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

van den Kommer, T.N.

2011

document version
Publisher's PDF, also known as Version of record

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

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 5

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

Tessa N. van den Kommer
Miranda G. Dik
Hannie C. Comijs
Dieter Lütjohann
Paul Lips
Cees Jonker
Dorly J.H. Deeg

Neurobiology of Aging (submitted)

Abstract

We examined the associations between extracerebral markers of cholesterol homeostasis and cognitive decline over six years of follow-up, and the modifying effect of ApoE e4.

Data were collected in the Longitudinal Aging Study Amsterdam (N = 967, with longitudinal data on cognition, ≥ 65 years) and analyzed using linear mixed models. General cognition (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. The following significant associations were found, only in ApoE e4 non-carriers (N = 718). A non-linear negative association between the ratio of lanosterol to cholesterol (≤ 189.96 ng/mg), a marker for cholesterol synthesis, and general cognition was found. A lower ratio of campesterol and sitosterol to cholesterol, indicative of lower cholesterol absorption, was associated with lower information processing speed. Finally, a higher rate of cholesterol synthesis relative to absorption was predictive of lower information processing speed. Future research should focus on the interaction between (disturbed) cholesterol homeostasis and ApoE e4 status with respect to dementia and cognitive decline.

Keywords: Longitudinal population-based study; Extracerebral cholesterol homeostasis; ApoE e4; Cholesterol precursors; Plant sterols; Trajectory of cognitive functioning.

Introduction

Disturbed cerebral and extracerebral cholesterol homeostasis has been suggested to play a role in dementia and cognitive decline. However, studies 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 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 (CSF) 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 was a significant modifier of the longitudinal associations between total serum cholesterol, the ratio of 27-hydroxycholesterol and total 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 those without the ApoE e4 allele (Van den Kommer et al., 2009). In the present study, we focus further on the role of cholesterol homeostasis, namely extracerebral markers of cholesterol synthesis and absorption with respect to cognitive performance and decline in late-life, and the possible modifying role of ApoE e4.

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 two different pathways, which use either desmosterol or lathosterol as the predominant precursors for de novo synthesis of brain cholesterol (Lütjohann et al., 2002). A negative association between lanosterol and lathosterol at baseline and cognitive performance over six years of follow up has been found in a normal aging population (Teunissen et al., 2003). Another study showed that in persons with subjective cognitive impairment, lower levels of lanosterol and lathosterol were associated with higher brain volumes, but not in patients with mild cognitive impairment (MCI) or AD. Plasma levels of lanosterol and lathosterol were lowest in the AD group compared to MCI patients and those with subjective memory impairment, the latter showing the highest concentration of these markers (Solomon et al., 2009).

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). Previous studies focusing on the association between plant sterols and cardiovascular disease, an important risk factor for dementia and cognitive decline (Stampfer, 2006), have shown contrasting results. Some studies showed an association between high cholesterol absorption and increased severity (Silbernagel et al., 2009) and incidence (Assmann et al., 2003) of coronary heart disease, while others found neutral (Wilund et al., 2004) or even protective

effects (Fassbender et al., 2008) of elevated plant sterols on atherosclerosis and coronary heart disease. Recent studies have demonstrated the presence of plant sterols and stanols in the brain (Fricke et al., 2007; Heverin et al., 2004; Jansen et al., 2006). Jansen et al. (2006) have suggested an important role for high-density lipoprotein and/or ApoE in the transfer of plant sterols into the brain. Elevated levels of plant sterols (and stanols) in the central nervous system may affect brain functioning (Jansen et al., 2006). A study focusing on the concentration of plant sterols in the brain of Alzheimer's patients found slightly higher levels of plant sterols in most areas (Heverin et al., 2004). However, a randomized double-blind placebo-controlled dietary intervention trial found no effect of long-term supplementation with plant sterol or stanol esters on cognitive functioning in hypercholesterolemic statin-treated persons (Schiepers et al., 2009).

To our knowledge no study has focused on the modifying role of ApoE 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 and lathosterol, sitosterol and campesterol and cognitive functioning and the modifying role of ApoE e4 on these associations.

