The role of extracerebral cholesterol homeostasis and ApoE e4 in cognitive decline

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Abstract

We examined the associations between extracerebral markers of cholesterol homeostasis and cognitive decline over 6 years of follow-up, and studied the modifying effect of apolipoprotein E (ApoE) e4. Data were collected in the Longitudinal Aging Study Amsterdam ($n = 967$, with longitudinal data on cognition, ages ≥ 65 years) and analyzed using linear mixed models. General cognition (Mini-Mental State Examination; MMSE), memory (Auditory Verbal Learning Test), and information processing speed (Coding task) were measured. The results show that ApoE e4 was a significant effect modifier. Significant associations were found only in ApoE e4 noncarriers ($n = 718$). We found a nonlinear negative association between the ratio of lanosterol to cholesterol (≤ 189.96 ng/mg), a marker for cholesterol synthesis, and general cognition. Lower cholesterol absorption, i.e., lower ratios of campesterol and sitosterol to cholesterol, as well as a higher rate of cholesterol synthesis relative to absorption were associated with lower information processing speed. In ApoE e4 carriers, the negative association between the ratio of campesterol to cholesterol and memory reached borderline significance. Future research should focus on the interaction between (disturbed) cholesterol homeostasis and ApoE e4 status with respect to dementia.
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1. Introduction

Disturbed cerebral and extracerebral cholesterol homeostasis has been suggested to play a role in dementia and cognitive decline. Cholesterol homeostasis is the balance between cholesterol absorption, synthesis, metabolism, and excretion (Grundy, 1991). Lathosterol is a major precursor in cholesterol biosynthesis. The ratio of lathosterol to cholesterol is considered a reflection of cholesterol synthesis (Miettinen et al., 1990). Lanosterol is the first common

intermediate of 2 different pathways, which use either desmosterol or lathosterol as the predominant precursors for de novo synthesis of brain cholesterol (Lütjohann et al., 2002). The serum plant sterol sitosterol concentration is generally considered as a measure of cholesterol absorption (Miettinen et al., 1990). It has been shown that plant sterols such as sitosterol or campesterol are effective as cholesterol lowering agents (Law, 2000; Plat and Mensink, 2001). Thus far several studies have examined the association between cholesterol homeostasis and cognitive decline. However, they show inconsistent results and causality and mechanisms underlying these associations remain to be further clarified (Anstey et al., 2008; Helzner et al., 2009; Heverin et al., 2004; Kölsch et al., 2004; Solomon et al., 2007, 2009).

Apolipoprotein E (ApoE) is the major lipid transport

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protein in the brain and is known to modulate cholesterol metabolism (Poirier, 1996). The ApoE system is important for the distribution of cholesterol in the brain and is partially involved in its transfer from brain tissue to the cerebrospinal fluid and from there to the circulation (Pitas et al., 1987). Furthermore, carriers of the ApoE e4 allele are at increased risk of cognitive decline and developing Alzheimer's disease (AD) (Dik et al., 2000). In a previous study we showed that ApoE e4 was a significant modifier of the longitudinal associations between total serum cholesterol, the ratio of 27-hydroxycholesterol to cholesterol, and cognitive functioning in late life. Lower cholesterol levels and a higher ratio of 27-hydroxycholesterol to cholesterol were predictive of worse cognitive functioning in older persons carrying the ApoE e4 allele, but not in e4 noncarriers (Van den Kommer et al., 2009).

To our knowledge no study has focused on the modifying role of ApoE e4 on the associations between markers for cholesterol synthesis and absorption and cognitive decline or dementia. The goal of the present study is to investigate the longitudinal associations between lanosterol, lathosterol, sitosterol, and campesterol, and cognitive functioning and the modifying role of ApoE e4 on these associations.

2. Methods

2.1. Study sample

Data were collected within the Longitudinal Aging Study Amsterdam (LASA), an ongoing population-based study (Deeg et al., 2002). A random sample of older men and women, stratified by age and sex according to the expected 5-year mortality, was drawn from the population registries of 11 municipalities in 3 areas of the Netherlands. Data collection started in 1992/1993, and includes follow-up measurements every 3 years. In total, 3107 subjects between the ages of 55 and 85 were enrolled during the first data collection of LASA, of whom 2545 (81.9%) were interviewed during the second data collection (1995/1996). Loss to follow-up was mainly due to mortality (74%). During the second data collection, blood samples were obtained in persons aged 65 years and older. Of the 1720 eligible respondents, 1352 persons agreed to take part in the blood drawing procedure. For the present study, persons were included of whom a blood sample was obtained and sterol levels could be determined ($n = 1248$). Subjects of whom blood samples could not be obtained were significantly older and had lower scores on cognition (all $p < 0.0001$) compared with subjects of whom blood samples were available. Persons using lipid-lowering drugs at the time of blood sampling were excluded ($n = 67$), resulting in 1181 subjects. Of these 1181 respondents, 1003 (84.9%) also participated in the 3-year follow-up interviews (1998/1999). Of the 178 subjects lost to follow-up, 149 had died (12.6%), 12 refused (1.0%), 11 were too frail (0.9%), and 6 could not be contacted (0.6%). Of the 1003 respondents participating in

the 3-year follow-up, 826 (82.4%) also participated in the interviews during the 6-year follow-up (2001/2002). Of those lost to follow-up, 154 had died (15.9%), 8 refused (0.8%), 9 were too frail (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 and to have cardiovascular disease (all $p < 0.0001$). Furthermore, they had significantly lower levels of cholesterol ($p = 0.003$), lower absolute levels of campesterol ($p = 0.027$), sitosterol ($p = 0.001$), and lathosterol ($p = 0.004$), and lower ratios of lathosterol to cholesterol ($p = 0.011$) at baseline compared with persons who remained in the study. In the present study, analyses were based on persons of whom data on cognitive functioning were available on at least 2 occasions, which resulted in a final sample of $n = 967$.

