry, VU University Medical Center, Amsterdam, The Netherlands*
[d]*Department of Clinical Genetics, Maastricht UMC+, Maastricht, The Netherlands*

Abstract

Introduction: Synaptic membrane formation depends on nutrients that fuel metabolic pathways for the synthesis of constituent phospholipids. Consequently, insufficient availability of such nutrients may restrict membrane formation and contribute to synaptic dysfunction in Alzheimer's disease (AD). We assessed whether blood and cerebrospinal fluid (CSF) concentrations of nutrients related to phospholipid synthesis differ among patients with AD, mild cognitive impairment (MCI), and control subjects.

Methods: Concentrations of uridine, choline, folate, homocysteine, and other related metabolites were analyzed in paired blood and CSF samples from subjects selected from the Amsterdam Dementia Cohort with AD ($n = 150$; age, 66 ± 7 years; 37% female), MCI ($n = 148$; age, 66 ± 8 years; 37% female), and control subjects ($n = 148$; age, 59 ± 8 years; 38% female).

Results: Age- and gender-adjusted analysis of variance revealed different concentrations of circulating uridine, choline, and folate and CSF uridine, folate, and homocysteine (all $P < .05$) among the three diagnostic groups. Post hoc pairwise comparison showed that subjects with AD had lower CSF uridine, plasma choline and higher CSF homocysteine concentrations, whereas subjects with MCI had lower plasma and CSF uridine, serum and CSF folate, and higher CSF homocysteine concentrations compared with control subjects (all $P < .05$), with differences ranging from −11 to +22%.

Discussion: AD and MCI patients have lower levels of nutrients involved in phospholipid synthesis. The current observations warrant exploration of the application of nutritional strategies in the early stages of AD.

Keywords: Nutritional status; Uridine; Choline; Folate; Homocysteine; Blood; Cerebrospinal fluid; Phospholipid precursors; Mild cognitive impairment; Alzheimer's disease

1. Introduction

Several interacting processes contribute to the neurodegenerative process of Alzheimer's disease (AD), including abnormal protein processing and neuronal membrane degeneration that lead to synaptic loss and synaptic dysfunction starting early in the disease course [1–3].

Nutrition is increasingly recognized as an important factor in the etiology and progression of AD. Epidemiologic studies have suggested that specific macronutrients and micronutrients are involved in the decline of cognitive function and risk of AD

*Corresponding author. Tel.: +31-30-2095000; Fax: +31-30-2100436.
E-mail address: nick.vanwijk@nutricia.com

http://dx.doi.org/10.1016/j.dadm.2017.04.005

[4,5]. Nutrients can affect normal functioning and maintenance of the brain via various mechanisms [6]. In particular, poor availability of certain nutrients in AD has been suggested to affect synaptic function [7,8]. The synthesis of synaptic membranes is dependent on several nutrients, for example, docosahexaenoic acid, uridine, choline, and folate, vitamin B12, vitamin B6, vitamin E, vitamin C, and selenium, which fuel the metabolic pathways for the formation of constituent phospholipids [9,10]. Consequently, insufficient availability of these nutrients hypothetically limits, among other processes, membrane formation and could contribute to synaptic dysfunction in AD.

Several studies have provided data on nutritional status in AD and results have generally shown lower blood levels of most nutrients that are required for phospholipid synthesis [11–13], but for some of these nutrients results are inconclusive. In addition, there is a lack of information in mild cognitive impairment (MCI), a predementia stage in which the scope for intervention is arguably higher. Most studies have focused only on one nutritional marker instead of a set of nutrients, which allow correlations between nutrients to be studied. Furthermore, only a limited number of studies reported paired blood and cerebrospinal fluid (CSF) nutritional markers. These data are important because blood levels are valuable in assessing nutritional status, whereas CSF levels give specific insights into the availability of nutrients in the brain.

The aims of this cross-sectional study were to assess whether blood and CSF concentrations of nutrients needed for phospholipid synthesis and related metabolites differ among AD, MCI, and control subjects. Concentrations of uridine, choline, betaine, folate, homocysteine, albumin, and bilirubin were measured in paired blood and CSF samples from subjects with MCI or AD and compared with control subjects. Revealing a disease-specific nutritional deficit would lend support to the application of nutritional supplementation in the management of AD.

