Nutrition and metabolic profiles in Alzheimer's disease

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Chapter 4.3

LDL cholesterol and uridine levels in blood are potential nutritional biomarkers for clinical progression in Alzheimer's disease: the NUDAD project

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Abstract

Introduction: We examined associations between nutritional biomarkers and clinical progression in individuals with subjective cognitive decline (SCD), mild cognitive impairment (MCI) and Alzheimer's disease (AD)-type dementia.

Methods: We included 528 individuals (64±8y, 46%F, follow-up 2.1±0.87y) with SCD (n=204), MCI (n=130) and AD (n=194). Baseline levels of cholesterol, triglycerides, glucose, homocysteine, folate, vitamin A, B12, E, and uridine were measured in blood and S-adenosylmethionine and S-adenosylhomocysteine in cerebrospinal fluid. We determined associations between nutritional biomarkers and clinical progression using Cox proportional hazard models.

Results: Twenty-two (11%) patients with SCD, 45 (35%) patients with MCI and 100 (52%) patients with AD showed clinical progression. In SCD, higher levels of LDL cholesterol were associated with progression (HR (95%CI) 1.88 (1.04-3.41)). In AD, lower uridine levels were associated with progression (0.79 (0.63-0.99)).

Discussion: Our findings suggest that LDL-cholesterol and uridine play a, stage-dependent, role in the clinical progression of AD.

Introduction

Changes in nutritional status including weight loss and lower nutrient levels are often prevalent before the onset of Alzheimer's disease (AD)-type dementia [1-4]. Impaired nutritional status has been associated with faster cognitive decline in community-based populations [5-7]. Nutritional biomarker levels in blood or cerebrospinal fluid (CSF) can be used to identify nutritional factors that may contribute to faster cognitive decline in AD.

Cross-sectional studies comparing nutritional biomarker levels in AD and controls have reported lower levels of several nutrients in AD [2, 3]. Moreover, large population-based studies have reported higher levels of homocysteine and cholesterol to be a risk factor for AD-type dementia [8, 9]. A recent study proposed a nutritional risk index including levels of omega-3 fatty acids, vitamin D and homocysteine that might help to identify non-demented elderly at risk for cognitive decline [6]. These findings suggest that nutritional biomarkers have potential to aid in the identification of targets for dietary interventions.

Further support that optimization of nutritional biomarker levels might be beneficial comes from studies of the Mediterranean diet. Higher adherence to the Mediterranean diet is associated with a reduced risk for AD and for mild cognitive impairment (MCI) conversion to AD [10-12]. The Mediterranean diet is rich in antioxidant nutrients that have been reported to be low in blood from AD patients [13]. Moreover, higher adherence to the Mediterranean diet reduces the intake of saturated fat, in comparison to the typical Western diet, and consequently lowers cholesterol levels [13, 14].

Memory-clinic patients are at increased risk for cognitive decline and eager to take benefit from dietary advice or interventions [15]. The role of nutritional biomarkers in the memory-clinic setting is, however, less clear. A previous cross-sectional study showed that lower levels of several nutritional biomarkers are already prevalent in patients with MCI [16]. In addition, in a retrospective study of patients with subjective cognitive decline (SCD) and MCI, we found modest associations between higher levels of high density lipoprotein (HDL) cholesterol and lower levels of cerebrospinal fluid (CSF) S-adenosylmethionine (SAM) and clinical progression [17]. The role of nutritional biomarkers across the complete cognitive spectrum of AD remains, however, largely unknown. Hence, in this prospective study, we studied the association of nutritional biomarkers with clinical progression, in a memory clinic population with SCD, MCI and AD-type dementia.

Methods

Patients

The NUDAD (Nutrition, the Unrecognized Determinant in Alzheimer's Disease) study is a prospective cohort that aims to identify nutritional determinants in AD-type dementia and predementia stages, with three year clinical follow-up. [4] The NUDAD study is nested within the Amsterdam Dementia Cohort and includes patients who visited the Alzheimer Center Amsterdam between September 2015 and August 2017, were diagnosed with SCD, MCI or AD and had a mini-mental state examination (MMSE) > 16. We excluded 23 patients that had no nutritional biomarker measurements and one patient whose initial AD diagnosis was retracted after three months, leaving 528 participants for data-analysis, including 204 individuals with SCD, 130 patients with MCI and 194 patients with AD. All participants underwent standardized dementia screening, including extensive neuropsychological assessment, neurological examination, magnetic resonance imaging, lumbar puncture, and blood sampling [18]. Diagnoses for MCI and AD were made in multidisciplinary consensus meetings according to the National Institute on Aging-Alzheimer's Association criteria [19, 20]. Individuals with SCD presented with memory complaints but performed normal on all clinical and cognitive examinations, i.e. did not fulfill criteria for MCI, dementia or any psychiatric diagnosis. Written informed consent was obtained from all participants and the protocol was approved by the local Medical Ethical Committee. Diabetes mellitus, hypertension and hypercholesterolemia were defined as self-reported medication use or a medical history for these conditions at baseline. 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 [21].

Follow-up

Follow-up took place by routine annual visits to our memory clinic, in which neuropsychological testing and medical examination were repeated. If participants were unable or did not want to attend annual clinical follow-up, the participant or a proxy was invited for a short telephone interview. In these telephone interviews we surveyed the following items: change of diagnosis or living situation (e.g. independent, institutionalized), and self-reported change in cognitive function (stable/improving/fluctuating/decline). Mean follow-up was 2.1±0.9 years. Main outcome was time to clinical progression, which was considered present when at least one of the following three criteria was met: 1) a follow-up syndrome diagnosis of MCI or dementia (in SCD/MCI); an increase of ≥1 point on the global clinical dementia rating scale (CDR) (in AD) [22], 2) deceased or admission to a nursing home, 3) subjective decline in cognitive

function as reported during the telephone interviews. Time to clinical progression was defined as time between baseline visit and first report of progression.