Methods

Study sample

Data for this study were collected within the Longitudinal Aging Study Amsterdam (LASA), an ongoing population-based study (Deeg et al., 2002). Procedures regarding sampling and data collection have been described previously 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 includes follow-up measurements every three years. Respondents were interviewed at home by means of a main and a medical interview, in which structured questionnaires were completed and tests were performed.

In total, 3,107 subjects between the ages of 55 and 85 were enrolled during the first data collection of LASA, of whom 2,545 (81,9%) completed the main interview 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 who participated in the medical interview. Of the 1,509 respondents participating in the medical interview, 1,352 persons agreed to take part in the blood drawing procedure. Blood samples were obtained either in the VU University Medical Center (VUmc) (respondents living in Amsterdam and vicinity), a health care center near their homes (respondents living in Zwolle or Oss and vicinity) or at home (respondents unable to come to the VUmc or a health care center near their home). For the present study, persons were included of whom a blood sample was obtained during the second data collection and sterol levels could be determined (N = 1,248). Subjects of whom blood samples could not be obtained were significantly older and had lower scores on

cognition (all $p < 0.0001$) compared to subjects participating in the medical interview of whom blood samples were available. Persons using lipid-lowering drugs at the time of blood sampling were excluded (N = 67), resulting in 1,181 eligible subjects. Of the 1,181 eligible respondents, 1,003 (84.9%) also participated in the three-year follow-up interviews (1998/1999). Of the 178 subjects who were lost to follow-up, 149 were 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 three-year follow-up, 826 (82.4%) also participated in the interviews during the six-year follow-up (2001/2002). Of those lost to follow-up, 154 were 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 and to have cardiovascular disease (all $p < 0.0001$). Furthermore, respondents lost to follow-up after three years 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. Respondents lost to follow-up after six years had significantly lower levels of cholesterol ($p = 0.008$) and lower absolute lathosterol levels ($p = 0.037$) at baseline compared to 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 two occasions, which resulted in a final sample of N = 967.

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 performance 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 (range, 1-42.7).

Measurement of sterol levels

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 was determined by highly sensitive gas chromatography-flame ionization detection. Plasma concentrations of plant sterols sitosterol and campesterol and cholesterol precursors lathosterol and lanosterol were assessed using an ultra-sensitive and highly specific gas chromatography-mass spectrometry selective ion-monitoring method as previously described (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 inter-assay coefficients of variation for all sterols were below 3% of the respective mean values (precision). Inter-assay 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.

Measurement of 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 non-carriers for subjects without the ApoE e4 isoform (phenotypes e2/2, e2/3, e3/3).

Potential confounders

The following variables were considered as potential confounders: age, sex, education, hypertension, diabetes mellitus, depressive symptoms, body mass index, alcohol intake, smoking status, use of lipid-lowering drugs at follow-up and ApoE e4 (if not a significant effect modifier).

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). Hypertension was defined by sitting blood pressure, ≥ 160/100 mm/Hg, use of anti-hypertensive medication or both. Diabetes mellitus was assessed by self-report (nearly perfect agreement with GP information,

$\kappa = 0.85$) (Kriegsman et al., 1996) and medication use. Depressive symptoms were assessed with the Center for Epidemiologic Studies Depression Scale (CES-D). The CES-D is a 20-item self-report scale (range, 0-60) designed to measure depressive symptoms in the general population (Beekman et al., 1997; 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 per week over the past year, and for the number of days in 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 smokers. Use of lipid-lowering drugs was determined by checking medication use. ApoE status was classified as described above.

Cardiovascular disease (cardiac disease, peripheral arterial disease, cerebrovascular accident) was evaluated as a potential mediator. The presence of cardiovascular disease at baseline and three-year follow-up was assessed by a combination of self-report data, medication use and records of the general practitioners in an algorithm previously described (Bremmer et al., 2006). At six-year follow-up self-report data and medication records were used.

Data analysis

Characteristics of the study sample by ApoE 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.

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 on these associations, data were analyzed using linear mixed models in SPSS for windows, version 15.0 (SPSS incorporated, 2007, Chicago, IL).

