Cognitive decline in late-life: biological markers and early identification of persons at risk for dementia

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2011

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Tessa Nicole van den Kommer

The studies presented in this thesis were conducted within the EMGO Institute for Health and Care Research (EMGO+) (www.emgo.nl). EMGO+ participates in the Netherlands School of Primary Care Research (CaRe) which was re-acknowledged in 2005 by the Royal Netherlands Academy of Arts and Sciences (KNAW).

Financial support for the production of this thesis has been kindly provided by:

Alzheimer Nederland
EMGO Institute for Health and Care Research
Internationale Stichting Alzheimer Onderzoek (ISAO)
Lundbeck B.V.
Novartis Pharma B.V.
Vrije Universiteit

ISBN: 9789086595198

Cover: 'Corvallis area, Oregon, USA'
Printed by: Gildeprint Drukkerijen, Enschede, www.gildeprint.nl

VRIJE UNIVERSITEIT

Cognitive decline in late-life: biological markers and early identification of persons at risk for dementia

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad Doctor aan
de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof.dr. L.M. Bouter,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de faculteit der Geneeskunde
op vrijdag 7 januari 2011 om 13.45 uur
in de aula van de universiteit,
De Boelelaan 1105

door

Tessa Nicole van den Kommer

geboren te Delft

promotoren:	prof.dr. D.J.H. Deeg
	prof.dr. C. Jonker
copromotoren:	dr. M.G. Dik
	dr. H.C. Comijs

“ ... It was the best of times, it was the worst of times, it was the age of wisdom, it was the age of foolishness, it was the epoch of belief, it was the epoch of incredulity, it was the season of light, it was the season of darkness, it was the spring of hope, it was the winter of despair ...”

Charles Dickens, A Tale of Two Cities
English novelist (1812 - 1870)

Contents

Chapter 4

Total cholesterol and oxysterols: early markers for cognitive decline in elderly?

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Neurobiology of Aging 2009;30(4):534–545
doi:10.1016/j.neurobiolaging.2007.08.005

Abstract

In this prospective study we examined whether total cholesterol and the oxysterols 24S- and 27-hydroxycholesterol were related to cognitive performance and rate of cognitive decline in elderly, and whether these associations were modified by ApoE e4. Data were collected during six years of follow-up as part of the Longitudinal Aging Study Amsterdam (N = 1,181, age ≥ 65 years), and analyzed using generalized estimating equations. Cognitive performance was measured with the Mini-Mental State Examination (general cognition), the Auditory Verbal Learning Test (memory) and the Coding Task (information processing speed).

Lower cholesterol at baseline was negatively associated with both general cognition ($p = 0.012$) and information processing speed ($p = 0.045$). ApoE modified the association between cholesterol and cognitive decline, and the association between the ratio of 27-hydroxycholesterol to cholesterol and cognitive functioning. In ApoE e4 carriers, lower cholesterol was related to a higher rate of decline on information processing speed ($p = 0.006$), and a higher ratio of 27-hydroxycholesterol to cholesterol was related to a lower level of general performance ($p = 0.002$) and memory functioning ($p = 0.045$). The results implicate that lower total cholesterol may be considered as a frailty marker, predictive of lower cognitive functioning in elderly.

Keywords: Cholesterol; Oxysterols; ApoE e4; Cognitive decline; Aging.

Introduction

Cholesterol and cholesterol metabolism have been increasingly linked to Alzheimer's disease (AD), yet evidence remains seemingly inconsistent. Although several epidemiological studies showed an association between higher levels of total cholesterol and higher dementia prevalence (Evans et al., 2000; Pappolla et al., 2003), some found an association between lower cholesterol levels and an increased risk of dementia (Kuuisto et al., 1997; Solfrizzi et al., 2002), whereas still others failed to find a relationship (Tan et al., 2003). These inconsistencies may be due to the clinical stage of dementia, small sample sizes, use of lipid-lowering drugs, and time of measurement over the life course of cholesterol. Also, the causality and mechanisms underlying the relationship between cholesterol and AD remain to be further clarified. High plasma concentrations of total cholesterol in midlife may be a risk factor for AD (Jarvik et al., 1995; Kivipelto et al., 2001; Pappolla et al., 2003), as opposed to later life where it may be considered an indicator of better health status, and may be associated with decreased dementia risk (Mielke et al., 2005). Furthermore, lower total cholesterol in later life could be an effect rather than a cause of dementia, as a consequence of decreased nutrition (Panza et al., 2006).

To gain more insight into the association between cholesterol and AD, it is necessary to look at transport and turnover of cholesterol in the brain, which is the most cholesterol-rich organ in the body containing about 25% of total body cholesterol. The major pathway of elimination of excessive brain cholesterol, derived from excessive synthesis or cell death, is the conversion of cholesterol into 24S-hydroxycholesterol (24S-OH cholesterol). This oxysterol is highly interesting because it passes through the blood-brain barrier into the peripheral blood, it can be determined in bloodserum, and it is brain-specific. It has been demonstrated that nearly all of the circulating 24S-OH cholesterol in the human body originates in the brain (Lütjohann et al., 1996). Concentrations of 24S-OH cholesterol, of which the ratio to cholesterol is considered a surrogate marker of brain cholesterol metabolism (Björkhem and Meaney, 2004), appear to be highest in the early stages of AD and subsequently decrease in severely demented patients (Lütjohann et al., 2000; Papassotiropoulos et al., 2000). 27-Hydroxycholesterol (27-OH cholesterol) is another oxidated product of cholesterol, and is also able to cross the blood-brain barrier (Leoni et al., 2003). It has been shown that patients with AD, vascular dementia (VaD) and mild cognitive impairment (MCI) had significantly lower ratios of 27-OH cholesterol to cholesterol compared with non-demented subjects (K□lsch et al., 2004).