Nutritional biomarker measures

Blood and CSF samples were obtained within one year from baseline visit (median (range) 0 (0-360) days) and before first report of progression (median (range) 475 (73-1355) days). Blood was collected in 6 mL tubes (BD, Plymouth, United Kingdom) for EDTA plasma or serum separation. CSF was obtained by lumbar puncture using a 25-gauge needle, and collected in 10 mL polypropylene tubes (Sarstedt, Nümbrecht, Germany). Within 2 hours, blood and CSF was centrifuged at 1800x *g* for 10 minutes at room temperature, aliquoted in portions of 0.5 mL and stored at -80°C until further analysis. In total 13 nutritional biomarkers were measured in serum or plasma (range n=451-516, 85-98%), except for SAM and S-adensoylhomocysteine (SAH) that were measured in CSF (n=284-285, 54%) [17]. The number of samples that could be measured for each nutritional biomarker, depended on the available sample volume. Nutritional biomarker concentrations were considered regardless of fasted (n=73) or non-fasted state (n=405 serum; n=399 plasma). Most analyses were performed at the Amsterdam UMC (Amsterdam, the Netherlands). The uridine measurements were performed at Maastricht UMC+ (Maastricht, the Netherlands). Detailed information on measurement methods of the nutritional biomarkers can be found in **Supplementary text 1.**

Amyloid status

Amyloid status as assessed by either positron tomography (PET) and/or CSF analysis was available within one year of baseline visit in 423/528 participants (PET n=51; CSF n=234; PET and CSF n=138). Patients were classified as having a positive amyloid status as at least one of the modalities (i.e. CSF or PET) revealed amyloid positivity. Amyloid positivity on amyloid PET scans was evaluated by an experienced nuclear medicine physician [23]. Amyloid β peptide$_{1\text{-}42}$(Aβ_{42}) were measured in CSF on a routine basis using commercially available enzyme-linked immunosorbent assays (Innotest β-amyloid$_{(1\text{-}42)}$, Ghent, Belgium) as previously described [24]. For Aβ_{42}, we used a drift corrected cut-off <813 pg/mL for amyloid positivity [25].

Statistical analysis

Nutritional biomarkers were log-transformed when not normally distributed and subsequently converted to z-scores to enable comparison of effect sizes. Descriptive characteristics and nutritional biomarkers were compared on their outcome (clinical progression yes/no) in the total group and stratified for baseline syndrome diagnosis (SCD/MCI/AD) using χ^2 tests, Mann-Whitney U tests and t-tests when appropriate. Cox proportional hazard models were used to investigate

if baseline nutritional biomarkers (continuous determinants) were associated with time to clinical progression (outcome) in the total group. We present an unadjusted model (model 1) and a model adjusted for sex, age, diagnosis, and lipid lowering medication (model 2). Hazard ratios (HR) are presented with 95% confidence intervals (CI). Subsequently, we repeated the models 1) stratified for baseline diagnosis, 2) in amyloid positive patients only and 3) stratified for lipid lowering medication (yes/no). Lastly, we focused our analysis on predementia stages, combining individual patient data from the current cohort and from the previously published, retrospective cohort. [17] Eight patients were included in both cohorts, and were therefore excluded from the retrospective cohort. For this analysis, we included only patients with SCD or MCI and we restricted the definition of clinical progression in both cohorts to clinical progression to MCI or dementia (to ensure uniformity over cohorts). The retrospective cohort included 142 patients with SCD (age 61±10y, F 43%) and 149 patients with MCI (age 66±8y, F 38%), mean follow-up was 3.4±2.2 years. We used Cox proportional hazard models in two models; a model only adjusted for cohort (model 1) and a model adjusted for cohort, sex, age, diagnosis, and lipid lowering medication (model 2). To assess if associations differed per cohort, we added interaction terms to the model (nutritional biomarkers*cohort). If there was an interaction between cohort and nutritional biomarker ($p<0.10$), results are additionally reported for the two cohorts separately. Analyses were performed in R version 3.6.1. Results are reported at the threshold of $p<0.05$

Results

Prospective cohort

Clinical progression was observed in 22 (11%) patients with SCD, 45 (35%) patients with MCI and 100 (52%) patients with AD. Patients who showed clinical progression were older, had lower MMSE scores and body mass index (BMI), were more often APOE ε4 carriers and were more often amyloid positive than patients without clinical progression (**Table 1**).

In the total cohort, patients with clinical progression had higher homocysteine levels than patients without clinical progression (**Table 2**). Cox proportional hazard models similarly showed that higher homocysteine levels were associated with a higher risk of clinical progression (HR (95%CI) 1.20 (1.02-1.41)), but this association was lost after adjustment for covariates (**Table 3**).

Stratified for baseline clinical diagnosis, we found that in SCD, higher levels of LDL cholesterol were associated with clinical progression (HR (95%CI) 1.88 (1.04-3.41), model 2) (**Table 3**). In AD, lower uridine levels were associated with clinical progression (HR (95%CI) 0.79 (0.63-0.99), model 2). No significant

associations with clinical progression were observed in the MCI group. An exploratory analysis additionally adjusting for APOE ε4 genotype and having ≥1 risk factor for cardiovascular disease (i.e. former or current smoking, hypertension, hypercholesterolemia, diabetes mellitus) showed comparable associations between higher LDL cholesterol levels and clinical progression in SCD (HR (95%CI) 1.63 (0.88-3.02)) and between lower uridine levels and clinical progression in AD (HR (95%CI) 0.78 (0.62-0.99)) (**Supplementary table 1**).

To evaluate whether the observed associations between nutritional markers and clinical progression were present in the Alzheimer's pathologic spectrum we repeated the models in the subgroup of 253 patients with positive amyloid status (age 66±8, 130 (51%) females(F), 45 SCD, 63 MCI, 145 AD, 110 (43%) clinical progression). Effect sizes remained comparable for homocysteine in the total cohort (HR (95%CI) 1.08 (0.89-1.31), model 1), LDL cholesterol in SCD (HR (95%CI) 2.46 (0.89-6.81), model 2) and uridine in AD (HR (95%CI) 0.82 (0.63-1.07), model 2), although significance was lost probably due to lower power (**Supplementary table 2**).