2.2. Cognitive functioning

General cognitive performance was measured with the Mini-Mental State Examination (MMSE) (Folstein et al., 1975). 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 3 instead of 5 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 3 trials; range, 0–15) was derived from this test. At follow-up, a parallel version of the Auditory Verbal Learning Test 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 task consisted of 3 identical 1-minute trials. The score on each trial consisted of the number of correctly completed characters. The mean score of the 3 trials was used in the analyses (range, 1–42.7).

2.3. Sterol levels

Morning blood samples were obtained. Participants were allowed to take tea and toast, but no dairy products. Serum samples were obtained and frozen at -80 °C until analysis. Cholesterol was determined by highly sensitive gas chromatography-flame ionization detection. Plasma concentrations of sitosterol, campesterol, lathosterol, lanosterol, and 27-hydroxycholesterol were assessed using an ultrasensitive and highly specific gas chromatography-mass spectrometry selective ion-monitoring method (Sudhop et al., 2002). Identity of all sterols was confirmed by comparison with the full scan mass spectra of the authentic compounds. The

intra-assay and interassay coefficients of variation for all sterols were below 3% of the respective mean values (precision). Interassay accuracy was lower than 3% of the respective nominal values. The limit of quantification was < 0.001 mg/dL for each sterol. The sterol to cholesterol ratios were defined as the absolute amount of lathosterol, lanosterol, sitosterol, and campesterol divided by the absolute amount of cholesterol. The ratios of lathosterol to the plant sterols were computed by dividing the absolute amount of lathosterol by the absolute amount of the campesterol and sitosterol respectively.

2.4. Apolipoprotein E phenotype

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 (ApoE e2/2: 0.8%; e2/3: 11.8%; e3/3: 60.9%; e2/4: 2.7%; e3/4: 20.6%; e4/4: 3.0%; missing: 0.3%). ApoE status was classified as e4 carriers for subjects with the ApoE e4 isoform (phenotypes e2/4, e3/4, e4/4) and as e4 noncarriers for subjects without the ApoE e4 isoform (phenotypes e2/2, e2/3, e3/3).

2.5. Potential confounders

Age, sex, education, hypertension, diabetes mellitus, depressive symptoms, body mass index (BMI), alcohol intake, smoking status, and use of lipid-lowering drugs at follow-up were considered as potential confounders.

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). Hypertension was defined by sitting blood pressure, ≥ 160/100 mm/Hg, use of antihypertensive medication or both. Diabetes mellitus was assessed by self-report (nearly perfect agreement with general practitioners (GP) information, kappa = 0.85) (Kriegsman et al., 1996) and medication use. Depressive symptoms were assessed with the Center for Epidemiologic Studies Depression Scale (Beekman et al., 1997; Radloff, 1977), a 20-item self-report scale (range, 0–60) designed to measure depressive symptoms in the general population. BMI was calculated as weight (kg)/ height (m^2). Alcohol consumption was assessed by asking the number of alcoholic units per week over the past year, and the number of days in the week in which alcohol was consumed, and was classified as no, middle, and high consumption according to the Netherlands Economic Institute (NEI) index (Reinhard and Rood-Bakker, 1998). Smoking status was classified as never, former, and current smokers. Use of lipid-lowering drugs was determined by checking medication use.

Cardiovascular disease (cardiac disease, peripheral arterial disease, cerebrovascular accident) was evaluated as a potential mediator. The presence of cardiovascular disease

at baseline and 3-year follow-up was assessed by a combination of self-report data, medication use, and GP records in an algorithm previously described (Bremmer et al., 2006). At 6-year follow-up self-report data and medication records were used.

2.6. Data analysis

Characteristics of the study sample by ApoE e4 status were compared using Mann-Whitney *U* tests for independent samples. The MMSE score was transformed (ln (31-MMSE score)) to obtain a near-normal distribution. Correlations between absolute values of all markers, ratios of all markers to cholesterol, and total cholesterol, absolute values of 27-hydroxycholesterol and its ratio to cholesterol were studied by means of Pearson correlation coefficients.

To study the longitudinal associations between the ratio of lathosterol, lanosterol, sitosterol, and campesterol to cholesterol and cognitive functioning, and the modifying effect of ApoE e4 on these associations, data were analyzed using linear mixed models in SPSS version 15.0. All markers were studied as continuous variables. Quadratic terms and interactions were tested at the 0.10 significance level. Main effects were tested at the 0.05 significance level.

First, the trajectories of cognitive functioning as indicated by scores on the MMSE, immediate recall, and information processing speed were modeled as a function of time. Time was defined as the number of years (i.e., 0, 3, and 6 years) between sterol determination and follow-up cycle. Second, in separate analyses the main predictors were added to each model and their quadratic term was tested for significance to study the presence of a nonlinear association. If significant ($p < 0.10$), the quadratic term was retained in the model. Third, the interaction between the main predictor and time was tested to study whether the main predictor was associated with rate of cognitive decline. If significant ($p < 0.10$) the interaction with time was retained in the model. Hereafter, all potential confounders were added 1 by 1 to the model. Variables that showed a significant confounding effect on the associations studied, i.e., ≥10% change in the unstandardized regression coefficient (B) of the main predictor, were retained in the model.

Effect modification by ApoE e4 status (yes, no) was tested in the fully adjusted models by adding the product terms (main predictor*ApoE e4 status) in separate analyses. If significant ($p < 0.10$), the additive effect of the main predictor centered at different percentiles (10th, 25th, 50th, 75th, and 90th) and the presence of the ApoE e4 allele on cognitive functioning was tested. Predictors were centered to test for significance of the main effects at these different levels and thus improve interpretability. If a significant modifying effect was not found, ApoE e4 was added to the model to test for potential confounding.