2. Methods

2.1. Subjects

Subjects for this cross-sectional study were selected from the Amsterdam Dementia Cohort of the Alzheimer Center of the VU University Medical Center (VUmc) [14]. The study included patients with probable AD (n = 150), MCI (n = 148), and control subjects with subjective cognitive decline (n = 148), with all baseline biomaterial available, that is, paired blood plasma, blood serum, and CSF. The three diagnostic groups were matched for gender but not for age, as this was not feasible. All subjects underwent dementia screening at baseline, including physical and neurologic examination, electroencephalography, brain magnetic resonance imaging, and laboratory tests. Cognitive screening included a Mini–Mental State Examination (MMSE) and comprehensive neuropsychological test battery. Diagnoses were made by consensus in a multidisciplinary team, without knowledge of AD CSF biomarker results. Probable AD was diagnosed according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association up to the beginning of 2012, and subsequently, using the National Institute on Aging-Alzheimer's Association criteria for AD. MCI was diagnosed using "the Petersen criteria" up to the beginning of 2012 and the National Institute on Aging-Alzheimer's Association criteria for MCI after this date [14]. As control subjects, we used subjects who presented with cognitive complaints at our memory clinic, but who performed normal on clinical examinations, that is, the criteria for MCI were not fulfilled, and there were no psychiatric or neurologic diseases contributing to cognitive complaints. In addition, if follow-up diagnosis was available, control subjects were selected only if they remained stable. All subjects gave written informed consent for the use of their clinical data and samples for research purposes, and the study was approved by the medical ethics committee of the VUmc (protocol 00/211).

2.2. Blood and CSF collection

CSF and blood samples were collected from nonfasted subjects during diagnostic workup. CSF was collected by lumbar puncture between the L3/L4 or L4/L5 intervertebral space, using a 25-gauge needle and syringe, and collected in polypropylene tubes (Sarstedt, Nümbrecht, Germany) in agreement with international consensus protocols [15]. Within 2 hours, CSF samples were centrifuged at 1800g for 10 minutes at 4°C. Aliquots were either frozen at −20°C until routine analysis of Alzheimer biomarkers or frozen and stored at −80°C until further analysis. Venous blood was drawn (clotted blood for serum and ethylenediaminetetraacetic acid [EDTA] blood for plasma), centrifuged at 1800g for 10 minutes at 4°C, aliquoted, and stored at −80°C.

2.3. Blood and CSF analyses

Analyses of CSF amyloid-β 1–42 (Aβ42), total tau, and tau phosphorylated at threonine 181 (p-tau-181) were routinely done at the Neurochemistry laboratory of the VUmc Department of Clinical Chemistry using sandwich enzyme-linked immunoassays (ELISAs, Innotest, beta-amyloid1–42, Innotest hTAU-Ag, and Innotest PhosphoTAU-181p; Fujirebio Europe, Gent, Belgium) [16]. The interassay coefficients of variation (CVs) were 10.9% for Aβ42, 9.9% for tau, and 9.1% for p-tau-181 [14].

Concentrations of nutrients needed for phospholipid synthesis and related metabolites were analyzed in paired blood and CSF samples. All compounds, except bilirubin, were measured in CSF. Uridine, choline, betaine, and homocysteine concentrations were measured in blood plasma, whereas folate, albumin, and bilirubin concentrations were measured in blood serum. The Department of Clinical Chemistry of the VUmc, Amsterdam, the Netherlands, performed

all analyses except the uridine analyses (plasma and CSF), which were performed at the Department of Clinical Genetics, Maastricht UMC+, Maastricht, The Netherlands. Overall, there were a minimum number of missing values per parameter. The reasons for missing values were missing or empty vials, or exceeding the lower or upper detection limits.

Plasma and CSF uridine were measured by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). $^{15}N_2$-uridine was used as an internal standard. Samples were deproteinized with acetonitrile before quantification. Uridine was detected in electrospray ionization positive mode and quantified with multiple reaction monitoring using the *m/z* transition 245 → 113 for uridine and 245.2 → 201.2 for $^{15}N_2$-uridine. Interassay CV for uridine in plasma and CSF was 9.6%–14%. Lower limit of quantification (LOQ) for plasma and CSF uridine was estimated at 0.2 μmol/L.

Choline and betaine in plasma were determined simultaneously, as well as choline and betaine in CSF, using liquid chromatography-tandem mass spectrometry (LC-MS/MS) according to a previously published method [17]. $^2H_9$-betaine and $^2H_9$-choline were used as internal standards. Samples were deproteinized with acetonitrile before quantification. Choline and betaine were detected in electrospray ionization positive mode and quantified with multiple reaction monitoring using the following *m/z* transition: 104.1 → 60 for choline, 113.1 → 69 for $^2H_9$-choline, 118.1 → 59.1 for betaine, and 127.1 → 68.1 for $^2H_9$-betaine. Interassay CVs for choline and betaine in plasma were 0.9%–2.6% and 1.3–2.1%, respectively, and in CSF 1.5%–2.9% and 0.7%–1.9%, respectively. Lower LOQs for choline and betaine in plasma were 0.3 and 0.8 μmol/L, respectively, and in CSF 0.09 μmol/L for both choline and betaine.

Serum and CSF folate were determined by competitive luminescence immunoassay on an Architect analyzer (Abbott Diagnostics, Abbott Laboratories, Abbott Park, IL, USA). The interassay CV for folate in serum and CSF was 6%–15%.