Finally, we reanalyzed the associations between cholesterol levels and clinical progression stratified for lipid lowering medication (yes, n=115; no, n=348). Lower total cholesterol and LDL cholesterol levels were associated with clinical progression in medication users (HR (95%CI) 0.57 (0.40-0.81), 0.83 (0.70-0.97), model 2), while higher levels of total cholesterol and LDL cholesterol were associated with clinical progression in patient who did not use lipid lowering medication (HR (95%CI) 1.15 (0.91-1.44), 1.18 (0.90-1.54), model 2) (**Supplementary table 3**). These associations were largely similar across diagnostic groups, but strongest for SCD patients who did not use lipid lowering medication (HR (95%CI) 2.11 (1.10-4.06), 1.73 (1.04-2.86), model 2).

Table 1 Baseline characteristics of the NUDAD study population

Baseline diagnosis	All		SCD		MCI		AD	
	No progression	Progression	No progression	Progression	No progression	Progression	No progression	Progression
	361	167	182	22	85	45	94	100
Age, y	63.65 ± 8.13	66.26 ± 8.39*	60.42 ± 7.45	62.61 ± 8.38	65.74 ± 7.34	66.85 ± 8.16	68.02 ± 7.44	66.79 ± 8.37
Female	166 (46)	78 (47)	86 (47)	9 (41)	34 (40)	19 (42)	46 (49)	50 (50)
Education (Verhage scale)	5 (5, 6)	5 (4, 6)	6 (5, 6)	6 (5, 6)	5 (5, 6)	5 (4, 6)	5 (5, 6)	5 (4, 6)
MMSE	27 (25, 29)	24 (21, 27)*	29 (27, 29)	29 (27, 29)	27 (25, 28)	26 (25, 28)	24 (22, 26)	22 (20, 24)*
APOE ε4 carrier[a]	173 (51)	102 (63)*	70 (41)	13 (62)	46 (56)	25 (60)	57 (66)	64 (65)
Follow-up duration, y	2.17 ± 0.90	2.11 ± 0.82	2.18 ± 0.93	2.23 ± 0.79	2.28 ± 0.69	2.09 ± 0.90	2.04 ± 0.99	2.09 ± 0.79
BMI, kg/m^2	26.24 ± 4.22	24.97 ± 3.86*	27.04 ± 4.72	25.79 ± 4.25	25.65 ± 3.72	24.99 ± 3.50	25.23 ± 3.24	24.77 ± 3.93
Smoker								
Current	51 (14)	25 (15)	25 (14)	2 (9)	15 (18)	8 (18)	11 (12)	15 (15)
Former	141 (39)	62 (37)	69 (38)	13 (59)	31 (36)	17 (38)	41 (44)	32 (32)
No	169 (47)	80 (48)	88 (48)	7 (32)	39 (46)	20 (44)	42 (45)	53 (53)
Alcohol, glasses per day	0.5 (0.0, 2.0)	0.5 (0.0, 2.0)	0.5 (0.0, 1.4)	0.8 (0.0, 2.0)	0.5 (0.0, 2.0)	0.5 (0.0, 1.0)	0.5 (0.0, 1.9)	0.5 (0.0, 1.6)
Diabetes Mellitus	35 (10)	13 (8)	11 (6)	3 (14)	15 (18)	2 (4)	9 (10)	8 (8)
Hypertension	131 (36)	51 (31)	64 (35)	6 (27)	33 (39)	16 (36)	34 (36)	29 (29)
Hypercholesterolemia	109 (30)	42 (25)	43 (24)	4 (18)	30 (35)	15 (33)	36 (38)	23 (23)*
Lipid lowering medication	98 (27)	35 (22)	39 (21)	2 (9)	25 (29)	13 (29)	34 (36)	21 (21)*
Amyloid positive[b]	143 (49)	110 (83)*	34 (24)	10 (69)*	37 (53)	26 (65)	72 (92)	73 (95)

Data in mean ± SD, n(%), median (IQR), groups were compared on their outcome (stable vs. progression) in the total cohort and within subgroups of their baseline syndrome diagnosis (SCD/MCI/AD). Education was rated using Verhage's scale ranging from 1 (low) to 7 (high) [26] *p<0.05 values correspond to t-test, χ^2 test or Mann-Whitney U tests when appropriate. Abbreviations: AD, Alzheimer's disease; APOE, apolipoprotein E; BMI, body mass index; IQR, interquartile range; MCI, mild cognitive impairment; SCD, subjective cognitive decline; SD, standard deviation.

[a] APOE genotype was available in 500 participants (95%)

[b] amyloid status (CSF or PET) was available in 423 (80%) participants

Table 2 Nutritional biomarker levels according to baseline diagnosis and clinical progression