Another important factor that could play a role in the association between cholesterol metabolism and cognitive decline is Apolipoprotein E (ApoE). ApoE is the major lipid transport protein in the brain and is known to modulate cholesterol metabolism (Poirier, 1996). A small amount of cholesterol is transported out of the brain into the periphery via an ApoE-dependent mechanism (Pitas et al., 1987). Furthermore, it is well-known that carriers of the APOE ε4 allele are at increased risk of cognitive decline and developing AD (Dik et al., 2000b). The few studies that focused on the interaction between APOE genotype, cholesterol and dementia (risk) found conflicting results. Some studies reported a significant interaction between APOE and

cholesterol (Evans et al., 2000, 2004; Hall et al., 2006), while others found no interaction (Romas et al., 1999). One study suggested that cholesterol mediates the effect of APOE ε4 on AD (Notkola et al., 1998). Even fewer studies looked at the interactions between APOE genotype, oxysterols, and dementia risk. It has been found that in a group of mild to severe Alzheimer patients, carriers of the APOE ε4 allele had significantly lower plasma 24S-OH cholesterol to cholesterol levels than non-carriers (Papassotiropoulos et al., 2000).

Taken together, so far no definitive and satisfying answers regarding the associations between cholesterol, its metabolites and cognitive decline and dementia, and the possible mediating or modifying role of ApoE have been given. Since previous studies suggest that total cholesterol and oxysterol levels may be early markers for AD, a longitudinal study within a sample of elderly drawn from the population is preferential when investigating risk factors for cognitive decline. In the present large population-based study we examined whether total cholesterol and the oxysterols 24S-OH cholesterol and 27-OH cholesterol were related to the rate of cognitive decline in elderly and whether these associations were modified by ApoE e4.

Methods

Study sample

Data for this study were collected within the Longitudinal Aging Study Amsterdam (LASA), an ongoing population-based study among 3,107 subjects between the ages of 55 and 85 (Deeg and Westendorp-de Serière, 1994). Procedures regarding sampling and data collection have previously been described in detail (Van den Heuvel et al., 1996). In short, a random sample of older men and women, stratified by age and sex according to the expected five-year mortality, was drawn from the population registries of eleven municipalities in areas in the west (Amsterdam and vicinity), northeast (Zwolle and vicinity) and the south (Oss and vicinity) of the Netherlands. Data collection started in 1992/1993, and included follow-up measurements every three years. Main and medical interviews were conducted in the homes of the respondents by specially trained and intensively supervised interviewers. Informed consent was obtained from all respondents, and the study was approved by the Ethical Review Board of the VU University Medical Center (VUmc).

In total, 3,107 subjects were enrolled in the LASA study. During the second measurement in 1995/1996, 2,204 of the 3,107 respondents (70.9%) completed the main interview. Loss to follow-up was mainly due to mortality. For the present study subjects were included of whom blood samples were obtained during the second data collection, sterol levels could be determined and who were not taking lipid-lowering drugs at the time of blood sampling. Blood samples were obtained either in the VUmc (respondents living in Amsterdam and vicinity), a health care center near the respondents' home (respondents living in Zwolle or Oss and vicinity) or in their home (respondents unable to come to the VUmc or a health care center near their home). Blood samples were asked from respondents who had participated in the medical interview (N = 1,509) which was restricted to subjects 65 years and older, of whom 1,332 (88.3%)

agreed to participate in the blood drawing procedure. In 1,248 respondents sterol levels could be defined, of whom 67 were taking lipid-lowering drugs, resulting in 1,181 eligible subjects. The respondents participating in the medical interview who refused to take part in the blood drawing procedure, were significantly older and had lower scores on cognition (all $p < 0.0001$) compared with subjects of whom blood samples were available. Respondents using lipid-lowering drugs were significantly younger ($p < 0.0001$), more likely to be female ($p = 0.033$), and had higher scores on cognition (MMSE: $p = 0.018$, immediate recall: $p = 0.002$) compared with subjects who were not using lipid-lowering drugs.

Of the 1,181 eligible respondents, 1,003 also participated in the three-year follow-up interviews (84.9%). Of the 178 subjects who were lost to follow-up, 149 deceased (12.6%), 12 refused (1.0%), 11 were ineligible (0.9%), and 6 could not be contacted (0.6%). Of the 1,003 respondents participating in the first follow-up, 826 participated in the interviews during the six-year follow-up (82.4%). Of those lost to follow-up, 154 deceased (15.9%), 8 refused (0.8%), 9 were ineligible (0.9%) and 1 could not be contacted (0.1%). Subjects who were lost to follow-up were significantly older, had lower scores on cognition, and were more likely to be men (all $p < 0.001$). Furthermore, respondents lost to follow-up after three years and six years had significantly lower cholesterol ($p = 0.004$ and $p = 0.001$, respectively) levels at baseline.

Cognitive functioning

Objective cognitive tasks were selected, in order to measure cognitive functions sensitive to decline with aging, and which can be used for screening of cognitive dysfunction and dementia.

General cognitive functioning was measured with the Mini-Mental State Examination (MMSE) (Folstein et al., 1975). The MMSE is a widely used, brief instrument used for screening of cognitive impairment. Scores range from 0 to 30, a higher score indicating better performance.