First, the trajectories of cognitive functioning as indicated by scores on the MMSE, immediate recall and information processing speed were modelled as a function of time. Time was defined as the number of years (i.e. 0, 3 and 6 years) between sterol analyses 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 non-linear 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 one by one to the model. Variables that showed a significant confounding effect on the studied associations, i.e. $\geq 10\%$ 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 x 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 the associations between the sterols, ApoE e4 and cognition.

Results

Table 1 shows the baseline characteristics of the total study sample and separately for ApoE e4 carriers (N = 247) and e4 non-carriers (N = 618). The results show that the amount of cholesterol as well as the absolute levels of the plant sterols, were significantly higher in ApoE e4 carriers compared to those not carrying the ApoE e4 allele.

Table 1 Baseline characteristics for the total study sample with longitudinal data on cognitive functioning and separately for ApoE e4 carriers and ApoE e4 non-carriers

Characteristic	Total sample	ApoE e4+	ApoE e4-
	(N = 967)	(N = 247)	(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) [1]	9.02 (6.39)	9.14 (3.22)	8.98 (3.28)
Depressive symptoms, mean (SD) [1]	7.80 (7.51)	7.21 (7.20)	8.01 (7.61)
Hypertension, % (N) [1]	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) [1]	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) [1]			
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	Unit	Total sample (N = 967)	ApoE e4+ (N = 247)	ApoE e4- (N = 718)
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)

[1] missing values; * indicate whether value distributions significantly vary between the ApoE e4 carriers and e4 non-carriers; * $p \leq 0.05$; ** $p \leq 0.01$. *ApoE* Apolipoprotein E; *MMSE* Mini-Mental State Examination.

Main effects

In Table 2 the results of the longitudinal analyses are shown 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. To test for non-linearity of the studied associations, 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 in which the associations with general cognitive performance (ln transformed MMSE score) and information processing speed (Coding task) were studied (see Table 2). If the quadratic term was not significant, the term was removed from the model.

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. In Table 2, only the strength and significance level of the linear and quadratic term of the ratio of lanosterol to cholesterol centered at the 10th percentile are shown. 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 to cholesterol absorption was significantly associated with lower general cognitive performance, memory performance and information processing speed. No significant interactions with time were found.

In the fully-adjusted models, only the (non-linear) 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 six years of follow-up independent of relevant confounders. Further analyses showed that this association was significant up to a ratio of lanosterol to cholesterol of 205.00 (ng/mg). In Figure 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.

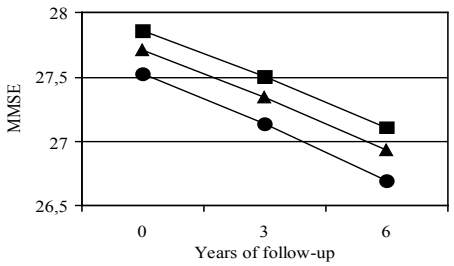

Figure 1 Six-year cognitive decline according to the ratio of lanosterol to cholesterol in total sample
Model adjusted for time, age, sex and education. Ratio of lanosterol to cholesterol: (●) perc. 90; (▲) perc. 50; (■) perc. 10. *MMSE* Mini-Mental State Examination.

Effect modification by ApoE e4

The results of the longitudinal analyses in which effect modification by ApoE was studied in the fully adjusted models, show a significant interaction (p = 0.054) 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 results show that the positive non-linear association between the ratio of lanosterol to cholesterol and the ln transformed MMSE score was significant only in ApoE e4 non-carriers (see Figure 2). Analyses show that this association was significant up to a ratio of 189.95 (ng/mg).