In addition, analyses were repeated after exclusion of persons with cardiovascular disease at baseline or follow-up to study the mediating effect of cardiovascular disease on

Table 1
Baseline characteristics for the total study sample with longitudinal data on cognitive functioning and separately for apoe e4 carriers and apoe e4 noncarriers

Characteristic	Unit	Total sample ($n = 967$)	ApoE e4+ ($n = 247$)	ApoE e4− ($n = 718$)
Age, years, mean (SD)		75.00 (6.39)	74.76 (6.45)	75.08 (6.38)
Female, % (n)		53.30 (515)	50.60 (125)	54.20 (389)
Education, years, mean (SD)[a]		9.02 (6.39)	9.14 (3.22)	8.98 (3.28)
Depressive symptoms, mean (SD)[a]		7.80 (7.51)	7.21 (7.20)	8.01 (7.61)
Hypertension, % (n)[a]		56.10 (539)	59.20 (145)	55.20 (394)
Diabetes mellitus, % (n)		6.40 (62)	4.50 (11)	7.10 (51)
Cardiovascular disease, % (n)		28.50 (275)	29.60 (73)	28.30 (203)
Body mass index, mean (SD)[a]		26.84 (4.09)	26.93 (3.88)	26.82 (4.15)
Smoking, % (n)				
No		37.20 (360)	36.00 (89)	37.60 (270)
Former		45.3 (438)	47.80 (118)	44.60 (320)
Current		17.50 (169)	16.20 (40)	17.80 (128)
Alcohol consumption, % (n)[a]				
No		23.60 (238)	21.50 (53)	24.40 (175)
Middle		66.60 (643)	68.80 (170)	65.70 (471)
High		9.80 (95)	9.70 (24)	9.90 (71)
MMSE score, mean (SD) ($n = 967$)		27.14 (2.57)	27.01 (2.61)	27.20 (2.50)
Immediate recall, mean (SD) ($n = 912$)		8.42 (2.49)	8.63 (2.47)	8.34 (2.47)
Information processing speed, mean (SD) ($n = 898$)		24.00 (6.86)	23.95 (6.64)	24.03 (6.64)
Sterol concentrations in serum				
Cholesterol, mean (SD)	mg/dL	235.67 (45.55)	242.55 (44.10)	233.37 (45.82)**
Cholesterol precursors				
Lathosterol, mean (SD)				
Absolute	mg/dL	0.28 (0.12)	0.28 (0.12)	0.28 (0.12)
Ratio to cholesterol	µg/mg	1.22 (0.53)	1.18 (0.49)	1.24 (0.54)
Lanosterol, mean (SD)				
Absolute	µg/dL	32.82 (9.24)	33.03 (8.31)	32.77 (9.55)
Ratio to cholesterol	ng/mg	142.87 (46.80)	138.92 (38.70)	144.30 (49.26)
Plant sterols				
Campesterol, mean (SD)				
Absolute	mg/dL	0.36 (0.19)	0.38 (0.21)	0.35 (0.19)*
Ratio to cholesterol	µg/mg	1.50 (0.77)	1.57 (0.84)	1.48 (0.75)
Sitosterol, mean (SD)				
Absolute	mg/dL	0.33 (0.17)	0.35 (0.18)	0.32 (0.17)*
Ratio to cholesterol	µg/mg	1.41 (0.68)	1.45 (0.72)	1.39 (0.66)

P values indicate whether value distributions significantly vary between the ApoE e4 carriers and e4 noncarriers *$p \leq 0.05$, **$p \leq 0.01$.
Key: ApoE, apolipoprotein E; MMSE, Mini-Mental State Examination.
[a] Missing values.

the associations between the sterols, ApoE e4, and cognition.

Finally, in additional analyses (see Supplementary data) we studied the associations between the absolute values of all markers and cognitive functioning. Also, the interaction between hyperlipidemic status, defined as total cholesterol levels ≥250.90 mL/dL (6.5 mmol/L) (yes, no), and absolute levels and ratios to cholesterol of all markers were studied in the fully adjusted models.

3. Results

Table 1 shows the baseline characteristics for the total study sample, ApoE e4 carriers, and e4 noncarriers. The amounts of cholesterol as well as the absolute levels of the plant sterols proved significantly higher in ApoE e4 carriers compared with e4 noncarriers.

Correlations between absolute levels and the ratios to cholesterol of all markers, and total cholesterol, absolute

levels, and the ratio of 27-hydroxycholesterol to cholesterol are shown in Table 2.

Table 3 shows the results of the longitudinal mixed models analyses in which the associations between the sterol ratios to cholesterol, and the ratios of lathosterol to campesterol and sitosterol, and cognitive functioning were studied in the total sample, as well as effect modification of these associations by ApoE e4 in the fully-adjusted models.

3.1. Main effects

We studied all markers as continuous variables in the total study sample. To test for nonlinearity of the associations studied, the quadratic term of each main predictor was added to the time-adjusted models in separate analyses for each outcome measure and predictor. The results show that only the quadratic term of the ratio of lanosterol to cholesterol reached significance in both the time- and fully-adjusted models of the associations with general cognitive

Table 2
Correlations between markers, total cholesterol and 27-hydroxycholesterol cholesterol levels

	Total cholesterol	27-hydroxycholesterol	Ratio of 27-hydroxycholesterol to cholesterol
Cholesterol precursors			
Absolute levels of lanosterol	0.270**	0.382**	0.253**
Ratio of lanosterol to cholesterol	−0.399**	0.067*	0.462**
Absolute levels of lathosterol	0.306*	0.266**	0.086**
Ratio of lathosterol tot cholesterol	−0.167**	0.064*	0.255**
Plant sterols			
Absolute campesterol levels	0.348**	0.298**	0.053
Ratio of campesterol to cholesterol	0.016	0.141**	0.135**
Absolute sitosterol levels	0.358**	0.318**	0.076*
Ratio of sitosterol to cholesterol	0.000	0.160**	0.180**
Cholesterol synthesis relative to absorption			
Ratio of lathosterol to campesterol	−0.127**	−0.076*	0.021
Ratio of lathosterol to sitosterol	−0.119**	−0.085**	0.005

* $p < 0.05$.
** $p < 0.01$.

performance (ln transformed MMSE score) and information processing speed. This indicates that the association between the ratio of lanosterol to cholesterol and the trajectory of information processing speed and general cognitive performance was nonlinear (i.e., a threshold was found above which the association is no longer significant), thus the quadratic term of the ratio of lanosterol to cholesterol was retained only in these models (see Table 3). By subsequently centering the ratio of lanosterol to cholesterol at different percentiles we were able to identify the inflexion point.