Plasma homocysteine was measured by competitive luminescence immunoassay on an Architect analyzer (Abbott Diagnostics, Abbott Laboratories). Interassay CV for homocysteine in plasma was 2%–4%. Homocysteine in CSF was measured using LC-MS/MS adapted from a previously published method [18]. $^2H_4$-homocysteine was used as an internal standard. Samples were reduced dithiotreitol, followed by a solid phase extraction cleanup. CSF homocysteine was detected in electrospray ionization positive mode and quantified with multiple reaction monitoring using the *m/z* transitions 136.1 → 90.1 for homocysteine and 140.1 → 94.1 for $^2H_4$-homocysteine. The interassay CV for this assay was 5.1%–28.1%. Lower LOQ for CSF homocysteine was 0.005 μmol/L.

Serum and CSF albumin concentrations were both determined by rate nephelometry on a Beckman Coulter Immage 800 immunochemistry system (ALB test, Beckman Coulter, Danaher, Washington DC, USA). The interassay CV for serum and CSF albumin was 2.3%–4.3%.

Serum bilirubin was measured by a colorimetric assay on a Cobas 8000 system (Bilirubin Total Gen, Roche Diagnostics, Roche, Basel, Switzerland). The interassay CV for serum bilirubin was 1.3%–1.6%.

2.4. Statistical analyses

All statistical analyses were performed using SPSS (version 19, SPSS Inc, Chicago, IL, USA). All continuous variables, except for CSF uridine, lacked normal distribution as indicated by the Kolmogorov-Smirnov test. Ln transformation was applied to obtain a normal distribution, where appropriate. Baseline characteristics were compared using analysis of variance or chi-square test, where appropriate. Differences in blood and CSF concentrations of nutrients and metabolites and CSF to serum albumin ratio were analyzed using an analysis of variance with diagnosis as the between-subject factor, and age and sex as covariates, after removal of the largest five absolute residuals per variable to exclude outliers. Post hoc pairwise comparison (Bonferroni adjusted *P* values) was performed when comparing individual diagnostic groups. Spearman's rank correlation coefficients were calculated to assess correlations between the concentrations of nutrients and metabolites and AD CSF biomarkers. *P* values $<.05$ were considered to reflect statistical significance.

3. Results

3.1. Subject characteristics

Table 1 shows the characteristics of subjects, including age, gender, MMSE, and AD CSF biomarkers (Aβ42, total tau, and p-tau-181), for each diagnostic category. Matching for gender resulted in an equal distribution of males and females between the three diagnostic groups. Control subjects were younger than subjects with MCI and AD. AD CSF biomarker levels were within the normal range (<550 pg/mL for Aβ42; >375 pg/mL for total tau; and >52 pg/mL for p-tau-181 [16]) in all control subjects. MMSE, Aβ42, total tau, and p-tau-181 differed between the three diagnostic categories.

3.2. CSF and blood levels of the nutritional markers

Table 2 shows the concentrations of all measured nutrients and metabolites in blood and CSF by diagnostic category. Age- and gender-adjusted analyses revealed differences in concentrations of plasma uridine, CSF uridine, plasma choline, serum folate, CSF folate, and CSF homocysteine between diagnostic groups (all $P < .05$). Post hoc testing showed that patients with AD had lower concentrations of CSF uridine and plasma choline and a higher concentration of homocysteine in CSF compared with control subjects. Subjects with MCI had lower concentrations of uridine in plasma and CSF and folate in serum and CSF and a higher concentration of homocysteine in CSF compared with control subjects. Concentrations of CSF choline, plasma betaine, CSF betaine, plasma homocysteine, serum

Table 1
Patient characteristics per diagnostic category

	Controls	MCI	AD
Total *n*	148	148	150
Age* (years)	59 (8)	66 (8)†	66 (7)†
Gender, female	56 (38%)	55 (37%)	56 (37%)
MMSE*	28.3 (1.8)	26.7 (2.2)†	20.5 (4.4)†‡
CSF Aβ42* (pg/mL)	936 (211)	677 (275)†	480 (145)†‡
CSF total tau* (pg/mL)	209 (67)	488 (382)†	735 (449)†‡
CSF p-tau-181* (pg/mL)	38 (10)	71 (37)†	91 (44)†‡

Abbreviations: Aβ42, amyloid-β 1–42; AD, Alzheimer's disease; ANOVA, analysis of variance; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; MMSE, Mini–Mental State Examination; p-tau-181, tau phosphorylated at threonine 181; SD, standard deviation.

NOTE. Data are expressed as the mean (SD) or number (%). Chi-squared test and ANOVA with post hoc pairwise comparison (Bonferroni adjusted *P* values) were performed, where appropriate. ANOVA for CSF Aβ42 and tau were performed after Ln transformation.

*$P < .001$ for ANOVA.

†$P < .001$ post hoc comparison with control subjects.

‡$P < .001$ post hoc comparison with MCI.

albumin, CSF albumin, and serum bilirubin did not differ between groups. No differences were found between subjects with MCI and AD. The covariates age and gender had profound effects on levels of most, but not all, nutrients and metabolites. Supplementary Table 1 shows the *P* values of the statistical analyses.