Table 2 Associations between sterols and cognitive performance over 6 years of follow-up in total study sample

	Ln transformed MMSE score [1]		Immediate recall		Information processing speed	
Models adjusted for time	B	p value	B	p value	B	p value
Precursors						
r_lath	0.060	.072	-0.091	.533	-0.60	.157
r_lano centered at p10	0.0018	.002	-0.0025	.140	-0.016	.039
r_lano x r_lano	-0.44×10^{-5}	.020	-	-	0.63×10^{-4}	.008
Plant sterols						
r_camp	-0.050	.028	0.20	.051	1.09	.000
r_sito	-0.072	.006	0.26	.021	1.25	.000
Cholesterol synthesis relative to absorption						
lath_camp	0.072	.000	-0.19	.019	-1.06	.000
lath_sito	0.070	.000	-0.17	.042	-1.01	.000
Fully adjusted models	B	p value	B	p value	B	p value
Precursors						
r_lath	0.016 [a]	.598	0.13 [b]	.313	-0.096 [c]	.783
r_lano centered at p10	0.0014 [d]	.008	0.00011 [e]	.937	-0.012 [d]	.066
r_lano x r_lano	-2.97×10^{-6}	.072	-	-	0.47×10^{-4}	.016
Plant sterols						
r_camp	.0058 [f]	.785	-0.16 [g]	.853	0.25 [d]	.313
r_sito	-0.008 [h]	.773	0.036 [i]	.723	0.34 [d]	.214
Cholesterol synthesis relative to absorption						
lath_camp	0.013 [j]	.438	0.034 [i]	.640	-0.32 [k]	.107
lath_sito	0.020 [l]	.232	0.028 [m]	.707	-0.31 [k]	.124

[1] direction of associations is reversed because of ln (natural log) transformation of the MMSE; *B* Unstandardized regression coefficient; [a] Adjusted for time, age, sex, education, diabetes mellitus, BMI, alcohol, and ApoE e4; [b] Adjusted for time, age, sex, education, diabetes mellitus, BMI, alcohol; [c] Adjusted for time, age, sex, education, diabetes mellitus, BMI, depressive symptoms, and ApoE e4; [d] Adjusted for time, age, sex, education; [e] Adjusted for time, age, sex, education, diabetes mellitus, hypertension, BMI, alcohol, smoking and ApoE e4; [f] Adjusted for time, age, education, diabetes mellitus, BMI, depressive symptoms; [g] Adjusted for time, age, sex, education, diabetes mellitus; [h] Adjusted for time, age, education, diabetes mellitus, BMI, depressive symptoms, alcohol, smoking and ApoE e4; [i] Adjusted for time, age, sex, education, diabetes mellitus, BMI, depressive symptoms, alcohol; [j] Adjusted for time, age, education, diabetes mellitus, BMI, alcohol and ApoE e4; [k] Adjusted for time, age, education; [l] Adjusted for time, age, education, diabetes mellitus; [m] Adjusted for time, age, sex, education, diabetes mellitus, BMI, depressive symptoms, alcohol, and smoking. *BMI* Body mass index; *ApoE* Apolipoprotein E; *MMSE* Mini-Mental State Examination; *r_lath* ratio of lathosterol to cholesterol (µg/mg); *r_lano* ratio of lanosterol to cholesterol (ng/mg); *r_camp* ratio of campesterol to cholesterol (µg/mg); *r_sito* ratio of sitosterol to cholesterol (µg/mg); *lath_camp* ratio of lathosterol to campesterol (mg/mg); *lath_sito* ratio of lathosterol to sitosterol (mg/mg); *p10* 10^{th} percentile.

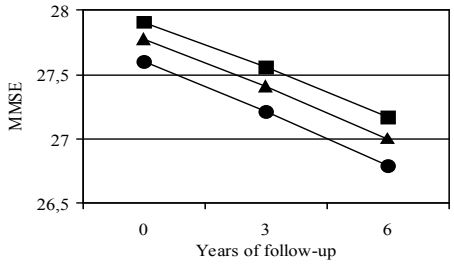

Figure 2 Six-year cognitive decline according to the ratio of lanosterol to cholesterol in e4 non-carriers
Model adjusted for time, age, sex and education. Ratio of lanosterol to cholesterol: (●) perc. 90; (▲) perc. 50; (■) perc. 10. *MMSE* Mini-Mental State Examination.