Memory was measured with an abbreviated version of the Auditory Verbal Learning Test (AVLT) (Rey, 1964). We used three instead of five learning trials to reduce the burden for the respondent. In each trial, the interviewer read aloud a list of 15 words, after which the respondents summed up as many words as they could remember. Immediate recall (highest score out of three trials; range, 0-15) was derived from this test. At follow-up, a parallel version of the AVLT was used. The parallel versions, which are used in treatment-research (Moller et al., 1998), were validated and tested on parallelism (Jolles et al., 1995).

Information processing speed was measured by an adapted version of a timed letter substitution task, the Alphabet Coding Task-15 (Piccinin and Rabbitt, 1999). The respondent had to combine as many characters as possible according to a given example (the substitution key). The substitution key showed 15 combinations of 2 characters in a row of double boxes. The test itself showed rows of double boxes, in which only the upper box contained characters and the lower box was empty. The respondent had to name the missing characters corresponding to the characters in the upper box (using the substitution key) as quickly and accurately as possible.

The task consisted of three identical one-minute trials. The score on each trial consisted of the number of correctly completed characters. The mean score of the three trials was used in the analyses.

Sterol analysis

Morning blood samples were obtained. The participants were allowed to take tea and toast, but no dairy products. Serum samples were obtained and frozen at - 80°C until analysis. Cholesterol and its metabolites 24S- and 27-OH cholesterol were extracted from serum by chloroform/methanol and determined after derivatization to the corresponding trimethylsilyl-ethers by gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS) as reported previously (Lütjohann et al., 2004). Identity of all sterols was confirmed by comparison with the full-scan mass spectra of the authentic compounds. The intra-assay and inter-assay coefficients of variation for all sterols were less than 4% of the respective mean values (precision). Inter-assay accuracy was lower than 3% of the respective nominal values. The limit of quantification was 1 mg/dL for cholesterol and 5 ng/mL for the oxysterols. The ratio of 24S- and 27-OH cholesterol to cholesterol were defined as the absolute amount of 24S- and 27-OH cholesterol divided by the absolute amount of cholesterol.

Putative modifier

Apolipoprotein E

Serum samples were obtained and frozen at - 80°C until determination of ApoE phenotype. The ApoE phenotype was determined by isoelectric focusing of delipidated serum samples, followed by immunoblotting (Havekes et al., 1987). The distribution of ApoE phenotypes was in Hardy-Weinberg equilibrium ($\chi^2_{df=3} = 0.67$, $p = 0.44$). ApoE status was classified as e4 carriers for subjects with the ApoE e4 isoform (phenotypes e2/4, e3/4, e4/4) and as non-e4 carriers for subjects without the ApoE e4 isoform (phenotypes e2/2, e2/3, e3/3).

Putative confounders

The relevant confounders that were included in the analyses were: time, age, sex, education, ApoE status, cardiovascular disease, hypertension, diabetes mellitus, intake of lipid-lowering drugs at follow-up, depressive symptoms, body mass index, alcohol intake, and smoking status.

The time variable was defined as the number of years (i.e. 0, 3 and 6 years) between the determination of the sterol levels and time of additional data measurement. Data on age and sex were derived from the population registries at baseline. Education was assessed by asking the respondent for the highest educational level completed, which was converted into the total number of years of education (range, 5-18 years). At baseline and first follow-up, cardiovascular disease (cardiac disease and stroke) was assessed by combining self-report data, medication use and records of the general practitioners (GP) in an algorithm previously described (Bremmer et

al., 2006). At second follow-up GP records were not yet available. Hypertension was assessed by measurement of blood pressure (≥ 160/100 mm/Hg), use of anti-hypertensive drugs or both. Diabetes mellitus was assessed by means of self-report (nearly perfect agreement with GP information, $\kappa = 0.85$) (Kriegsman et al., 1996). Intake of lipid-lowering drugs was determined by checking medication use. Depressive symptoms were assessed with the Center for Epidemiologic Studies Depression Scale (CES-D). This is a 20-item self-report scale (range, 0-60) designed to measure depressive symptoms in the general population (Radloff, 1977). Body mass index (BMI) was calculated as weight (kg) / (height (m))^2. Alcohol consumption was assessed by asking for the number of alcoholic units consumed per week over the past year, and the number of days of the week in which alcohol was consumed, and was thereafter classified as no, middle, and high consumption according to the NEI index (Reinhard and Rood-Bakker, 1998). Smoking status was classified as never, former, and current.

Data analysis

ApoE allele and phenotype distribution were calculated. Characteristics of the study sample by ApoE status were compared using Mann-Whitney U tests for independent samples. Because the MMSE score was not normally distributed, a transformation (ln (31-MMSE score)) was performed.

To evaluate whether sterol levels were associated with the rate of cognitive decline, cognitive change scores were calculated for each individual as the difference between baseline score and first follow-up score, as well as first follow-up and second follow-up score. Associations between sterols, cognitive performance and rate of cognitive decline during six years of follow-up, and the possible modifying role of ApoE e4, were analyzed using generalized estimating equations (GEE) (Twisk and De Vente, 2000). An important feature, necessary for longitudinal analyses, is that the dependency of repeated observations within subjects is taken into account by GEE. This dependency is added to the analyses by assuming a certain correlation structure in the repeated observations of the outcome variable. Here, an exchangeable correlation structure was assumed, meaning that the correlation was constant between any two cognitive scores attained by one person. Moreover, an advantage of GEE is that subjects are included regardless of missing values. Thus, subjects who were lost to follow-up after two measurements were also included in the analyses. This reduces bias that might have arisen from a differential loss to follow-up of the more cognitively impaired elderly. As we were interested in (the rate of) cognitive decline, the ascertainment of which requires two measurements, subjects with cognitive data on only one measurement were excluded from the analyses.