CSF to serum albumin ratios were calculated per subject to assess the intactness of blood-CSF barrier function. Adjusted for age and gender, ratios of CSF to serum albumin concentrations did not differ between diagnostic groups ($P = .78$, after Ln transformation).

In each diagnostic group, nutrient and metabolite concentrations in blood correlated positively ($P < .05$) with corresponding levels in CSF, except for albumin (see Table 3). The strength of these correlations differed among diagnostic categories. Similarly, several, albeit weak, correlations were found between levels of nutrients and metabolites and markers of disease, that is, AD CSF biomarkers and MMSE and between levels of nutrients and metabolites in blood or CSF (see Supplementary Tables 2–4). These correlations also differed between the diagnostic categories.

4. Discussion

The main findings of this study were that blood and/or CSF concentrations of uridine, folate, choline, and homocysteine differed between subjects with MCI or AD compared with control subjects. Specifically, subjects with MCI had lower concentrations of plasma and CSF uridine and serum and CSF folate, whereas concentration of CSF homocysteine was higher than in control subjects. In subjects with AD, concentrations of CSF uridine, plasma choline, and serum folate were lower, and concentration of CSF homocysteine was higher than in control subjects. Blood and CSF concentrations of these metabolites did not differ between subjects with MCI and AD.

Lower levels of the nutrients measured in the present study could affect the AD brain through several mechanisms. For example, circulating choline is a precursor for the biosynthesis of the neurotransmitter acetylcholine, and folate influences the availability of methyl groups for numerous methylation reactions, such as those involved in DNA

Table 2
Plasma or serum (A) and CSF (B) concentrations of all measured nutrients and metabolites in control, MCI, and AD subjects

	Control subjects	MCI	AD
(A) Plasma or serum			
Total *n*	142–147	142–147	144–148
Plasma uridine* (μmol/L)	4.08 (1.50)	3.64 (1.25)†	3.92 (1.26)
Plasma choline* (μmol/L)	10.48 (2.02)	10.74 (2.35)	10.24 (2.20)†
Plasma betaine (μmol/L)	39.98 (10.09)	40.33 (10.49)	40.06 (10.57)
Serum folate* (μmol/L)	0.020 (0.009)	0.018 (0.009)†	0.019 (0.008)
Plasma homocysteine (μmol/L)	11.44 (3.02)	12.61 (3.67)	12.65 (3.82)
Serum albumin (g/L)	39.69 (3.14)	38.80 (2.96)	38.92 (2.90)
Serum bilirubin (μmol/L)	7.18 (2.84)	7.53 (2.81)	7.28 (2.66)
(B) CSF			
Total *n*	144–146	145–147	144–150
CSF uridine* (μmol/L)	3.07 (0.59)	2.90 (0.60)†	2.87 (0.49)†
CSF choline (μmol/L)	2.72 (0.44)	2.90 (0.49)	2.83 (0.48)
CSF betaine (μmol/L)	2.29 (0.54)	2.32 (0.54)	2.30 (0.57)
CSF folate* (μmol/L)	0.033 (0.005)	0.030 (0.005)†	0.031 (0.006)
CSF homocysteine* (μmol/L)	0.062 (0.021)	0.072 (0.023)†	0.076 (0.028)†
CSF albumin (g/L)	0.23 (0.08)	0.24 (0.10)	0.23 (0.09)

Abbreviations: AD, Alzheimer's disease; ANOVA, analysis of variance; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; SD, standard deviation.

NOTE. Data are expressed as the mean (SD). ANOVA adjusted for age and gender and post hoc pairwise comparison (Bonferroni adjusted *P* values) were performed. ANOVA for plasma choline and betaine, serum bilirubin, and CSF choline and albumin were performed after Ln transformation. The concentrations of all nutrients and metabolites in control subjects in the range of absolute values that are typically reported, except for CSF betaine for which no previous reported values were found.

*$P < .05$ for ANOVA.

†$P < .05$ post hoc comparison with control subjects.

Table 3
Correlations between blood and CSF concentrations of each nutrient/metabolite in control, MCI, and AD subjects

	Control subjects	MCI	AD
Uridine, ρ	0.401*	0.295*	0.189*
Choline, ρ	0.316*	0.402*	0.385*
Betaine, ρ	0.368*	0.483*	0.359*
Folate, ρ	0.452*	0.451*	0.294*
Homocysteine, ρ	0.517*	0.499*	0.554*
Albumin, ρ	0.100	0.048	0.126

Abbreviations: AD, Alzheimer's disease; MCI, mild cognitive impairment; ρ, Spearman's rank correlation coefficient.
*$P < .05$.