In the time-adjusted models the results show that a higher ratio of lanosterol to cholesterol was significantly associated with a higher ln transformed MMSE score (i.e., worse general cognitive performance) and lower information processing speed. Table 3 shows only the strength and significance level of the linear and quadratic term of the ratio of lanosterol to cholesterol centered at the 10th percentile. No significant associations were found between the ratio of lathosterol to cholesterol and cognitive functioning. Furthermore, a significant association was found between a higher ratio of the plant sterols to cholesterol and a lower ln transformed MMSE score (i.e., better general cognitive performance) and higher information processing speed. In addition, a higher ratio of sitosterol to cholesterol was significantly associated with better immediate recall. Finally, the time-adjusted models showed a significant positive association between the ratios of lathosterol to the plant sterols and the ln transformed MMSE score and a significant negative association between these ratios and immediate recall and information processing speed. This indicates that a relatively higher rate of cholesterol synthesis compared with cholesterol absorption was significantly associated with lower general cognitive performance, memory performance, and information processing speed. No significant interactions with time were found, indicating that none of the markers of cholesterol homeostasis were significantly associated with rate of cognitive decline. Furthermore, the absence of significant interactions between the markers of cholesterol homeostasis and time indicates that the strength of the associations found remained constant over time.

In the fully-adjusted models, only the (nonlinear) association between the ratio of lanosterol to cholesterol and the ln transformed MMSE score remained significant. The results indicate that a higher ratio of lanosterol to cholesterol was significantly associated with lower general cognitive performance over 6 years of follow-up independent of relevant confounders. Further analyses show that this association was significant up to a ratio of lanosterol to cholesterol of 205.00 (ng/mg). In Fig. 1, the trajectory of performance on the MMSE score is shown according to the ratio of lanosterol to cholesterol. The negative association between the ratio of lanosterol of cholesterol (centered at the 10th percentile) and information processing speed reached borderline significance ($p = 0.066$). All other associations lost statistical significance after adjustment for relevant confounders.

3.2. Effect modification by ApoE e4

The results as presented in Table 3 show a significant interaction between the quadratic term of the ratio of lanosterol to cholesterol and ApoE e4 status in the model predicting the ln transformed MMSE score. The positive nonlinear association between the ratio of lanosterol to cholesterol and the ln transformed MMSE score was significant only in ApoE e4 noncarriers (Fig. 2). Analyses showed that this association was significant up to a ratio of 189.95 (ng/mg).

The negative nonlinear association between the ratio of lanosterol to cholesterol and information processing speed reached significance in ApoE e4 noncarriers; although no significant effect modification by ApoE e4 status was found (see Table 3). However, further analyses showed that only

Table 3

Associations between sterols and cognitive performance over 6 years of follow-up separately for total study sample, e4 carriers, and e4 noncarriers

Ln transformed MMSE (direction of associations is reversed because of ln [natural log] transformation)

	Total sample ($n = 967$)				ApoE e4− ($n = 718$)		ApoE e4+ ($n = 247$)		
	B time adjusted	p value	B fully adjusted	p value	B fully adjusted	p value	B fully adjusted	p value	p interaction term
Precursors									
r_lath	0.060	0.072	0.016[a]	0.598	0.019[a]	0.564	0.0032[a]	0.958	0.814
r_lano at p10	0.0018	0.002	0.0014[b]	0.008	0.0014[b]	0.018	0.00027[b]	0.837	0.434
r_lano*r_lano	$-0.44*10^{-5}$	0.020	$-0.30*10^{-5}$	0.072	$-0.34*10^{-5}$	0.048	$0.96*10^{-5}$	0.141	0.054
Plant sterols									
r_camp	−0.050	0.028	0.0058[c]	0.785	−0.0033[c]	0.895	0.033[c]	0.390	0.422
r_sito	−0.072	0.006	−0.0081[d]	0.773	−0.020[d]	0.480	0.021[d]	0.625	0.421
Cholesterol synthesis relative to absorption									
lath_camp	0.072	0.000	0.013[e]	0.438	0.018[e]	0.336	−0.0055[e]	0.874	0.549
lath_sito	0.070	0.000	0.020[f]	0.232	0.027[f]	0.134	−0.0085[f]	0.809	0.362

Immediate recall

	Total sample ($n = 918$)				ApoE e4 ($n = 683$)		ApoE e4+ ($n = 234$)		
	B time adjusted	p value	B fully adjusted	p value	B fully adjusted	p value	B fully adjusted	p value	p interaction term
Precursors									
r_lath	−0.091	0.533	0.13[g]	0.313	0.12[g]	0.416	0.22[g]	0.407	0.725
r_lano	−0.0025	0.140	0.00011[h]	0.937	−0.00018[h]	0.909	.0015[h]	0.647	0.638
Plant sterols									
r_camp	0.20	0.051	−0.16[i]	0.853	0.11[i]	0.305	−0.29[i]	0.052	0.029
r_sito	0.26	0.021	0.036[j]	0.723	0.11[j]	0.354	−0.17[j]	0.364	0.199
Cholesterol synthesis relative to absorption									
lath_camp	−0.19	0.019	0.034[j]	0.640	0.00040[j]	0.996	0.17[j]	0.244	0.304
lath_sito	−0.17	0.042	0.028[k]	0.707	−0.0045[k]	0.957	0.16[k]	0.285	0.331