The regression models were adjusted for time (model 1), and additionally adjusted for age, sex, education, use of lipid lowering drugs at follow-up, cardiovascular disease, hypertension, diabetes mellitus, depressive symptoms, body mass index, alcohol intake, smoking status, and ApoE status (model 2).

Whether the associations between serum sterol levels at baseline and cognitive performance over the six-year follow-up period were significantly modified by time, was evaluated by including product terms of the sterol levels with time in the models. Effect modification by ApoE status was investigated using product terms of the sterol levels with ApoE status. In addition, separate analyses were performed according to ApoE status, adjusted for the above mentioned relevant confounders.

All analyses were tested at the 0.05 level of significance, except for effect modification for which a level of significance of 0.10 was tolerated, because of the multiplication of the measurement error.

Table 1 ApoE allele and phenotype distribution in the total study sample

e2		e3		e4	
.081		.772		.147	
e2/2	e2/3	e3/3	e2/4	e3/4	e4/4
0.8%	11.8%	61.0%	2.7%	20.6%	3.0%

ApoE Apolipoprotein E.

Results

ApoE allele and phenotype distribution in the total study sample are shown in Table 1.

Table 2 shows the baseline characteristics of the whole study sample and by ApoE status. Mann-Whitney U tests for independent samples show that the ApoE e4 carriers had significantly higher cholesterol levels than the ApoE e4 non-carriers. In addition, ApoE e4 carriers had a significantly lower ratio of 24S-OH cholesterol to cholesterol (r_24S-OH chol) and higher absolute levels of 27-OH cholesterol compared with ApoE e4 non-carriers.

Table 3 shows the results of the longitudinal analyses for the whole sample. The analyses show a significantly positive association between cholesterol and performance on the MMSE, immediate recall and coding task over time. After additional adjustment for the relevant confounders, the associations between cholesterol and the MMSE score as well as coding remained significant. The cholesterol level at baseline was positively associated with performance on the MMSE and on coding over the whole follow-up period, independent of the mentioned confounders. These results indicate a relatively better cognitive performance over a six-year period when total cholesterol level is higher at baseline, and thus a relatively worse cognitive performance when total cholesterol is lower at baseline.

None of the associations between the metabolites and cognitive performance reached statistical significance, except for the negative association between the ratio of 27-OH cholesterol to cholesterol (r_27-OH chol) and immediate recall over time. However, after additional adjustment for the mentioned confounders this association was no longer significant.

Table 2 Baseline characteristics, and sterol concentrations, absolute levels and sterol to cholesterol ratio, for the total study sample and separately for ApoE e4 carriers and ApoE e4 non-carriers

Characteristic		Total group (N = 1181)	ApoE e4+ (N = 310)	ApoE e4- (N = 868)
Age, years, mean (SD)		75.65 (6.58)	75.32 (6.49)	75.75 (6.61)
Men, % (N)		49.2 (581)	51.6 (160)	48.4 (420)
Education, years, mean (SD)		8.96 (3.33)	8.97 (3.28)	8.96 (3.36)
MMSE, mean (SD)		26.80 (3.01)	26.59 (3.07)	26.89 (2.95)
Immediate recall, mean (SD)		8.06 (2.60)	8.16 (2.61)	8.03 (2.59)
Information processing speed, mean (SD)		22.97 (7.35)	22.73 (7.33)	23.06 (7.31)
CES-D Depressive symptoms, mean (SD)		8.08 (7.60)	7.52 (7.18)	8.30 (7.74)
Hypertension % (N)		57.3 (677)	60.0 (186)	56.5 (490)
Diabetes mellitus % (N)		7.6 (90)	5.8 (18)	8.3 (72)
Cardiovascular disease, % (N)		31.7 (374)	30.6 (95)	32.0 (278)
BMI, mean (SD)		26.65 (4.18)	26.69 (4.13)	26.65 (4.18)
Smoking, % (N)				
No		35.7 (422)	34.2 (106)	36.2 (314)
Former		46.1 (544)	49.0 (152)	45.2 (392)
Current		18.2 (215)	16.8 (52)	18.7 (162)
Alcohol consumption, % (N)				
No		24.6 (290)	24.2 (75)	24.8 (215)
Middle		65.8 (777)	67.4 (209)	65.2 (566)
High		9.6 (113)	8.4 (26)	9.9 (86)
Sterol concentrations in serum	Unit	Total group (N = 1181)	ApoE e4+ (N = 310)	ApoE e4- (N = 868)
Total cholesterol, mean (SD)				
Absolute	mg/dL	234.14 (45.72)	239.83 (45.31)	232.17 (45.75) **
Metabolites				
24S-hydroxycholesterol, mean (SD)				
Absolute	ng/mL	96.59 (27.01)	96.23 (25.89)	96.80 (27.41)
Ratio	ng/mg	41.73 (11.05)	40.39 (9.01)	42.23 (11.67)*
27-hydroxycholesterol, mean (SD)				
Absolute	ng/mL	252.47 (76.46)	260.42 (75.84)	249.90 (76.49)**
Ratio	ng/mg	109.27 (32.21)	109.85 (27.73)	109.19 (33.67)

p values indicate whether value distributions vary between the ApoE e4 carriers and e4 non- carriers; * $p \leq 0.05$; ** $p \leq 0.01$; *ApoE* Apolipoprotein E; *MMSE* Mini-Mental Status Examination; *CES-D* Center of Epidemiological Studies Depression Scale; *BMI* Body mass index.