methylation and neurotransmitter synthesis. One common denominator for the current set of nutrients is their involvement in phospholipid synthesis. Uridine and choline are important nutritional precursors that act by enhancing the substrate saturation of the enzymes that catalyze the rate-limiting steps in the synthesis of new phospholipids via the Kennedy pathway [9]. Folate (together with vitamins B6 and B12) can increase the bioavailability of the phospholipid precursors docosahexaenoic acid and choline [10]. Phospholipids are the main constituents of membranes and also of neuronal membrane structures, like the presynaptic and postsynaptic membranes of synapses. In AD, degeneration of neuronal membranes and increased breakdown of membrane phospholipids have been linked to synapse loss [19,20]. The lower circulating and CSF nutrient levels found in the present study indicate lower availability nutrients for phospholipid synthesis, which could restrict synaptic membrane formation and contribute to synaptic dysfunction and loss in AD. This lower nutritional status already occurs in a predementia stage, because lower levels were also found in MCI patients. Differences ranged from 2% to 11% of the control subjects and are in the order of magnitude of what generally was found in previous studies [11,12]. Although these differences are relatively small, they could all add up to have a significant effect on rate-limiting phospholipid synthesis, because most enzymes involved in phospholipids synthesis have low affinities for their substrates [9].

This putative nutritional deficit supports the application of nutritional strategies in the early stages of AD, before synaptic pathologic changes have accumulated to an irreversible degree. In accordance with this early intervention hypothesis, randomized controlled studies of a multinutrient intervention containing the nutrients needed for phospholipid synthesis showed improved cognitive function in MCI [21] and mild AD [22], but not in mild-moderate AD [22].

The most consistent finding was the lower plasma and/or CSF uridine concentration in subjects with MCI and AD compared with control subjects. This is the first study to report plasma uridine concentrations in subjects with MCI. The lower concentrations of uridine accord with previous studies reporting lower uridine levels in plasma [12,23] and CSF [24] in AD compared with control subjects. Another study, however, reported relatively higher peak area of uridine in CSF of patients with MCI and AD compared with control subjects [25]. Differences in population, sample size, state of fasting, analytical methods (relative vs. absolute values), and confounder adjustment could possibly explain this contrasting result; however, these differences also account for the aforementioned studies showing lower levels of uridine. Overall, the results of this current large cohort, together with most studies from the literature, clearly indicated a lower uridine status and MCI and AD.

Subjects with AD had lower plasma choline concentrations than control subjects, whereas plasma choline did not differ between MCI and control subjects or AD. CSF choline concentrations did not differ among the three groups. This is the first study presenting plasma and CSF choline concentrations in MCI. Previous studies in AD provide inconsistent results, reporting lower levels of choline in plasma [13] or no differences in plasma [26,27] and higher levels of choline in CSF [28,29] or no differences in CSF [30] in AD patients. Despite this inconsistency in the literature, perhaps ascribable to methodological difficulties in measuring free choline, the current findings are at least confirmative for a defective choline metabolism in AD.

Serum and CSF folate concentrations were lower in subjects with MCI than in control subjects, but did not differ between AD and control subjects or MCI. Previous studies also reported lower serum folate concentration in MCI [31,32], although this has not been consistently found [33,34]. Similarly, some studies found no differences in CSF folate in AD compared with control subjects [35,36], whereas other found lower CSF folate in AD than in control subjects [37–39]. In addition, according to a meta-analysis, AD patients generally show lower serum folate levels compared with control subjects [11]. Plasma homocysteine may be considered a functional indicator of vitamin B status, including folate [40], and is a strong independent risk factor for dementia and AD [41]. In the present study, CSF homocysteine concentrations were higher in subjects with AD and MCI than in control subjects, and plasma homocysteine concentrations did not differ among the three groups. These results are in line with two studies that observed no difference in plasma homocysteine between MCI and control subjects [31,33]. However, current observations contrast with previous meta-analysis, systematic reviews, and studies showing higher plasma homocysteine in AD [42] and MCI [32,34,43], and no difference in CSF homocysteine in MCI [43] and AD [42] compared with control subjects. A range of differences in study characteristics could explain the different outcomes in the present and previous studies, such as differences in population, sample size, state of fasting, and confounder adjustment. It should be noted, however, that plasma and CSF concentrations of folate and plasma concentrations of homocysteine in AD tended to be lower than control subjects, which is more in line with existing data.

Factors contributing to a lower nutritional status in AD may include disease-related changes in nutrient intake, uptake, metabolism, and utilization [7,8], for example, altered

eating behavior [44], aberrant nutrient absorption [45], compromised uptake into the brain [38], and reduced endogenous biosynthesis of nutritional compounds [46]. Additional research is needed to understand the extent of AD-specific changes in eating behavior and nutrient metabolism.

Blood concentrations positively correlated with CSF concentrations for uridine, choline, betaine, folate, and homocysteine in all groups. Nevertheless, correlations between blood and CSF concentrations of uridine and folate were weaker in subjects with AD than in control subjects, perhaps indicating decreased transport function and uptake into the brain. The positive correlations between blood and CSF uridine, choline, and folate occur because these compounds are passively or actively transported into the brain [9,47], and hence the availability of nutrients in the blood largely influences their levels in the brain [9]. A previous study similarly showed positive correlations between serum and CSF folate and between plasma and CSF homocysteine in subjects without cognitive impairment [48]. The lack of correlation in any group for CSF and serum albumin is plausible because albumin typically does not cross the blood-brain barrier. Moreover, subjects with MCI and AD did not have an increased CSF to serum albumin ratio compared with control subjects, which indicates the blood-CSF barrier function remains intact [49]. These results are consistent with previous reports [37,49].