In addition, the negative non-linear association between the ratio lanosterol to cholesterol and information processing speed reached significance ($B_{\text{linear term centered at p10}}$ = -0.014; p = 0.044, $B_{\text{quadratic}}$ = 0.54 x 10^{-4}; p = 0.007) in persons not carrying the ApoE e4 allele, although no significant effect modification by ApoE e4 status was found. However, further analyses showed that only 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 (B = -0.29; p = 0.052). Also, a significant interaction between ApoE e4 and the ratio of campesterol (p = 0.007) and sitosterol (p = 0.014) to cholesterol and lathosterol to campesterol (p = 0.017) and sitosterol (p = 0.034) in the models predicting information processing speed was found. Only in non-carriers of the ApoE e4 allele, a significant association was found between a higher ratio of the plant sterols to cholesterol and higher information processing speed over six years of follow-up, independent of relevant confounders. Figure 3 shows the trajectory of information processing speed according to the ratio of the plant sterols to cholesterol in ApoE e4 non-carriers.

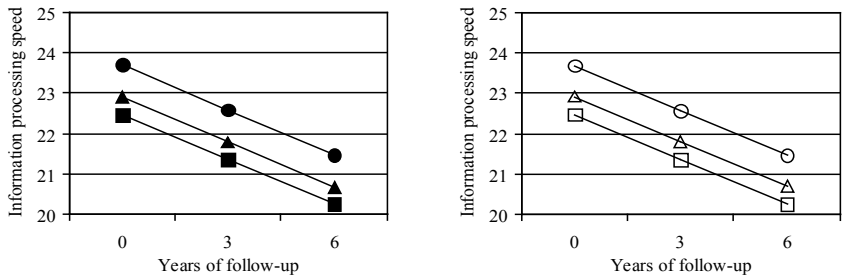

Figure 3 Six-year cognitive decline according to the ratio of plant sterols to cholesterol in e4 non-carriers
Models adjusted for time, age, sex and education. Ratio of campesterol to cholesterol: (●) perc. 90; (▲) perc. 50; (■) perc. 10. Ratio of sitosterol to cholesterol: (○) perc. 90; (Δ) perc. 50; (□) perc. 10.

In addition, a significant association between a higher ratio of lathosterol to the plant sterols and lower information processing speed was found, only in ApoE e4 non-carriers. 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 Figure 4.

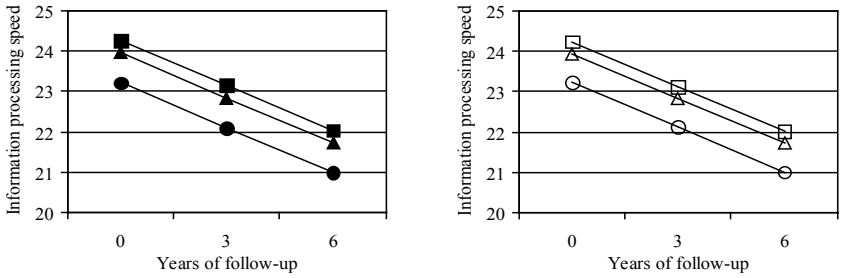

Figure 4 Six-year cognitive decline according to the rate of cholesterol synthesis relative to absorption in e4 non-carriers

Models adjusted for time, age and education. Ratio of lathosterol to campesterol: (●) perc. 90; (▲) perc. 50; (■) perc. 10. Ratio of lathosterol to sitosterol: (○) perc. 90; (Δ) perc. 50; (□) perc. 10.

Sensitivity analyses

In order to study whether the presence of cardiovascular disease at baseline or follow-up was a significant mediator of the associations studied, 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.

Discussion

This prospective population-based study focused on the associations between extracerebral markers of cholesterol synthesis, i.e. the ratios of cholesterol precursors lathosterol and lanosterol to cholesterol, and markers of cholesterol absorption, i.e. the ratios of plant sterols campesterol and sitosterol to cholesterol, and cognitive functioning over six years of follow-up in older persons. We showed a significant independent non-linear 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 ratio of lanosterol to cholesterol of 205.00 ng/mg. Furthermore, this association was significant in ApoE e4 non-carriers (up to 189.95 ng/mg), but not in ApoE e4 carriers. It was shown that ApoE e4 non-carriers with a ratio of lanosterol to cholesterol in the 10th percentile performed at approximately the same level of cognitive functioning after three years of follow-up as those with a ratio in the 90th percentile did at baseline. 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 non-carriers. Again, there was a three-year difference in level of functioning between ApoE e4 non-carriers with a ratio of in the 90th percentile and those in the 10th percentile, i.e. on average, persons with a ratio in the 90th percentile were functioning at approximately the same level of information processing speed after three years of follow-up as those with a ratio in the 10th percentile did at baseline. 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 non-carriers a higher rate of cholesterol synthesis relative to cholesterol absorption was shown to predict lower information processing speed over six years of follow-up. Again, the mean level of information processing speed after three years of follow-up in ApoE e4 non-carriers with a rate of cholesterol synthesis relative to absorption in the 10th percentile, was comparable to the mean level of information processing speed in persons with a rate in the 90th percentile at baseline.