Baseline diagnosis	**N**	**All**	**N**	**All**	**N**	**SCD**	**N**	**SCD**	**N**	**MCI**	**N**	**MCI**	**N**	**AD**	**N**	**AD**
Progression (-/+)		**-**		**+**		**-**		**+**		**-**		**+**		**-**		**+**
Serum HDL, mmol/L	318	1.33 ± 0.41	145	1.33 ± 0.39	161	1.28 ± 0.40	22	1.30 ± 0.39	71	1.35 ± 0.42	41	1.32 ± 0.42	86	1.42 ± 0.43	82	1.34 ± 0.38
Serum LDL, mmol/L	318	2.41 ± 0.82	145	2.62 ± 0.93	161	2.33 ± 0.83	22	2.85 ± 0.90*	71	2.45 ± 0.85	41	2.39 ± 0.77	86	2.54 ± 0.78	82	2.67 ± 0.99
Serum total cholesterol, mmol/L	318	4.39 ± 1.04	145	4.59 ± 1.16	161	4.28 ± 1.08	22	4.85 ± 1.24	71	4.41 ± 1.10	41	4.24 ± 0.94	86	4.59 ± 0.92	82	4.69 ± 1.21
Serum triglycerides, mmol/L	318	1.46 ± 0.95	145	1.43 ± 0.75	161	1.53 ± 1.06	22	1.55 ± 0.75	71	1.38 ± 0.76	41	1.18 ± 0.51	86	1.38 ± 0.87	82	1.52 ± 0.82
Plasma glucose, nmol/L	350	7.08 ± 1.91	164	7.16 ± 2.02	176	6.92 ± 1.75	21	7.07 ± 2.07	84	7.25 ± 2.16	44	7.15 ± 2.34	90	7.24 ± 1.95	99	7.18 ± 1.87
Plasma homocysteine, µmol/L	314	12.33 ± 3.63	143	13.16 ± 3.75*	158	11.58 ± 3.27	22	11.65 ± 2.26	70	12.99 ± 3.50	41	13.23 ± 3.39	86	13.20 ± 4.09	80	13.54 ± 4.16
Serum folate, nmol/L	352	19.03 ± 9.77	164	17.90 ± 8.86	178	19.83 ± 11.06	21	16.76 ± 6.30	84	18.45 ± 8.43	44	17.61 ± 8.57	90	18.00 ± 8.01	99	18.26 ± 9.48
Serum vitamin B12, pmol/L	351	372 ± 398	164	333 ± 224	177	364 ± 382	21	305 ± 187	84	359 ± 306	44	325 ± 151	90	398 ± 496	99	342 ± 258
CSF SAM, nmol/L	198	169 ± 37	86	164 ± 39	91	166 ± 34	13	169 ± 44	48	176 ± 48	24	156 ± 46	59	166 ± 32	49	167 ± 33
CSF SAH, nmol/L	198	15.79 ± 5.01	87	16.56 ± 6.27	91	14.96 ± 4.84	13	18.56 ± 9.83	48	16.75 ± 4.87	25	15.72 ± 5.64	59	16.30 ± 5.25	49	16.46 ± 5.37
Plasma vitamin A, µmol/L	314	2.18 ± 0.52	143	2.11 ± 0.47	158	2.19 ± 0.49	22	2.05 ± 0.44	70	2.17 ± 0.52	41	2.03 ± 0.43	86	2.18 ± 0.58	80	2.16 ± 0.50
Plasma vitamin E, µmol/L	314	35.66 ± 8.27	143	35.53 ± 7.92	158	35.02 ± 8.42	22	36.14 ± 6.66	70	35.61 ± 8.68	41	34.27 ± 7.75	86	36.87 ± 7.56	80	36.02 ± 8.33
Plasma uridine, nmol/L	308	4216 ± 1348	143	3957 ± 1176	156	4146 ± 1291	22	4290 ± 1449	67	4404 ± 1632	41	4386 ± 1163	85	4196 ± 1197	80	3646 ± 1009*

Data in mean ± SD, groups were compared on their outcome (stable vs. progression) in the total cohort and within subgroups of their baseline syndrome diagnosis (SCD/MCI/AD). *p<0.05 values correspond to t-tests on log-tranformed nutritional biomarker levels. Abbreviations: AD, Alzheimer's disease; HDL, high density lipoprotein; LDL, low density lipoprotein; MCI, mild cognitive impairment; SAH, S-adenosylhomocysteine; SAM, S-adensoylmethionine; SCD, subjective cognitive decline.

Table 3 Cox proportional hazard models for the association of nutritional biomarkers with clinical progression during follow-up

Baseline diagnosis	N	All		N	SCD		N	MCI		N	AD	
		Model 1	Model 2		Model 1	Model 2		Model 1	Model 2		Model 1	Model 2
HDL	463	1.01 (0.86-1.19)	0.93 (0.77-1.11)	183	1.07 (0.71-1.62)	1.04 (0.66-1.65)	112	0.93 (0.69-1.26)	0.84 (0.59-1.19)	168	0.9 (0.72-1.12)	0.9 (0.7-1.15)
LDL	463	1.11 (0.92-1.34)	0.94 (0.78-1.12)	183	2.01 (1.15-3.5)*	1.88 (1.04-3.41)*	112	0.93 (0.77-1.11)	0.91 (0.76-1.09)	168	0.93 (0.7-1.23)	0.8 (0.58-1.09)
Total cholesterol	463	1.12 (0.94-1.32)	0.95 (0.78-1.15)	183	1.64 (1.05-2.55)*	1.57 (0.99-2.51)	112	0.85 (0.63-1.15)	0.79 (0.57-1.09)	168	0.95 (0.74-1.22)	0.86 (0.65-1.14)
Triglycerides	463	0.99 (0.84-1.17)	1.02 (0.86-1.21)	183	1.11 (0.75-1.64)	1.2 (0.79-1.83)	112	0.81 (0.59-1.12)	0.83 (0.59-1.16)	168	1.12 (0.89-1.41)	1.12 (0.89-1.41)
Glucose	514	1.03 (0.88-1.19)	0.97 (0.83-1.13)	197	1.08 (0.69-1.71)	1.21 (0.74-1.97)	128	0.98 (0.75-1.28)	0.97 (0.74-1.27)	189	0.9 (0.74-1.1)	0.95 (0.77-1.16)
Homocysteine	457	1.2 (1.02-1.41)*	1.09 (0.92-1.29)	180	1.03 (0.64-1.64)	1.02 (0.61-1.68)	111	1.09 (0.78-1.53)	1.13 (0.79-1.6)	166	1.07 (0.88-1.3)	1.08 (0.87-1.33)
Folate	516	0.9 (0.77-1.05)	0.93 (0.79-1.08)	199	0.77 (0.49-1.2)	0.67 (0.41-1.09)	128	0.94 (0.7-1.27)	0.9 (0.66-1.23)	189	0.99 (0.82-1.2)	0.98 (0.81-1.2)
Vitamin B12	515	0.97 (0.83-1.14)	0.93 (0.79-1.1)	198	0.84 (0.52-1.38)	0.82 (0.49-1.38)	128	0.96 (0.7-1.3)	0.91 (0.66-1.26)	189	0.95 (0.77-1.18)	0.96 (0.77-1.19)
CSF SAM	284	0.92 (0.75-1.13)	0.9 (0.73-1.12)	104	0.99 (0.59-1.65)	1.12 (0.65-1.93)	72	0.76 (0.55-1.05)	0.73 (0.52-1.02)	108	0.98 (0.69-1.39)	1 (0.69-1.43)
CSF SAH	285	1.13 (0.91-1.40)	1.01 (0.79-1.29)	104	1.48 (0.93-2.34)	1.34 (0.79-2.26)	73	0.83 (0.52-1.31)	0.66 (0.36-1.2)	108	0.96 (0.7-1.31)	0.98 (0.7-1.38)
Vitamin A	457	0.93 (0.80-1.09)	0.96 (0.81-1.13)	180	0.82 (0.56-1.19)	0.81 (0.54-1.22)	111	0.83 (0.62-1.12)	0.85 (0.62-1.15)	166	1.08 (0.88-1.33)	1.11 (0.89-1.39)
Vitamin E	457	0.98 (0.83-1.16)	0.85 (0.7-1.02)	180	1.23 (0.8-1.91)	1.18 (0.75-1.87)	111	0.89 (0.67-1.19)	0.79 (0.56-1.11)	166	0.86 (0.69-1.08)	0.79 (0.61-1.02)
Uridine	451	0.88 (0.75-1.03)	0.9 (0.76-1.06)	178	1.04 (0.69-1.58)	0.98 (0.65-1.49)	108	1 (0.75-1.35)	0.98 (0.72-1.33)	165	0.79 (0.63-0.99)*	0.79 (0.63-0.99)*