Information processing speed

	Total sample ($n = 898$)				ApoE e4− ($n = 667$)		ApoE e4+ ($n = 230$)		
	B time adjusted	p value	B fully adjusted	p value	B fully adjusted	p value	B fully adjusted	p value	p interaction term
Precursors									
r_lath	−0.60	0.157	−0.096[l]	0.783	−0.25[l]	0.528	0.43[l]	0.545	0.398
r_lano at p10	−0.016	0.039	−0.012[b]	0.066	−0.014[b]	0.044	0.0070[b]	0.650	0.210
r_lano*r_lano	$0.63*10^{-4}$	0.008	$0.47*10^{-4}$	0.016	$0.54*10^{-4}$	0.007	$-0.53*10^{-4}$	0.478	0.167
Plant sterols									
r_camp	1.09	0.000	0.25[b]	0.313	0.71[b]	0.017	−0.67[b]	0.111	0.007
r_sito	1.25	0.000	0.34[b]	0.214	0.79[b]	0.016	−0.65[b]	0.185	0.014
Cholesterol synthesis relative to absorption									
lath_camp	−1.06	0.000	−0.32[m]	0.107	−0.57[m]	0.011	0.54[m]	0.189	0.017
lath_sito	−1.01	0.000	−0.31[m]	0.124	−0.54[m]	0.018	0.46[m]	0.267	0.034

Key: ApoE, apolipoprotein E; B, unstandardized regression coefficient; BMI, body mass index; lath_camp, ratio of lathosterol to campesterol (mg/mg); lath_sito, ratio of lathosterol to sitosterol (mg/mg); MMSE, Mini-Mental State Examination; p10, 10th percentile; r_camp, ratio of campesterol to cholesterol (μg/mg); r_lano, ratio of lanosterol to cholesterol (ng/mg); r_lath, ratio of lathosterol to cholesterol (μg/mg); r_sito, ratio of sitosterol to cholesterol (μg/mg).

[a] Adjusted for time, age, sex, education, diabetes mellitus, BMI, alcohol, and ApoE e4.

[b] Adjusted for time, age, sex, and education.

[c] Adjusted for time, age, education, diabetes mellitus, BMI, and depressive symptoms.

[d] Adjusted for time, age, education, diabetes mellitus, BMI, depressive symptoms, alcohol, smoking, and ApoE e4.

[e] Adjusted for time, age, education, diabetes mellitus, BMI, alcohol, and ApoE e4.

[f] Adjusted for time, age, education, and diabetes mellitus.

[g] Adjusted for time, age, sex, education, diabetes mellitus, BMI, and alcohol.

[h] Adjusted for time, age, sex, education, diabetes mellitus, hypertension, BMI, alcohol, smoking, and ApoE e4.

[i] Adjusted for time, age, sex, education, and diabetes mellitus.

[j] Adjusted for time, age, sex, education, diabetes mellitus, BMI, depressive symptoms, and alcohol.

[k] Adjusted for time, age, sex, education, diabetes mellitus, BMI, depressive symptoms, alcohol, and smoking.

[l] Adjusted for time, age, sex, education, diabetes mellitus, BMI, depressive symptoms, and ApoE e4.
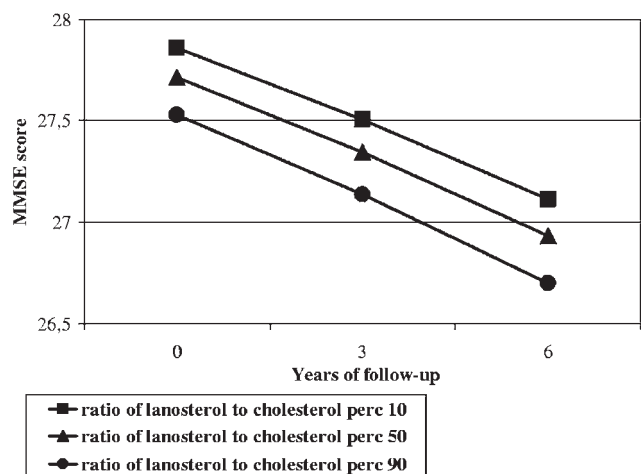
[m] Adjusted for time, age, and education.

Figure 1. Six-year cognitive decline according to the ratio of lanosterol to cholesterol in total sample, adjusted for time, age, sex, and education. MMSE, Mini Mental State Examination.

in persons with the lowest ratios of lanosterol to cholesterol (<111.14 ng/mg) the association with information processing speed was significant.

Furthermore, in the model predicting immediate recall, a significant interaction between the ratio of campesterol to cholesterol and ApoE e4 was found. In ApoE e4 carriers, the main effect of the ratio of campesterol to cholesterol on memory performance approached significance ($p = 0.052$). Also, a significant interaction between ApoE e4 and the ratio of campesterol and sitosterol to cholesterol, and lathosterol to campesterol and sitosterol in the models predicting information processing speed was found. Only in e4 noncarriers, a significant association was found between a higher ratio of the plant sterols to cholesterol and higher information processing speed over 6 years of follow-up, independent of relevant confounders. Fig. 3 shows the trajectory of information processing speed according to the ratio of the plant sterols to cholesterol in ApoE e4 noncarriers.

Furthermore, a significant association between a higher ratio of lathosterol to the plant sterols and lower information processing speed was found, only in ApoE e4 noncarriers. This indicates that a relatively higher rate of cholesterol synthesis relative to absorption was significantly associated with lower information processing speed. This result is shown in Fig. 4.