The present study has several limitations. First, mean age differed between the diagnostic categories, which could have masked existing differences between the clinical subgroups, although the analyses were corrected for this confounder. Second, blood and CSF samples were taken from nonfasted subjects at the time of diagnostic workup. Postprandial increases in concentrations of the measured compounds probably increased the variance in each group and, in general, intraindividual variation on nutrient concentration is unknown. Finally, it could be argued that the SCD control group may not be a healthy control reference because some of subjects may have preclinical AD or, at least, an increased risk of developing AD [50]. Nevertheless, the proportion of individuals with SCD who develop MCI and AD is low, and, in the present study, only those subjects meeting rigorous criteria were selected. Most importantly, all had AD CSF biomarker values in the normal ranges, which is a strong indicator for not developing AD [50].

In conclusion, this study showed that compared with control subjects, subjects with MCI and AD have lower blood and CSF concentrations of uridine, folate, and choline, which are involved in synaptic membrane formation. Such disease-specific nutritional deficits, which could exacerbate synaptic dysfunction and could affect other functional processes, are already evident in MCI. In addition, physiological correlations between nutrients and biomarkers are apparently weaker at end-stage, AD dementia. The putative nutritional deficit may be addressed by specific dietary management with low risk of adverse effects, which may be most effective when applied to earlier stages in the development of AD.

Acknowledgments

This work is part of the Nutrition the Unrecognized Determinant for Alzheimer's Disease (NUDAD) project, which received a grant from the Food, Brain and Cognition program of the Nederlandse Organisatie voor Wetenschappelijk Onderzoek, NWO (grant number FCB14-28). Nutricia Advanced Medical Nutrition, Nutricia Research funded the laboratory analyses of the blood and CSF samples.
Conflicts of interest: N.v.W., E.B., and J.W.C.S. are employees of Nutricia Research. P.S. has received consultancy fees from Nutricia Research. All other authors have no conflicts of interest to declare.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.dadm.2017.04.005.

RESEARCH IN CONTEXT

1. Systematic review: Review of the clinical literature on nutritional status in mild cognitive impairment and Alzheimer's disease (AD) revealed lower blood and cerebrospinal fluid levels of nutrients that are required for membrane phospholipid synthesis, but for some of these nutrients results are inconclusive.
2. Interpretation: The current cross-sectional study showed that compared with control subjects, subjects with mild cognitive impairment and AD have lower blood and cerebrospinal fluid concentrations of uridine, folate, and/or choline, which are involved in membrane formation. This putative nutritional deficit may be addressed by specific dietary management with low risk of adverse effects, which may be most effective when applied to earlier stages of AD.
3. Future directions: Additional research is needed to understand the extent of AD-specific changes in nutritional status and to which extent these changes are affected by alterations in eating behavior and nutrient metabolism.

References

[1] Eckert GP, Wood WG, Muller WE. Lipid membranes and beta-amyloid: a harmful connection. Curr Protein Pept Sci 2010;11:319–25.
[2] Selkoe DJ. Alzheimer's disease is a synaptic failure. Science 2002; 298:789–91.
[3] de Wilde MC, Overk CR, Sijben JW, Masliah E. Meta-analysis of synaptic pathology in Alzheimer's disease reveals selective molecular vesicular machinery vulnerability. Alzheimers Dement 2016;12:633–44.