Cox proportional hazard model 1 unadjusted, model 2 adjusted for age, sex and lipid-lowering medication and in total group also for diagnosis. Data are presented as hazard ratio (95% confidence interval). Nutritional markers were log-transformed and converted to z-scores prior to analysis. Abbreviations: AD, Alzheimer's disease; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; MCI, mild cognitive impairment; SAM, S-adesonsylmethionine; SAH, S-adenosylhomocysteine; SCD, subjective cognitive decline.

* $p<0.05$

Analysis of predementia stages across two cohorts

In an additional analysis, we focused our analysis on predementia stages (i.e. SCD and MCI) combining individual patient data from the retrospective cohort and prospective cohort. Clinical progression was observed in 37 (11%) patients with SCD and 71 (25%) patients with MCI. Higher homocysteine levels were associated with clinical progression in model 1 (HR (95%CI) 1.21 (1.01-1.47)), but this association was lost in model 2 (**Table 4**). Higher HDL cholesterol levels were associated with clinical progression (HR (95%CI) 1.31 (1.04-1.64), model 2). Interactions between cohort and nutritional biomarkers were only found for LDL cholesterol (HR (95%CI) retrospective cohort: 0.97 (0.75-1.25); prospective cohort: 1.52 (0.91-2.55), model 2).

Stratified for syndrome diagnosis, higher HDL cholesterol levels were associated with clinical progression in SCD (HR (95%CI) 1.48 (1.02-2.15), model 2) while, lower CSF SAM and SAH levels were associated with clinical progression in MCI (HR (95%CI) 0.72 (0.58-0.90), 0.74 (0.54-1.00), model 2) (**Table 4**).

Discussion

The main finding of this memory-clinic cohort study is that higher LDL cholesterol levels in SCD and lower uridine levels in AD were associated with clinical progression. The association for higher LDL cholesterol levels in SCD, was driven by individuals that did not use lipid lowering medication. Our findings extend on previous work in population-based studies by showing associations of nutritional biomarkers with clinical progression in a memory-clinic sample [8, 9].

In our prospective study, we found that higher LDL cholesterol levels were associated with clinical progression in SCD. This association was driven by SCD patients who did not use lipid lowering medication. Mid-life hypercholesterolemia is often reported as risk factor for cognitive decline and dementia [8, 27, 28]. This association is less clear in late-life and in the symptomatic phase of AD [29-32]. This might explain why the association between higher LDL cholesterol and clinical progression is restricted to SCD and not found in MCI or AD. Our findings indicate that the relation between cholesterol and clinical progression is complex and seemingly dependent on disease stage and medication use.

Table 4 Cox proportional hazard models for the association of nutritional biomarkers with clinical progression in predementia stages in two independent cohorts restricting the definition of clinical progression to progression to MCI or dementia

Baseline diagnosis	**N**	**All**		**N**	**SCD**		**N**	**MCI**	**MCI**
		Model 1	Model 2		Model 1	Model 2		Model 1	Model 2
HDL	586	1.24 (1.03-1.49)*	1.31 (1.04-1.64)*	325	1.14 (0.84-1.54)	1.48 (1.02-2.15)*	261	1.38 (1.09-1.76)*	1.23 (0.92-1.66)
LDL	586	1.06 (0.87-1.28)	1.08 (0.88-1.33)	325	1.13 (0.8-1.62)	1.11 (0.75-1.64)	261	1.08 (0.86-1.36)	1.03 (0.81-1.3)
Total cholesterol	586	1.07 (0.88-1.3)	1.1 (0.88-1.36)	325	1.2 (0.85-1.68)	1.23 (0.85-1.79)	261	1.07 (0.84-1.37)	0.96 (0.74-1.26)
Triglycerides	586	0.87 (0.72-1.05)	0.86 (0.7-1.06)	325	1.04 (0.76-1.42)	0.87 (0.61-1.24)	261	0.76 (0.59-0.97)*	0.79 (0.61-1.02)
Glucose	517	1.21 (0.99-1.49)	1.19 (0.96-1.47)	296	1.31 (0.92-1.87)	1.22 (0.82-1.82)	221	1.12 (0.87-1.43)	1.13 (0.89-1.45)
Homocysteine	568	1.21 (1.01-1.47)*	1.05 (0.84-1.3)	311	1.14 (0.82-1.58)	0.87 (0.59-1.28)	257	1.11 (0.87-1.43)	1.25 (0.96-1.62)
Folate	618	0.99 (0.81-1.2)	1 (0.83-1.21)	341	1.2 (0.84-1.74)	1.2 (0.83-1.74)	277	0.94 (0.76-1.16)	0.91 (0.73-1.14)
Vitamin B12	544	1.02 (0.83-1.27)	1.02 (0.82-1.25)	296	1.18 (0.82-1.68)	1.05 (0.72-1.53)	248	0.95 (0.73-1.25)	0.93 (0.7-1.22)
CSF SAM	466	0.86 (0.7-1.04)	0.86 (0.71-1.04)	245	1.11 (0.77-1.62)	1.23 (0.85-1.79)	221	0.76 (0.61-0.94)	0.72 (0.58-0.9)*
CSF SAH	468	1.06 (0.87-1.29)	0.87 (0.7-1.09)	246	1.31 (0.95-1.79)	1.01 (0.72-1.42)	222	0.77 (0.57-1.03)	0.74 (0.54-1)*
Vitamin A	582	1.03 (0.86-1.24)	1.05 (0.85-1.28)	322	1.1 (0.79-1.52)	1.07 (0.76-1.51)	260	0.94 (0.75-1.18)	1.07 (0.83-1.39)
Vitamin E	582	1.04 (0.86-1.27)	1.07 (0.87-1.31)	322	1.11 (0.79-1.56)	1.1 (0.77-1.56)	260	1.09 (0.86-1.38)	0.99 (0.77-1.28)
Uridine	571	1.03 (0.86-1.24)	0.99 (0.83-1.2)	316	1.05 (0.77-1.42)	1.09 (0.8-1.48)	255	0.94 (0.74-1.19)	0.91 (0.72-1.15)