3.3. Sensitivity analyses

In order to study whether the presence of cardiovascular disease at baseline or follow-up was a significant mediator of the studied associations, all analyses were repeated in the sample excluding persons with cardiovascular disease ($n = 501$). Overall, the results show no substantial change in the unstandardized B of the main predictors with respect to the association with cognitive functioning (data not shown).

This indicates that the presence of cardiovascular disease could not explain the associations found.

4. Discussion

This prospective population-based study focused on the associations between extracerebral markers of cholesterol synthesis, markers of cholesterol absorption, and cognitive functioning over 6 years of follow-up in older persons. We showed a significant independent nonlinear association between a higher rate of cholesterol synthesis as reflected by a higher ratio of lanosterol to cholesterol, and worse general cognitive performance. It was shown that this association was significant up to a threshold value of the ratio of lanosterol to cholesterol of 205.00 ng/mg. Furthermore, this association was significant in ApoE e4 noncarriers (up to a threshold value of the ratio of lanosterol to cholesterol of 189.95 ng/mg), but not in ApoE e4 carriers. It was shown that the mean level of cognitive performance after 3 years of follow-up in ApoE e4 noncarriers with a ratio of lanosterol to cholesterol in the 10th percentile was approximately the same as the baseline level in those with a ratio in the 90th percentile. In addition, it was shown that a higher rate of cholesterol absorption as reflected by a higher ratio of campesterol and sitosterol to cholesterol was independently associated with higher information processing speed, only in ApoE e4 noncarriers. On average, level of information processing speed after 3 years of follow-up in ApoE e4 noncarriers with a rate of cholesterol absorption in the 90th percentile was similar to baseline information processing speed in those in the 10th percentile. In ApoE e4 carriers, the association between a higher rate of cholesterol absorption as reflected by the ratio of campesterol to cholesterol, and worse memory performance approached statistical significance. Finally, in ApoE e4 noncarriers a higher rate of cholesterol synthesis relative to cholesterol absorption was
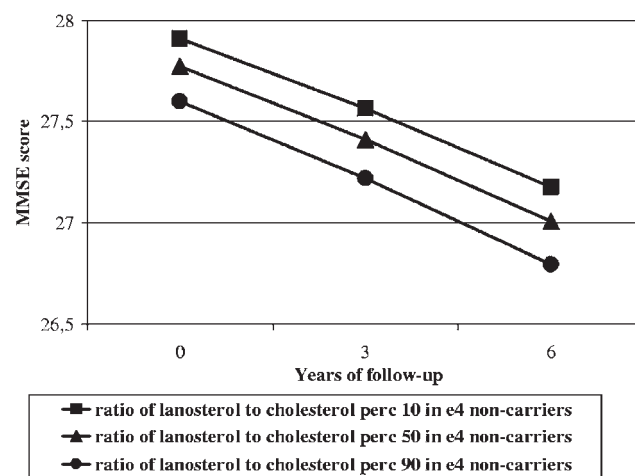

Figure 2. Six-year cognitive decline according to the ratio of lanosterol to cholesterol in ApoE e4 noncarriers, adjusted for time, age, sex, and education. MMSE, Mini Mental State Examination.
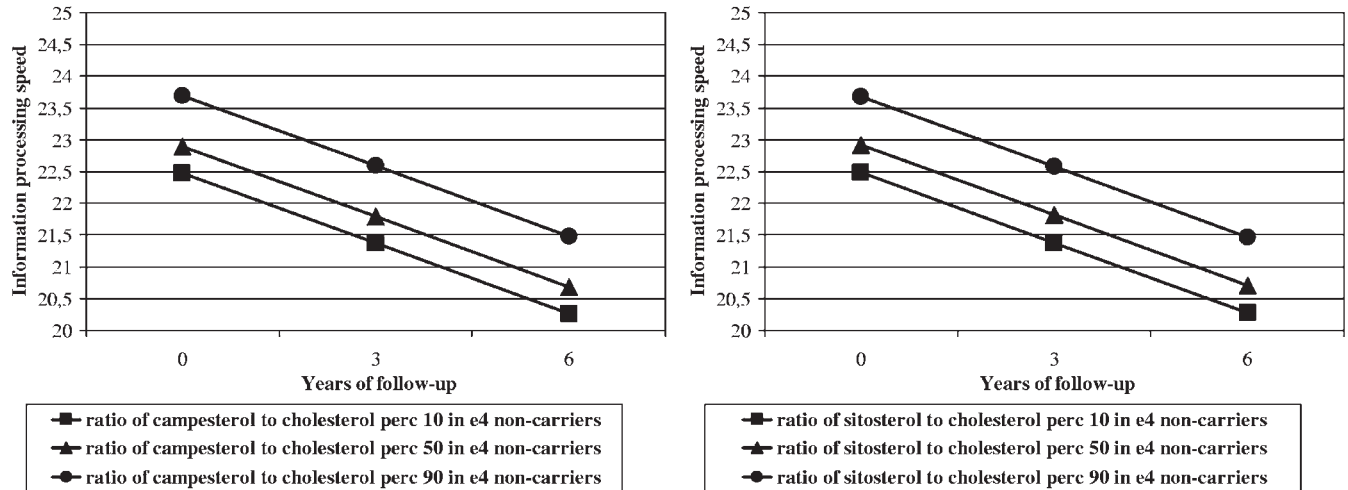
Figure 3. Six-year cognitive decline according to the ratio of plant sterols to cholesterol in ApoE e4 noncarriers, adjusted for time, age, sex, and education.

shown to predict lower information processing speed over 6 years of follow-up. The mean level of information processing speed after 3 years of follow-up in ApoE e4 noncarriers with a rate of cholesterol synthesis relative to absorption in the 10th percentile was comparable to the mean level of baseline information processing speed in persons with a rate in the 90th percentile. In addition, the present study showed that the strength of the associations between these markers and level of cognitive functioning remained unchanged over time. No significant associations between the studied markers of cholesterol metabolism and a faster rate of cognitive decline were found.