[4] Reitz C, Mayeux R. Alzheimer disease: epidemiology, diagnostic criteria, risk factors and biomarkers. Biochem Pharmacol 2014; 88:640–51.
[5] Scarmeas N, Luchsinger JA, Schupf N, Brickman AM, Cosentino S, Tang MX, et al. Physical activity, diet, and risk of Alzheimer disease. JAMA 2009;302:627–37.
[6] Bourre JM. Effects of nutrients (in food) on the structure and function of the nervous system: update on dietary requirements for brain. Part 1: micronutrients. J Nutr Health Aging 2006;10:377–85.
[7] Mi W, van Wijk N, Cansev M, Sijben JW, Kamphuis PJ. Nutritional approaches in the risk reduction and management of Alzheimer's disease. Nutrition 2013;29:1080–9.
[8] Gustafson DR, Clare Morris M, Scarmeas N, Shah RC, Sijben J, Yaffe K, et al. New perspectives on Alzheimer's disease and nutrition. J Alzheimers Dis 2015;46:1111–27.
[9] Wurtman RJ, Cansev M, Sakamoto T, Ulus IH. Use of phosphatide precursors to promote synaptogenesis. Annu Rev Nutr 2009;29:59–87.
[10] van Wijk N, Broersen LM, de Wilde MC, Hageman RJ, Groenendijk M, Sijben JW, et al. Targeting synaptic dysfunction in Alzheimer's disease by administering a specific nutrient combination. J Alzheimers Dis 2014;38:459–79.
[11] Lopes da Silva S, Vellas B, Elemans S, Luchsinger J, Kamphuis P, Yaffe K, et al. Plasma nutrient status of patients with Alzheimer's disease: systematic review and meta-analysis. Alzheimers Dement 2014; 10:485–502.
[12] Olde Rikkert MG, Verhey FR, Sijben JW, Bouwman FH, Dautzenberg PL, Lansink M, et al. Differences in nutritional status between very mild Alzheimer's disease patients and healthy controls. J Alzheimers Dis 2014;41:261–71.
[13] Blass JP, Hanin I, Barclay L, Kopp U, Reding MJ. Red blood cell abnormalities in Alzheimer disease. J Am Geriatr Soc 1985;33:401–5.
[14] van der Flier WM, Pijnenburg YA, Prins N, Lemstra AW, Bouwman FH, Teunissen CE, et al. Optimizing patient care and research: the Amsterdam Dementia Cohort. J Alzheimers Dis 2014; 41:313–27.
[15] Teunissen CE, Petzold A, Bennett JL, Berven FS, Brundin L, Comabella M, et al. A consensus protocol for the standardization of cerebrospinal fluid collection and biobanking. Neurology 2009; 73:1914–22.
[16] Mulder C, Verwey NA, van der Flier WM, Bouwman FH, Kok A, van Elk EJ, et al. Amyloid-beta(1-42), total tau, and phosphorylated tau as cerebrospinal fluid biomarkers for the diagnosis of Alzheimer disease. Clin Chem 2010;56:248–53.
[17] Imbard A, Smulders YM, Barto R, Smith DE, Kok RM, Jakobs C, et al. Plasma choline and betaine correlate with serum folate, plasma S-adenosyl-methionine and S-adenosyl-homocysteine in healthy volunteers. Clin Chem Lab Med 2013;51:683–92.
[18] Smith DE, Hornstra JM, Kok RM, Blom HJ, Smulders YM. Folic acid supplementation does not reduce intracellular homocysteine, and may disturb intracellular one-carbon metabolism. Clin Chem Lab Med 2013;51:1643–50.
[19] Prasad MR, Lovell MA, Yatin M, Dhillon H, Markesbery WR. Regional membrane phospholipid alterations in Alzheimer's disease. Neurochem Res 1998;23:81–8.
[20] Nitsch RM, Blusztajn JK, Pittas AG, Slack BE, Growdon JH, Wurtman RJ. Evidence for a membrane defect in Alzheimer disease brain. Proc Natl Acad Sci U S A 1992;89:1671–5.
[21] Soininen H, Visser P, Kivipelto M, Hartmann T, Grp LS. A clinical trial investigating the effects of Fortasyn Connect (Souvenaid) in prodromal Alzheimer's disease: results of the Lipididiet Study. Neurobiol Aging 2016;39:S23.
[22] Cummings J, Scheltens P, McKeith I, Blesa R, Harrison JE, Bertolucci PH, et al. Effect size analyses of Souvenaid in patients with Alzheimer's disease. J Alzheimers Dis 2017;55:1131–9.
[23] Wang G, Zhou Y, Huang FJ, Tang HD, Xu XH, Liu JJ, et al. Plasma metabolite profiles of Alzheimer's disease and mild cognitive impairment. J Proteome Res 2014;13:2649–58.
[24] Czech C, Berndt P, Busch K, Schmitz O, Wiemer J, Most V, et al. Metabolite profiling of Alzheimer's disease cerebrospinal fluid. PLoS One 2012;7:e31501.
[25] Ibanez C, Simo C, Barupal DK, Fiehn O, Kivipelto M, Cedazo-Minguez A, et al. A new metabolomic workflow for early detection of Alzheimer's disease. J Chromatogr A 2013;1302:65–71.
[26] Miller BL, Read S, Tang C, Jenden D. Differences in red blood cell choline and lipid-bound choline between patients with Alzheimer disease and control subjects. Neurobiol Aging 1991;12:61–4.
[27] Kanof PD, Greenwald BS, Mohs RC, Davis KL. Red blood cell choline. II: Kinetics in Alzheimer's disease. Biol Psychiatry 1985;20:375–83.
[28] Walter A, Korth U, Hilgert M, Hartmann J, Weichel O, Hilgert M, et al. Glycerophosphocholine is elevated in cerebrospinal fluid of Alzheimer patients. Neurobiol Aging 2004;25:1299–303.
[29] Elble R, Giacobini E, Higgins C. Choline levels are increased in cerebrospinal fluid of Alzheimer patients. Neurobiol Aging 1989; 10:45–50.
[30] Jia JP, Jia JM, Zhou WD, Xu M, Chu CB, Yan X, et al. Differential acetylcholine and choline concentrations in the cerebrospinal fluid of patients with Alzheimer's disease and vascular dementia. Chin Med J (Engl) 2004;117:1161–4.
[31] Quadri P, Fragiacomo C, Pezzati R, Zanda E, Forloni G, Tettamanti M, et al. Homocysteine, folate, and vitamin B-12 in mild cognitive impairment, Alzheimer disease, and vascular dementia. Am J Clin Nutr 2004; 80:114–22.
[32] Siuda J, Gorzkowska A, Patalong-Ogiewa M, Krzystanek E, Czech E, Wiechula B, et al. From mild cognitive impairment to Alzheimer's disease—influence of homocysteine, vitamin B12 and folate on cognition over time: results from one-year follow-up. Neurol Neurochir Pol 2009;43:321–9.
[33] Irizarry MC, Gurol ME, Raju S, Diaz-Arrastia R, Locascio JJ, Tennis M, et al. Association of homocysteine with plasma amyloid beta protein in aging and neurodegenerative disease. Neurology 2005;65:1402–8.
[34] Kim J, Park MH, Kim E, Han C, Jo SA, Jo I. Plasma homocysteine is associated with the risk of mild cognitive impairment in an elderly Korean population. J Nutr 2007;137:2093–7.
[35] Nijst TQ, Wevers RA, Schoonderwaldt HC, Hommes OR, de Haan AF. Vitamin B12 and folate concentrations in serum and cerebrospinal fluid of neurological patients with special reference to multiple sclerosis and dementia. J Neurol Neurosurg Psychiatry 1990;53:951–4.
[36] Popp J, Lewczuk P, Linnebank M, Cvetanovska G, Smulders Y, Kolsch H, et al. Homocysteine metabolism and cerebrospinal fluid markers for Alzheimer's disease. J Alzheimers Dis 2009;18:819–28.
[37] Hagnelius NO, Wahlund LO, Nilsson TK. CSF/serum folate gradient: physiology and determinants with special reference to dementia. Dement Geriatr Cogn Disord 2008;25:516–23.
[38] Serot JM, Christmann D, Dubost T, Bene MC, Faure GC. CSF-folate levels are decreased in late-onset AD patients. J Neural Transm 2001; 108:93–9.
[39] Smach MA, Jacob N, Golmard JL, Charfeddine B, Lammouchi T, Ben Othman L, et al. Folate and homocysteine in the cerebrospinal fluid of patients with Alzheimer's disease or dementia: a case control study. Eur Neurol 2011;65:270–8.
[40] Selhub J, Jacques PF, Wilson PW, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. JAMA 1993;270:2693–8.
[41] Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med 2002;346:476–83.
[42] Shen L, Ji HF. Associations between homocysteine, folic acid, vitamin B12 and Alzheimer's disease: insights from meta-analyses. J Alzheimers Dis 2015;46:777–90.
[43] Trojanowski JQ, Vandeerstichele H, Korecka M, Clark CM, Aisen PS, Petersen RC, et al. Update on the biomarker core of the Alzheimer's Disease Neuroimaging Initiative subjects. Alzheimers Dement 2010; 6:230–8.