No significant associations between the studied markers of cholesterol metabolism and a faster rate of cognitive decline were found.

Cholesterol synthesis

The present findings on cholesterol synthesis are inconsistent with the results from a previous population-based study examining the associations between cholesterol precursors and cognitive performance in a healthy aging sample (Teunissen et al., 2003). We did not observe significant associations between the ratio of lathosterol to cholesterol and cognitive functioning, while Teunissen et al. (2003) showed that a higher ratio of lathosterol to cholesterol at baseline was predictive of relatively lower cognitive functioning. Furthermore, while they found a significant linear association between a higher ratio of lanosterol to cholesterol at baseline and lower memory performance and executive functioning, the current study showed a non-linear association with lower general cognitive performance, but not memory performance. These inconsistent results may be due to differences in study sample. The present study sample was older (mean age of 75.0 versus 57.4 years) and had higher cholesterol (median levels of 231 mg/dL versus 155 mg/dL), lanosterol (median levels of 30.85 µg/dL versus 11.9 µg/dL) and lathosterol (median levels of 0.26 mg/dL versus 0.18 mg/dL) levels at baseline. Also, in the present study the non-linear association between a higher ratio of lanosterol to cholesterol and lower general cognitive performance was significant in ApoE e4 non-carriers but not in e4 carriers. Teunissen et al. (2003) did not study the modifying role of ApoE e4 status on these associations.

In a recent cross-sectional study it was shown that in persons with subjective memory impairment (mean age 57.5), but not in 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 post-mortem study it was concluded that the novo synthesis of cholesterol but not the cholesterol amount itself declines with aging in the hippocampus (Thelen et al., 2006). However, another recent post-mortem 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).

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 non-carriers, 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 ($p = 0.052$). Previously, in a study among healthy middle-aged men it has been 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, this potentially adverse effect of a higher ratio of plant sterols to cholesterol on cognition in ApoE e4 carriers only approached significance in the model predicting memory performance. The present findings may indicate different pathways in ApoE e4 carriers versus non-carriers. Also, it may be suggested 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.

Cholesterol synthesis versus absorption

The current findings did not show significant associations between the ratio of lathosterol and the plant sterols and cognitive performance in the total study sample, which is in contrast with the findings in 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 inconsistent result may be due to differences in characteristics between samples, such as age, general health and level of the studied markers. 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 non-carriers but not in e4 carriers. Again, it may be hypothesized that the negative effect of carrying the ApoE e4 allele prevails over the potential negative effect of rate of cholesterol synthesis and absorption on cognitive functioning.

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

Strengths

The major strengths of the present study are that the longitudinal associations between several markers for cholesterol homeostasis, ApoE phenotype and cognitive functioning and decline were studied. Cognitive performance was examined on three different cognitive tests most sensitive to age-related decline, on three occasions over six years of follow-up. In addition, these associations were studied in a large population-based study with data on 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 towards increased cholesterol absorption and reduced cholesterol synthesis is associated with increased cardiovascular risk (Weingärtner et al., 2010). 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 (i.e. heart disease, peripheral and cerebral artery 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 (strength of the) associations studied could not be fully excluded.

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 to those who participated in the three-year follow-up measurement. 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 to 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 reflect disease or a gradual decline in overall health (Abbott et al., 1997; Ferrara et al., 1997).