Additional analyses (see Supplementary data) showed that the direction of the associations found when studying the absolute levels of the sterols was consistent with the direction of the associations found when studying the effect

of the ratios of the sterols to cholesterol on cognitive functioning. Also, in some instances a modifying effect of hyperlipidemia was found, indicating that some of the significant positive associations between the absolute levels of the plant sterols and cognitive functioning were significant only in nonhyperlipidemic persons.

4.1. Cholesterol synthesis

The present findings on cholesterol synthesis are inconsistent with the results from a previous population-based study (Teunissen et al., 2003). In contrast with our findings, Teunissen et al. (2003) showed that a higher ratio of lathosterol to cholesterol at baseline was predictive of lower cognitive functioning. Furthermore, while they found a significant linear association between a higher ratio of lanos-
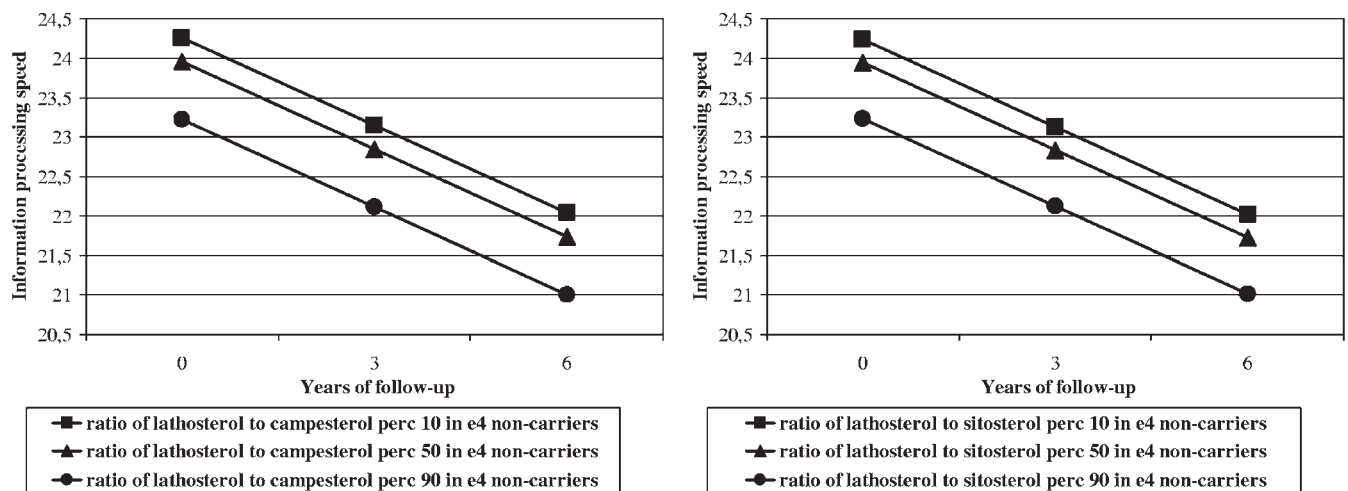

Figure 4. Six-year cognitive decline according to the rate of cholesterol synthesis relative to absorption in ApoE e4 noncarriers adjusted for time, age, and education.

terol to cholesterol at baseline and lower memory performance and executive functioning, the current study showed a nonlinear association with lower general cognitive performance, but not with memory performance. These inconsistent results may be due to differences in study sample. The present study sample was older (mean age of 75.0 vs. 57.4 years) and had higher cholesterol, lanosterol, and lathosterol levels at baseline. Also, in the present study the nonlinear association between a higher ratio of lanosterol to cholesterol and lower general cognitive performance was significant in ApoE e4 noncarriers but not in e4 carriers. Teunissen et al. (2003) did not study the modifying role of ApoE e4.

In a recent cross sectional study it was shown that in persons with subjective memory impairment (mean age 57.5 years), but not in mild cognitive impairment (MCI) and AD patients, a higher ratio of lanosterol and lathosterol to cholesterol was significantly associated with lower brain volumes (Solomon et al., 2009). In a postmortem study it was concluded that de novo synthesis of cholesterol but not the cholesterol amount itself declines with aging in the hippocampus (Thelen et al., 2006). However, another recent postmortem study (mean age = 85 years, SD = 6) showed this age-related decrease of cholesterol synthesis only in the brains of AD patients, and the opposite effect in persons with no cognitive impairment (Hascalovici et al., 2009). Thus, current evidence suggests a complex interaction between age and (brain) sterol homeostasis (Hascalovici et al., 2009; Thelen et al., 2006).

4.2. Cholesterol absorption

In line with the study by Teunissen et al. (2003), no significant associations were found between the ratio of campesterol and sitosterol to cholesterol and cognitive functioning in the total study sample. However, in the model predicting information processing speed and immediate recall, we found a significant interaction between these markers and ApoE e4. In ApoE e4 noncarriers, a significant association between higher cholesterol absorption and faster information processing speed was found, while in ApoE e4 carriers, a higher rate of cholesterol absorption was associated with worse memory performance. In a previous study we found that especially in ApoE e4 carriers, lower cholesterol was associated with faster decline in information processing speed. Levels of total cholesterol were however not significantly associated with memory performance (Van den Kommer et al., 2009). Also, the ratio of campesterol to cholesterol, indicative of cholesterol absorption, was not significantly correlated with total cholesterol. This in contrast to absolute campesterol levels, which showed a positive correlation with cholesterol. Taken together, the borderline significant association between a higher rate of cholesterol absorption and worse memory performance in ApoE e4 carriers does not seem to be related to hypercholesterolemia. It may be speculated that this indicates that a higher rate of cholesterol absorption in ApoE e4 carriers is

associated with increased risk of AD. In a study among healthy middle-aged men it was suggested that especially those with the ApoE e4 allele are at increased risk of the putative adverse effects of plant sterols, for example, premature atherosclerosis (Nissinen et al., 2008). In the present study, additional analyses showed that this potentially adverse effect of plant sterols on cognition in ApoE e4 carriers only reached significance in the model predicting memory performance. Overall, the present findings may indicate different pathways in ApoE e4 carriers versus noncarriers. The findings also suggest that the negative effect of carrying the ApoE e4 allele on cognitive functioning prevails over the potential (negative) effects of plant sterols.