Table 3 Associations between sterols and cognitive performance over 6 years of follow-up

		ln-transformed MMSE score [1]			Immediate recall			Information processing speed		
		B		p value	B		p value	B		p value
Total cholesterol	Model 1 [a]	-.0014	**	.000	.0060	**	.001	.0149	**	.004
(mg/dL)	Model 2 [b]	-.0008	*	.012	.0002		.886	.0086	*	.045
Metabolites										
r_24S-OH chol	Model 1 [a]	.0019		.329	-.0124		.079	-.0006		.983
(ng/mg)	Model 2 [b]	-.0002		.878	-.0068		.216	.0201		.348
r_27-OH chol	Model 1 [a]	.0003		.678	-.0065	*	.019	.0078		.349
(ng/mg)	Model 2 [b]	.0006		.344	-.0025		.248	.0068		.400

[1] direction of associations is reversed because of ln (natural log) transformation of the MMSE; *B* Unstandardized regression coefficients; * $p \leq 0.05$; ** $p \leq 0.01$. [a] Adjusted for time. [b] Adjusted for time, age, sex, education, cardiovascular disease, diabetes mellitus, hypertension, Apolipoprotein E e4, use of lipid-lowering drugs at follow-up, depressive symptoms, alcohol consumption, smoking, and body mass index. *MMSE* Mini-Mental Status Examination; *r_24S-OH chol* 24S-hydroxycholesterol to cholesterol ratio; *r_27-OH chol* 27-hydroxycholesterol to cholesterol ratio.

Subsequently, the interactions between total cholesterol and time, and the oxysterols and time were explored to find out whether time had a significant influence on the association between the sterol levels and cognitive functioning during the follow-up period of six years. The analyses show that the interaction term cholesterol x time was significant in the models predicting performance on immediate recall ($p = 0.074$) and coding ($p = 0.035$) over time, suggesting that the association between cholesterol and cognitive performance during the six-year follow-up was significantly modified by time. The interactions between r_27-OH chol and time, and r_24S-OH chol and time were not significant, suggesting that the associations between these oxysterols and cognition were not significantly modified by time in the total study sample.

To be able to examine whether the baseline levels of total cholesterol and the oxysterols were related to the rate of cognitive decline over time, cognitive change scores were calculated. The longitudinal analyses showed no significant associations, except for the positive association ($B = 0.004$, $p = 0.035$) between total cholesterol at baseline and change in information processing speed, indicating that a higher total cholesterol level was associated with a slower rate of decline. However, after adjusting for all the relevant confounders, this association was no longer significant.

Furthermore, the interaction terms with ApoE e4 were included in the models and thereafter the associations between the different sterols and cognitive performance over time were analyzed separately for ApoE e4 carriers and non-carriers in order to investigate the effect of ApoE e4 on these associations. Table 4 shows the results of these separate longitudinal analyses.

After inclusion of the interaction term r_27-OH chol x ApoE e4 in the models, the results

reveal that the associations between r_27-OH chol and performance on the MMSE, as well as immediate recall were significantly modified by ApoE e4. In addition, the interaction term r_24S-OH x ApoE e4 was significant in the model predicting performance on coding, indicating a modifying role of ApoE e4. None of the other interaction terms reached significance.

Table 4 Associations between sterols and cognitive performance over 6 years of follow-up separately for ApoE e4 carriers and e4 non-carriers

	ln transformed MMSE score					
	ApoE e4+ (N=244)			ApoE e4- (N=711)		p interaction term
	B		p value	B	p value	
Total cholesterol (mg/dL)	-.0017	*	.019	-.0007	.077	.398
Metabolites						
r_24S-OH cholesterol (ng/mg)	.0037		.329	-.0010	.460	.235
r_27-OH cholesterol (ng/mg)	.0033	**	.002	.0001	.922	.009
	Immediate recall					
	ApoE e4+ (N=231)			ApoE e4- (N=678)		p interaction term
	B		p value	B	p value	
Total cholesterol (mg/dL)	-.0026		.341	.0012	.532	.236
Metabolites						
r_24S-OH cholesterol (ng/mg)	-.0182		.242	-.0043	.456	.394
r_27-OH cholesterol (ng/mg)	-.0094	*	.045	-.0010	.655	.070
	Information processing speed					
	ApoE e4+ (N=227)			ApoE e4- (N=664)		p interaction term
	B		p value	B	p value	
Total cholesterol (mg/dL)	.0112		.156	.0078	.123	.910
Metabolites						
r_24S-OH cholesterol (ng/mg)	-.0547		.197	.0337	.127	.081
r_27-OH cholesterol (ng/mg)	-.0091		.490	.0114	.173	.200

[1] direction of associations is reversed because of ln (natural log) transformation of the MMSE; *B* Unstandardized regression coefficients; * $p \leq 0.05$; ** $p \leq 0.01$. Adjusted for time, age, sex, education, cardiovascular disease, diabetes mellitus, hypertension, use of lipid-lowering drugs at follow-up, depressive symptoms, alcohol consumption, smoking, and body mass index. *MMSE* Mini-Mental Status Examination; *ApoE* Apolipoprotein E; *r_24S-OH cholesterol* 24S-hydroxycholesterol to cholesterol ratio; *r_27-OH cholesterol* 27-hydroxycholesterol to cholesterol ratio.