[44] Shatenstein B, Kergoat MJ, Reid I. Poor nutrient intakes during 1-year follow-up with community-dwelling older adults with early-stage Alzheimer dementia compared to cognitively intact matched controls. J Am Diet Assoc 2007;107:2091–9.

[45] McCaddon A, Davies G, Hudson P. Nutritionally independent B12 deficiency and Alzheimer disease. Arch Neurol 2000;57:607–8.

[46] Astarita G, Jung KM, Berchtold NC, Nguyen VQ, Gillen DL, Head E, et al. Deficient liver biosynthesis of docosahexaenoic acid correlates with cognitive impairment in Alzheimer's disease. PLoS One 2010; 5:e12538.

[47] Zhao R, Diop-Bove N, Visentin M, Goldman ID. Mechanisms of membrane transport of folates into cells and across epithelia. Annu Rev Nutr 2011;31:177–201.

[48] Smith DE, Smulders YM, Blom HJ, Popp J, Jessen F, Semmler A, et al. Determinants of the essential one-carbon metabolism metabolites, homocysteine, S-adenosylmethionine, S-adenosylhomocysteine and folate, in cerebrospinal fluid. Clin Chem Lab Med 2012;50:1641–7.

[49] Goos JD, Teunissen CE, Veerhuis R, Verwey NA, Barkhof F, Blankenstein MA, et al. Microbleeds relate to altered amyloid-beta metabolism in Alzheimer's disease. Neurobiol Aging 2012; 33:1011.e1–9.

[50] van Harten AC, Visser PJ, Pijnenburg YA, Teunissen CE, Blankenstein MA, Scheltens P, et al. Cerebrospinal fluid Abeta42 is the best predictor of clinical progression in patients with subjective complaints. Alzheimers Dement 2013;9:481–7.