Cox proportional hazard model 1 adjusted for cohort, model 2 adjusted for cohort, age, sex and lipid-lowering medication and in total group also for diagnosis. Data are presented as hazard ratio (95% confidence interval). Nutritional markers were log-transformed and converted to z-scores prior to analysis. Abbreviations: AD, Alzheimer's disease; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; MCI, mild cognitive impairment; SAM, S-adesonsylmethionine; SAH, S-adenosylhomocysteine; SCD, subjective cognitive decline.

* $p<0.05$

The finding that lower levels of uridine were associated with clinical progression in patients with AD extends on previous cross-sectional studies that described lower levels of CSF and plasma uridine in MCI and AD in comparison to controls [16, 33, 34]. Uridine is a precursor for phospholipids and is required for neuronal cell membrane formation. Higher uridine levels may have a positive effect on synaptic function and synapse membrane formation which might alleviate synaptic dysfunction in AD [35-37]. The association of lower levels of uridine in AD might be explained by a lower nutrient intake or an increased need for uridine to regenerate synaptic membranes in AD [35, 38]. Future studies that assess more nutritional biomarkers involved in the phospholipid metabolism, such as choline and docosahexaenoic acid, will help to gain more insight in the role of the phospholipid metabolism in the clinical progression of AD. Since our findings for uridine were restricted to the AD dementia stage the associations of lower uridine levels with increased risk of clinical progression might only develop in relation to homeostatic changes during the late symptomatic phase.

Additionally, in a combined analysis of predementia stages in our prospective and retrospective cohort, higher HDL cholesterol levels and lower CSF SAM and SAH levels in MCI with clinical progression. The role of HDL cholesterol levels in AD is inconclusive [17, 39]. One explanation for our findings could be that HDL is dysfunctional in AD, as also reported for type 2 diabetes and coronary heart disease, resulting in impaired protective effects while HDL cholesterol levels remain normal [40]. Our findings for higher homocysteine levels (prospective cohort and combined analysis) and lower CSF SAM and SAH levels in MCI (combined analysis) are in line with previous reports of low CSF SAM levels in AD vs. controls [41, 42], and with higher homocysteine levels as risk factor for dementia [9]. Hyperhomocysteinemia is one of the most widely studied nutritional risk factors in AD and can be caused by suboptimal levels of folate, vitamin B12 and vitamin B6 [43]. Elevated homocysteine levels are associated with an increased risk of atherosclerosis and stroke which can contribute to cognitive decline [44, 45]. Homocysteine is a metabolite in the one-carbon metabolism in which SAM is the direct methyl group donor [46]. SAM together with SAH, affects the methylation of DNA, RNA, neurotransmitters and phospholipids and therefore, might be biologically influencing the AD disease process [47]. For example, low levels of SAM have been suggested to influence expression of presenilin 1 and β-secretase and increase Aβ production [48, 49]. SAM and SAH levels in AD have mostly been investigated in brain tissue or CSF [41, 50]. Measurements in plasma are less invasive but require deproteinized blood samples that were unavailable in this study [51]. Future studies should examine SAM/SAH changes in deproteinized plasma as this will enhance the implementation of these markers in large population based studies that usually do not collect CSF.

The current prospective study was set up to extend on the findings from our previous retrospective cohort study [17]. Our previous findings for higher HDL cholesterol and lower CSF SAM levels and clinical progression, remained in our combined analyses, but could not be replicated in the prospective cohort alone. In our previous report on the retrospective cohort we additionally applied an integrative approach to study the associations between combinations of nutritional biomarkers to clinical progression. Since we measured only a subset of the nutritional biomarkers in the current prospective cohort, we could not validate the previously identified profiles, and we show associations for single biomarkers. Overall, the heterogeneous findings in the two cohorts stress that associations between nutritional biomarkers and clinical progression are highly complex. For both cohorts, patients were included from our tertiary memory-clinic and thus received similar clinical work-up. In the retrospective cohort, however, we oversampled patients with clinical progression to increase statistical power, which could perhaps explain the difference in findings between the two cohorts. Another explanation could be that other factors, such as medication use, cause this variability.

Strengths of this study is that our participants underwent standardized cognitive screening and follow-up, had CSF or PET amyloid status available and included measurements of several nutritional biomarkers. Furthermore, this study extended on our previous retrospective cohort, as we investigated the 13 nutritional biomarkers that showed most promising associations with clinical progression in retrospective cohort. This study also has some limitations. We defined deceased, nursing home admission and subjective progression of cognitive symptoms as clinical events in our outcome measure. One could argue that these events might not always be a consequence of neurodegenerative disease progression. This enabled us, however, to capture detrimental outcomes in a wider context than measured at the clinical visits to our memory-clinic. Furthermore, we used both fasting and non-fasting samples, however, we found no statistical evidence for effect modification or confounding by fasting status (data not shown).

In conclusion, we found associations between higher LDL cholesterol levels in SCD and lower uridine in AD with clinical progression. Our findings are biologically plausible and fit with previous findings in animal studies and cell studies of disturbed uridine and cholesterol metabolism in AD [35, 52]. Our findings suggest that further studies should investigate if dietary interventions that influence cholesterol and uridine metabolism can slow the rate of clinical progression in memory-clinic patients.