Separate analyses by ApoE status show a positive association between cholesterol and performance on the MMSE over the whole follow-up period independent of the relevant confounders. The results show that this association was significant for the ApoE e4 carriers and reached borderline significance for the ApoE e4 non-carriers. The associations between cholesterol and immediate recall or coding did not reach statistical significance in either ApoE e4 group.

A significant negative association between r_27-OH chol and performance on both the MMSE as well as on immediate recall over six years of follow-up was observed independent of the relevant confounders, only in the ApoE e4 carriers. No significant associations were found between r_24S-OH chol and cognitive performance over time in either group.

Subsequently, the sterol x time interactions were examined separately for ApoE e4 carriers and non-carriers. In the model predicting performance on the MMSE, the interactions between cholesterol and time ($p = 0.032$) and between r_27-OH chol and time ($p = 0.082$) were significant only in the ApoE e4 carrier group. This result suggests that time was a significant modifier of the associations between cholesterol and general cognitive performance and r_27-OH chol and general cognitive performance, only in the ApoE e4 carrier group. In the model predicting performance on coding, the interaction between cholesterol and time ($p = 0.008$) and the interaction between r_24S-OH chol and time ($p = 0.095$) were significant, only in the ApoE e4 carrier group. This suggests that only in the ApoE e4 carrier group, time was a significant modifier of the association between cholesterol and information processing speed and r_24S-OH chol and information processing speed. None of the other interactions with time reached statistical significance.

Finally, the modifying role of ApoE e4 was studied with respect to the associations between the baseline sterol levels and cognitive change scores, and these associations were analyzed separately for ApoE e4 carriers and non-carriers. The results show a significant interaction between ApoE e4 and change in general cognitive performance ($p = 0.056$) and ApoE e4 and change in information processing speed ($p = 0.015$), indicating a modifying role of ApoE e4. In the separate analyses by ApoE status, the association between total cholesterol and change in general cognition failed to reach significance in either ApoE e4 group. A significant positive association ($B = 0.010$, $p = 0.006$) between total cholesterol level and change in information processing speed was found only in the ApoE e4 carrier group, suggesting that a higher total cholesterol level at baseline was significantly associated with a slower rate of decline. No other significant associations were found, suggesting that the baseline levels of r_24S-OH chol and r_27-OH chol were not associated with the rate of cognitive decline over the follow-up period within the ApoE e4 carrier group as well as within the ApoE e4 non-carrier group.

In Figures 1 and 2 the main results are presented to visualize to what extent level of cognitive functioning and rate of decline were affected by the sterol levels at baseline. The figures show cognitive performance independent of the major confounders over six years of follow-up, for subjects with a mean sterol level (Figure 1: total cholesterol level, Figure 2: r_27-OH chol), and for those with sterol levels two standard deviations below or above the sample mean. Figure 1 clearly shows that level of cognitive performance was lowest in subjects with a lower cholesterol level, and highest in subjects with a higher cholesterol level. Only in ApoE e4 carriers, the rate of decline on information processing speed was affected by total cholesterol level. Figure 2 shows that level of cognitive performance was lowest in subjects with a higher r_27-OH chol, and highest in subjects with a lower r_27-OH chol.