A recent randomized double-blind placebo controlled trial in statin treated hypercholesterolemic persons showed that long term consumption of plant sterols and plant stanol esters did not have significant effects on cognitive functioning (Schiepers et al., 2009). In light of the current findings, it may be suggested that ApoE phenotype should be taken into account when studying the effect of dietary interventions on cognitive functioning.

4.3. Cholesterol synthesis versus absorption

The current study did not show significant associations between the ratio of lathosterol and the plant sterols and cognitive performance in the total study sample, which contrasts with the study by Teunissen et al. (2003). They showed a significant association between a higher lathosterol to campesterol and sitosterol ratio and worse memory performance, after adjustment for age, sex, and education. Again, this inconsistency may be due to differences in characteristics between samples, such as age, general health, and level of the markers studied. However, in the current study we showed that a higher rate of cholesterol synthesis relative to absorption was associated with lower information processing speed in ApoE e4 noncarriers but not in e4 carriers. Again, this suggests that the negative effect of carrying the ApoE e4 allele prevails over the effect of cholesterol synthesis and absorption on cognitive functioning.

In light of the present findings, future studies should focus on the potential differential effect of lipid-lowering compounds, affecting cholesterol synthesis and absorption markers, on cognition in ApoE e4 carriers and noncarriers.

See Supplementary data for a reflection on previous findings with respect to the associations between total cholesterol, 27-hydroxycholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol (Van den Kommer et al., 2009, 2010).

4.4. Strengths

The major strengths of the present study are that the associations between several markers for cholesterol homeostasis, ApoE phenotype, and both level and change in cognitive functioning were studied. Cognitive performance

was examined on 3 different cognitive tests most sensitive to age-related decline, on 3 occasions over 6 years of follow-up. In addition, these associations were studied in a large population-based sample including multiple relevant confounding factors. To our knowledge, the interaction between markers for cholesterol synthesis and absorption and ApoE e4 status on cognitive functioning and decline has not been studied before.

It has been well established that cardiovascular risk is related to dementia and cognitive decline. Weingärtner et al. (2010) reviewed the accumulating evidence that altered cholesterol homeostasis toward increased cholesterol absorption and reduced cholesterol synthesis is associated with increased cardiovascular risk. Therefore, in the present study sensitivity analyses were performed to exclude the influence of cardiovascular disease on the associations studied. We showed that excluding persons with cardiovascular disease at baseline or follow-up did not significantly change the strength (or direction) of the associations found. However, the influence of subclinical cardiovascular disease on the associations studied could not be fully excluded.

4.5. Limitations

A number of limitations need to be addressed. First, sterol levels were only determined once, at baseline, not during follow-ups. Thus, we were unable to study whether a change in sterol levels was associated with cognitive decline. In addition, similar to other population-based studies, the frailest persons with lower levels of cognitive functioning refused blood sampling and were lost to follow-up. Furthermore, there were some significant differences in sterol levels between those lost to follow-up and those who remained in the study. Persons lost to follow-up had significantly lower levels of cholesterol, lathosterol, plant sterols, and a lower ratio of lathosterol to cholesterol at baseline compared with those who remained in the study. This may have lead to an underestimation of the strength of the associations studied. Previous studies have shown that total cholesterol tends to increase with age up to midlife, but decreases over time after midlife (Abbott et al., 1997; Ferrara et al., 1997; Solomon et al., 2007). A reduced cholesterol absorption rate has been shown in older men compared with middle-aged men, which may be a major factor related to decrease in serum cholesterol after midlife (Gylling et al., 1994). It has been suggested that this decrease may be explained by physiologic aging and reflects disease or a gradual decline in overall health (Abbott et al., 1997; Ferrara et al., 1997).

Another potential limitation of the present study is the lack of data on dietary intake. It has been suggested that fatty fish and intake of n-3 fatty acids may play a protective role with respect to age-related decline and dementia, although studies have shown inconsistent results (Dullemeijer et al., 2007; Van de Rest et al., 2009; Van Gelder et al.,

2007). Thus, it would have been preferable to include dietary intake as a potential confounding factor.

Finally, the issue of reverse causality cannot be fully dismissed. In LASA, a clinical dementia diagnosis based on formal criteria is not available. However, at baseline we identified only 3 persons showing persistent cognitive decline, defined as clinically relevant decline over 3 years of follow-up (>2 SD below the mean) and continued decline over the subsequent 3 years (Van den Kommer et al., 2008), reducing the risk of reverse causality.

4.6. Conclusions

In sum, the present longitudinal population-based study showed that a higher rate of cholesterol synthesis as reflected by a higher ratio of lanosterol to cholesterol was predictive of lower general cognitive performance in the total sample. This association was significant in ApoE e4 noncarriers, not in ApoE e4 carriers. In addition, higher cholesterol absorption was significantly predictive of a relatively faster information processing speed, only in ApoE e4 noncarriers. Finally, only in ApoE e4 noncarriers, a higher rate of cholesterol synthesis relative to cholesterol absorption was significantly predictive of lower information processing speed. Although absolute differences are somewhat small, overall the current findings showed a persistent difference in level of cognitive functioning between ApoE e4 noncarriers with sterol levels in the 10th percentile versus those with levels in the 90th percentile. Future research should focus on the role of (disturbed) cholesterol homeostasis and effect modification by ApoE e4 status with respect to dementia.

Disclosure statement

No actual or potential conflicts of interest.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2011.02.019.

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