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Conflicts of interest

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Supplementary text 1

HDL cholesterol, total cholesterol and triglycerides were measured in serum with a colorimetric assay on a Cobas 8000 system (HDL-Cholesterol plus, Cholesterol gen, Triglycerides; Roche Diagnostics, Roche, Basel, Switzerland). For these lipids inter-assay coefficients of variations (CV's) were 0.9-2.0%. The lower limit of quantitation (LLOQ) was 0.08 mmol/L for HDL, 0.1 mmol/L for triglycerides and 0.1 mmol/L for total cholesterol. Low density lipoprotein (LDL) cholesterol was calculated from total cholesterol using the Friedewald formule LDL-cholesterol = total cholesterol – HDL cholesterol – (0.45x triglyceride). Plasma glucose was tested with UV detection on a Cobas 8000 system (Glucose HK Gen; Roche Diagnostics, Roche, Basel, Switzerland). Inter-assay CV for glucose was 1.1-1.3% and the LLOQ was 0.11 mmol/L. Plasma homocysteine, serum folate and serum vitamin B12 were measured by competitive luminescence immunoassay on an Architect analyzer (Abbott Diagnostics, Abbott Laboratories, Abbott Park, USA). Inter-assay CV's were 2%-4% for homocysteine in plasma, 9% for folate and 6.3% for vitamin B12. LLOQ was 1µmol/L for homocysteine in plasma, 2nmol/L for folate and 44 pmol/L for vitamin B12. CSF SAM and SAH were measured by positive electrospray LC-MS/MS [1]. Inter-assay CV's for SAM were 3.2% and for SAH 8.6%. Vitamin A and E were measured in plasma using high performance liquid chromatography (HPLC) with UV detection [2]. The LLOQ was 0.1 µmol/L for vitamin A and 1.0 µmol/L for vitamin E. Inter-assay variation was determined at two different concentrations and ranged from 0.7-1.0% for vitamin A and 0.8% - 1.6% for vitamin E. Plasma uridine was measured by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Inter-assay CV for uridine was 4.0%-14%, LLOQ was calculated at 0.2 µmol/L [3].

Supplementary table 1 Cox proportional hazard models for the association of nutritional biomarkers with clinical progression during follow-up adjusted for age, sex, lipid lowering medication, APOE Ɛ4 genotype, ≥1 cardiovascular risk factor

Baseline diagnosis	**N**	**All**	**N**	**SCD**	**N**	**MCI**	**N**	**AD**
Nutritional biomarker								
HDL	439	0.92 (0.77-1.11)	171	1.13 (0.69-1.88)	107	0.77 (0.52-1.13)	124	0.94 (0.73-1.21)
LDL	439	0.92 (0.77-1.09)	171	1.63 (0.88-3.02)	107	0.9 (0.75-1.09)	124	0.82 (0.6-1.13)
Total cholesterol	439	0.93 (0.77-1.13)	171	1.45 (0.9-2.33)	107	0.78 (0.56-1.08)	124	0.89 (0.67-1.19)
Triglycerides	439	1.03 (0.86-1.22)	171	1.13 (0.72-1.75)	107	0.85 (0.58-1.24)	124	1.11 (0.88-1.42)
Glucose	492	0.98 (0.84-1.14)	184	1.09 (0.65-1.82)	124	0.99 (0.74-1.31)	142	0.92 (0.74-1.13)
Homocysteine	434	1.08 (0.92-1.28)	169	0.97 (0.59-1.6)	106	1.12 (0.77-1.61)	122	1.09 (0.88-1.33)
Folate	493	0.94 (0.8-1.09)	185	0.77 (0.47-1.28)	124	0.91 (0.65-1.27)	142	0.98 (0.8-1.19)
Vitamin B12	493	0.93 (0.79-1.1)	185	0.93 (0.53-1.61)	124	0.89 (0.63-1.24)	142	0.95 (0.77-1.19)
CSF SAM	270	0.9 (0.72-1.12)	97	1.1 (0.62-1.97)	69	0.75 (0.53-1.05)	101	1.02 (0.72-1.45)
CSF SAH	271	1.02 (0.79-1.31)	97	1.24 (0.67-2.3)	70	0.71 (0.37-1.35)	101	1.05 (0.74-1.51)
Vitamin A	434	0.94 (0.8-1.11)	169	0.78 (0.51-1.19)	106	0.82 (0.6-1.13)	122	1.08 (0.86-1.35)
Vitamin E	434	0.84 (0.69-1.01)	169	1.12 (0.69-1.84)	106	0.82 (0.57-1.18)	122	0.79 (0.61-1.02)
Uridine	428	0.89 (0.76-1.06)	167	1.09 (0.72-1.65)	103	0.92 (0.66-1.27)	122	0.78 (0.62-0.99)*

Cox proportional hazard adjusted for age, sex, lipid-lowering medication, APOE Ɛ4 genotype and ≥1 cardiovascular risk factor and in total group also for diagnosis. Cardiovascular risk factors were former or current smoking, hypercholesterolemia, hypertension or diabetes mellitus. Data are presented as hazard ratio (95% confidence interval). Nutritional markers were log-transformed if not normally distributed and converted to z-scores prior to analysis. Abbreviations: AD, Alzheimer's disease; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; MCI, mild cognitive impairment; SAM, S-adesonsylmethionine; SAH, S-adenosylhomocysteine; SCD, subjective cognitive decline.