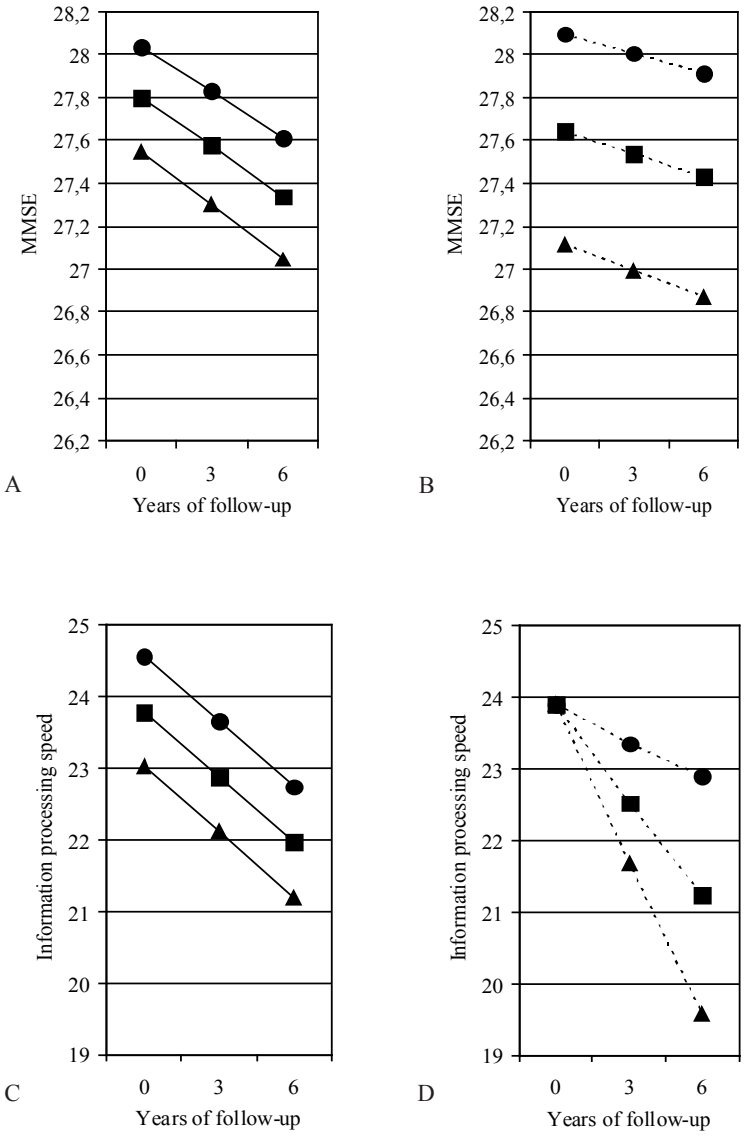

Figure 1 Six-year cognitive decline according to total cholesterol level

Models adjusted for time, age, sex, education, cardiovascular disease, diabetes mellitus, hypertension, use of lipid-lowering drugs at follow-up, depressive symptoms, alcohol consumption, smoking, body mass index, and ApoE e4 (in total study sample) in total study sample (solid line) and in ApoE e4 carriers only (dotted line). (A) and (B) MMSE; (C) and (D) Information processing speed. Total cholesterol level: (●) mean + 2 SD; (■) mean; (▲) mean - 2 SD. *MMSE* Mini-Mental State Examination; *ApoE* Apolipoprotein E.

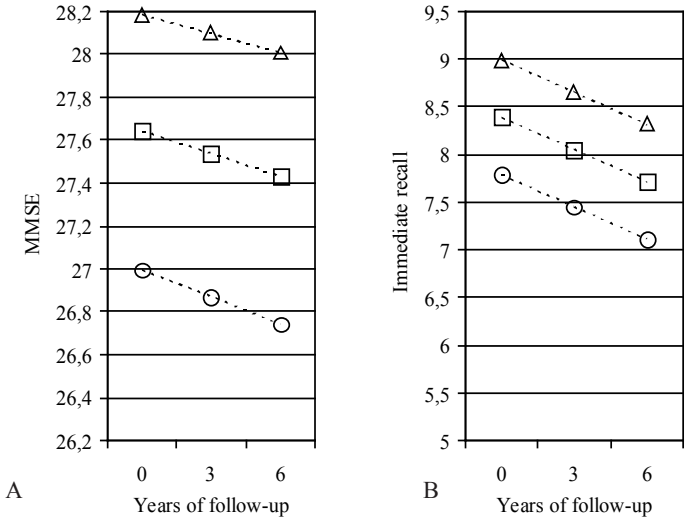

Figure 2 Six-year cognitive decline according to 27-hydroxycholesterol to cholesterol ratio
Models adjusted for time, age, sex, education, cardiovascular disease, diabetes mellitus, hypertension, use of lipid-lowering drugs at follow-up, depressive symptoms, alcohol consumption, smoking, body mass index in ApoE e4 carriers only. (A) MMSE and (B) Immediate recall. 27-hydroxycholesterol to cholesterol ratio: (○) mean + 2 SD; (□) mean; (Δ) mean – 2 SD. *MMSE* Mini-Mental State Examination; *ApoE* Apolipoprotein E.

Discussion

This population-based study showed that a higher cholesterol level at the age of 65 years and older was an independent predictor of a relatively better level of general cognitive performance, and a relatively higher level of information processing speed over a six-year period. These results are in line with previous studies which suggest that high cholesterol level in older persons is associated with a decreased risk for AD (Kuuisto et al., 1997; Mielke et al., 2005; Solfrizzi et al., 2002). Thus, a lower total cholesterol level in the elderly population should be considered as a frailty marker, predictive of worse cognitive functioning. Previously, in LASA, lower cholesterol has been described as a frailty marker for functional decline (Schalk et al., 2004). Also, a large 21-year follow-up study showed an association between serum total cholesterol changes from midlife to late-life and late-life cognitive status (Solomon et al., 2007). A moderate decrease was significantly associated with increased risk of having a more impaired late-life cognitive status after adjusting for major confounders. These results support the hypothesis that decreased serum cholesterol in late-life may reflect ongoing pathological processes in the brain and may represent a risk marker for cognitive impairment and dementia. In addition to the role of for example poor nutritional status and lifestyle changes, lower total cholesterol levels in elderly have been associated with higher levels of the serum inflammation marker Interleukin-6 (IL-6) (Lehtimäki et al., 2005). IL-6 has been associated with dementia, although results are inconclusive (Solfrizzi et al., 2006). Also, a study that examined aging in mouse models of AD,

proposed that a decrease in total cholesterol levels may reflect an increase in Aβ levels during aging (Wirths et al., 2006).

Furthermore, this prospective study did not show an interaction between serum total cholesterol and ApoE e4 on level of cognitive functioning. However, ApoE e4 tended to modify the association between total cholesterol and the rate of cognitive decline. Only in the ApoE e4 carrier group, a higher level of cholesterol was an independent predictor of a slower rate of decline on information processing speed. Thus, in elderly carriers of the ApoE e4 allele, in addition to being a frailty marker predictive of a poorer level of cognitive functioning, a lower total cholesterol level was also related to a faster rate of decline on information processing speed. Information processing speed appears to be the cognitive function most sensitive to aging (Salthouse, 1996), and decline of this cognitive function may be an even earlier indicator of AD than memory decline (Dik et al., 2000a).

These findings concerning the modifying role of ApoE are in contrast with previous research. One retrospective study reported that Alzheimer patients with high cholesterol levels and no ApoE e4 allele had the fastest rate of decline in cognitive and functional performance (Evans et al., 2004). Also, some reports indicated an independent role of cholesterol, while others concluded that only in ApoE e4 non-carriers higher levels of cholesterol were related to an increased risk of AD (Evans et al., 2000; Hall et al., 2006). Furthermore, some studies suggested a modifying or mediating role of cholesterol in the association between APOE genotype and AD (Jarvik et al., 1995; Notkola et al., 1998). The studies that have focused on the interaction between ApoE e4 status and cholesterol were mostly cross-sectional of design with dementia diagnosis as outcome measure, some using a relatively small sample size. Selective survival of the healthier ApoE e4 carriers may explain the more pronounced findings in the e4 non-carrier group. The longitudinal design of our study, the large sample size, and level of cognitive functioning and cognitive decline as outcome measures reduces biases caused by selective survival. In addition, it seems likely that the interaction between ApoE e4 and cholesterol will also depend on the time of measurement over the life course of cholesterol and stage of dementia. The results of the present study showed no differences in cognition between the ApoE e4 carriers and non-carriers, suggesting that the found associations in the ApoE e4 carrier group were not caused by differences in cognition.

Studying the cholesterol metabolites in the present study, no significant associations were found between the ratio of 24S-OH cholesterol to cholesterol and cognition over six years of follow-up, which is consistent with the conclusion of another population-based study (Teunissen et al., 2003). Also, ApoE status was no significant modifier with respect to the association between the ratio of 24S-OH cholesterol to cholesterol and cognition. However, it was shown that ApoE e4 was a significant modifier in the association between the ratio of 27-OH cholesterol to cholesterol and general cognitive performance as well as memory function. A higher ratio of 27-OH cholesterol to cholesterol was an independent predictor of a worse level of cognitive functioning in elderly carriers of ApoE e4 over a six-year period. The results

of another population-based study did not support an association between the ratio of 27-OH cholesterol to cholesterol and cognitive performance in older persons (Teunissen et al., 2003). This conflicting result might be explained by the fact that the modifying role of ApoE status was not taken into account. Another study concluded that in addition to an altered brain cholesterol metabolism, peripheral cholesterol metabolism also seems to be altered in dementia (Kölsch et al., 2004). However, they found a lower plasma ratio of 27-OH cholesterol to cholesterol in MCI patients and demented patients compared with healthy controls. These contrasting findings are possibly due to the fact that they used a cross-sectional design and focused on the association between dementia diagnosis and the ratios of the oxysterols to cholesterol, while the present study was prospective and focused on the influence of these ratios on cognitive decline and the modifying role of ApoE status.

The oxysterol 27-OH cholesterol originates from extracerebral and extrahepatic sources of cholesterol. Both 24S- and 27-OH cholesterol are able to pass the blood-brain barrier (Lütjohann et al., 1996). The level of 27-OH cholesterol in the circulation is proportional to the level of cholesterol and a net uptake of 27-OH cholesterol by the brain has been shown, which is considered by some authors to be of importance for intracerebral cholesterol homeostasis (Heverin et al., 2005). It has been speculated that the fluxes of 24S- and 27-OH cholesterol over the blood-brain barrier correspond to a to-and-fro communication between cerebral and extracerebral cholesterol pools (Björkhem et al., 2006). One study has shown that the brains of deceased AD patients contained increased levels of 27-OH cholesterol compared with the examined brains of deceased control subjects (Heverin et al., 2004). Furthermore, increased levels of 27-OH cholesterol may reduce the cellular cholesterol level (Michikawa, 2006), which in turn has been demonstrated to enhance Aβ generation (Abad-Rodriguez et al., 2004).

A major strength of this study is that longitudinal data from a large population-based study was used. In addition, multiple cognitive measures were studied, which allowed us to focus on a broad spectrum of cognitive functioning. Our outcome measures were both level of cognitive functioning, and rate of cognitive decline.

A limitation of this study is that the most frail and poorest functioning elderly were lost to follow-up. Individuals lost to follow-up had significantly lower cholesterol and lower cognition scores. Although bias caused by loss to follow-up is partly obviated by the use of the longitudinal data-analysis technique GEE, an underestimation of the found associations may be likely. Another possible limitation is the lack of information on food habits and intake. Some studies, although not all (Laurin et al., 2003), have shown a protective effect of fish or n-3 fatty acids on cognitive decline or dementia (Kalmijn et al., 1997; Van Gelder et al., 2007). Therefore, it would have been preferable to include food intake as a possible confounder. Also, the sterol levels were measured only once, at baseline not during follow-up, which could have diluted the found associations, and thus may have led to underestimation of the risk estimates. Although

the strength of the associations seems small and may not be clinically relevant, the results indicate that sterols may affect cognition independent of major confounders and may direct future research on etiology and risk factors of cognitive decline and AD.

In light of the results found in the present study, future research should focus on the use and effect of prescribing a class of cholesterol lowering drugs, the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, also termed statins, in older persons, especially elderly carriers of ApoE e4. Also, it seems important to study spontaneous changes in cholesterol and oxysterols over time. Furthermore, the roles of for example LDL, VLDL, HDL cholesterol and triglycerides may be more specific, and should be included in future research.

In sum, this population-based study confirms that a lower level of serum total cholesterol may be viewed as a frailty marker, predictive of lower cognitive functioning in older persons. In addition, in elderly carrying the ApoE e4 allele, a lower cholesterol level is also predictive of a faster rate of decline on information processing speed. Finally, in ApoE e4 carriers, a higher ratio of 27-OH cholesterol to cholesterol, which may be indicative of increased cholesterol breakdown, is an independent predictor of a worse level of cognitive functioning. These findings implicate that a low cholesterol level may be used as a marker for cognitive impairment, and early cognitive decline, especially in ApoE e4 carriers.

Conflict of interest

None.

Acknowledgements

The Longitudinal Aging Study Amsterdam is funded by the Dutch Ministry of Health, Welfare, and Sports and the Vrije Universiteit. The present study is supported by Hersenstichting Nederland (grant # 12F04.46).

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