* $p<0.05$

Supplementary table 2 Cox proportional hazard models for the association of nutritional biomarkers with clinical progression during follow-up in amyloid positive patients

Baseline diagnosis	N	All		N	SCD		N	MCI		N	AD	
		Model 1	Model 2		Model 1	Model 2		Model 1	Model 2		Model 1	Model 2
HDL	218	1.02 (0.83-1.27)	1.03 (0.81-1.3)	40	1.25 (0.61-2.56)	1.41 (0.51-3.88)	54	1 (0.66-1.5)	0.99 (0.61-1.6)	124	0.97 (0.74-1.27)	0.96 (0.71-1.29)
LDL	218	0.95 (0.8-1.13)	0.9 (0.75-1.07)	40	1.84 (0.91-3.75)	2.46 (0.89-6.81)	54	0.94 (0.78-1.13)	0.94 (0.77-1.15)	124	0.82 (0.57-1.18)	0.7 (0.47-1.06)
cholesterol	218	1 (0.8-1.25)	0.93 (0.71-1.21)	40	1.67 (0.93-2.99)	2.49 (0.98-6.36)	54	0.88 (0.6-1.3)	0.86 (0.56-1.32)	124	0.9 (0.66-1.22)	0.8 (0.56-1.15)
Triglycerides	218	1.04 (0.83-1.3)	1.04 (0.83-1.3)	44	1.11 (0.46-2.66)	1.01 (0.41-2.48)	54	0.9 (0.57-1.41)	0.88 (0.54-1.42)	124	1.12 (0.86-1.47)	1.13 (0.87-1.47)
Glucose	247	0.92 (0.76-1.11)	0.91 (0.75-1.1)	40	1.19 (0.59-2.42)	1.22 (0.6-2.51)	61	0.93 (0.64-1.36)	0.91 (0.62-1.35)	142	0.87 (0.69-1.09)	0.87 (0.69-1.11)
Homocysteine	215	1.08 (0.89-1.31)	1.07 (0.87-1.31)	44	1.06 (0.55-2.03)	0.98 (0.45-2.12)	53	1.35 (0.85-2.15)	1.44 (0.87-2.36)	122	0.98 (0.78-1.23)	1 (0.78-1.28)
Folate	247	1.02 (0.84-1.22)	1.02 (0.84-1.23)	44	0.92 (0.39-2.16)	0.65 (0.25-1.7)	61	0.86 (0.6-1.24)	0.83 (0.56-1.23)	142	1.11 (0.89-1.38)	1.09 (0.87-1.36)
Vitamin B12	247	1.01 (0.83-1.22)	0.99 (0.81-1.22)	44	1.1 (0.66-1.81)	1.98 (0.91-4.29)	61	1.08 (0.71-1.62)	1.07 (0.68-1.69)	142	0.96 (0.74-1.24)	0.93 (0.71-1.21)
CSF SAM	172	0.92 (0.73-1.16)	0.92 (0.72-1.16)	27	1.18 (0.58-2.42)	1.36 (0.6-3.08)	44	0.74 (0.53-1.03)	0.72 (0.51-1.02)	101	1.05 (0.73-1.52)	1.07 (0.73-1.56)
CSF SAH	172	0.99 (0.79-1.26)	0.99 (0.76-1.28)	27	1.19 (0.7-2.02)	1.17 (0.63-2.15)	44	0.73 (0.44-1.23)	0.57 (0.29-1.15)	101	1 (0.73-1.38)	1.01 (0.72-1.43)
Vitamin A	215	1.1 (0.9-1.36)	1.14 (0.92-1.42)	40	1.58 (0.81-3.08)	1.89 (0.83-4.27)	53	0.9 (0.58-1.4)	0.88 (0.55-1.42)	122	1.12 (0.87-1.45)	1.15 (0.88-1.52)
Vitamin E	215	0.94 (0.75-1.17)	0.88 (0.68-1.13)	40	1.35 (0.61-3.03)	1.28 (0.41-4.03)	53	0.95 (0.64-1.41)	0.92 (0.59-1.45)	122	0.88 (0.66-1.15)	0.8 (0.58-1.1)
Uridine	211	0.85 (0.7-1.04)	0.86 (0.7-1.05)	39	0.92 (0.52-1.65)	0.87 (0.47-1.62)	50	0.91 (0.62-1.33)	0.9 (0.61-1.34)	122	0.83 (0.64-1.06)	0.82 (0.63-1.07)

Cox proportional hazard model unadjusted, model adjusted for age, sex and lipid-lowering medication and in total group also for diagnosis. Data are presented as hazard ratio (95% confidence interval). Nutritional markers were log-transformed if not normally distributed and converted to z-scores prior to analysis. Abbreviations: AD, Alzheimer's disease; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; MCI, mild cognitive impairment; SAM, S-adesonsylmethionine; SAH, S-adenosylhomocysteine; SCD, subjective cognitive decline. * p<0.05

Supplementary table 3 Cox proportional hazard models for the association of nutritional biomarkers with clinical progression during follow-up; stratified for lipid lowering medication

	All		SCD		MCI		AD	
Use of lipid lowering medication	Yes (n=115)	No (n=348)	Yes (n=36)	No (n=147)	Yes (n=33)	No (n=79)	Yes (n=49)	No (n=122)
HDL	0.72 (0.46-1.12)	0.98 (0.8-1.2)	4.26 (0.3-60)	0.95 (0.59-1.53)	0.32 (0.12-0.88)*	1.03 (0.69-1.54)	0.8 (0.41-1.56)	0.96 (0.73-1.26)
LDL	0.83 (0.7-0.97)*	1.18 (0.9-1.53)	0.61 (0.1-3.82)	2.11 (1.1-4.06)*	0.9 (0.74-1.08)	1.04 (0.61-1.78)	0.56 (0.31-1)*	1.01 (0.69-1.47)
Total cholesterol	0.57 (0.4-0.81)*	1.15 (0.91-1.44)	0.49 (0.08-2.89)	1.73 (1.04-2.86)*	0.56 (0.34-0.92)*	0.95 (0.62-1.46)	0.54 (0.3-0.97)**	1.08 (0.78-1.5)
Triglycerides	0.69 (0.47-1)	1.13 (0.94-1.37)	0.48 (0.11-2.06)	1.4 (0.89-2.19)	0.82 (0.41-1.63)	0.81 (0.53-1.22)	0.7 (0.41-1.2)	1.23 (0.96-1.58)

Cox proportional hazard model 2 adjusted for age and sex and in total group also for diagnosis. Data are presented as hazard ratio (95% confidence interval). Nutritional markers were log-transformed and converted to z-scores prior to analysis. Abbreviations: AD, Alzheimer's disease; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; MCI, mild cognitive impairment; SAM, S-adesonsylmethionine; SAH, S-adenosylhomocysteine; SCD, subjective cognitive decline. * p<0.05

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