Another possible limitation of the present study is the lack of data on dietary intake in LASA. 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 reversed causality can not be fully dismissed. In LASA, a clinical dementia diagnosis based on formal criteria is not available. However, we identified persons showing persistent cognitive decline, defined as clinically relevant decline over three years of follow-up (> 2 SD below the mean) and continued decline over the subsequent three years (Van den Kommer et al., 2008). In the present study, only three persons were identified as persistent cognitive decliners at the time of blood sampling (baseline), reducing the risk of reverse causality.

Conclusion

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. Furthermore, this association was significant in ApoE e4 non-carriers, not in ApoE e4 carriers. In addition, higher cholesterol absorption was significantly predictive of faster information processing speed, only in ApoE e4 non-carriers. Finally, only in ApoE e4 non-carriers, a higher rate of cholesterol synthesis relative to cholesterol absorption was significantly predictive of lower information processing speed. Overall, the current findings showed a three-year difference in level of cognitive functioning between ApoE e4 non-carriers with sterol levels in the 10^{th} percentile versus those with levels in the 90^{th} percentile. Future research should focus on the role of (disturbed) cholesterol homeostasis and effect modification by ApoE e4 status with respect to dementia and cognitive decline.

Acknowledgements

The Longitudinal Aging Study Amsterdam is funded largely by the Dutch Ministry of Health, Welfare and Sports, and the Vrije Universiteit.

Disclosure statement

No actual or potential conflicts of interest.

References

Abbott, R.D., Sharp, D.S., Burchfiel, C.M., Curb, J.D., Rodriguez, B.L., Hakim, A.A., Yano, K., 1997. Cross-sectional and longitudinal changes in total and high-density-lipoprotein cholesterol levels over a 20-year period in elderly men: the Honolulu Heart Program. Ann. Epidemiol. 7, 417-424.

Anstey, K.J., Lipnicki, D.M., Low, L.F., 2008. Cholesterol as a risk factor for dementia and cognitive decline: A systematic review of prospective studies with meta-analysis. Am. J. Geriatr. Psychiatry 16, 343-354.

Assmann, G., Cullen, P., Erbey, J.R., Ramey, D.R., Kannenberg, F., Schulte, H., 2003. Elevation in plasma sitosterol concentration is associated with an increased risk for coronary events in the PROCAM study. Circulation 108, 730-730.

Beekman, A.T.F., Deeg, D.J.H., van Limbeek, J., Braam, A.W., de Vries, M.Z., van Tilburg, W., 1997. Criterion validity of the Center for Epidemiologic Studies Depression scale (CES-D): results from a community-based sample of older subjects in the Netherlands. Psychol. Med. 27, 231-235.

Bremmer, M.A., Hoogendijk, W.J.G., Deeg, D.J.H., Schoevers, R.A., Schalk, B.W.M., Beekman, A.T.F., 2006. Depression in older age is a risk factor for first ischemic cardiac events. Am. J. Geriatr. Psychiatry 14, 523-530.

Deeg, D.J., van Tilburg, T., Smit, J.H., de Leeuw, E.D., 2002. Attrition in the Longitudinal Aging Study Amsterdam. The effect of differential inclusion in side studies. J. Clin. Epidemiol. 55, 319-328.

Dik, M.G., Jonker, C., Bouter, L.M., Geerlings, M.I., van Kamp, G.J., Deeg, D.J.H., 2000. APOE-epsilon 4 is associated with memory decline in cognitively impaired elderly. Neurology 54, 1492-1497.

Dullemeijer, C., Durga, J., Brouwer, I.A., van de Rest, O.V., Kok, F.J., Brummer, R.J.M., van Boxtel, M.P.J., Verhoef, P., 2007. N 3 Fatty acid proportions in plasma and cognitive performance in older adults. Am. J. Clin. Nutr. 86, 1479-1485.

Fassbender, K., Lütjohann, D., Dik, M.G., Bremmer, M., Konig, J., Walter, S., Liu, Y., Letièmbre, M., von Bergmann, K., Jonker, C., 2008. Moderately elevated plant sterol levels are associated with reduced cardiovascular risk - The LASA study. Atherosclerosis 196, 283-288.

Ferrara, A., Barrett-Connor, E., Shan, J., 1997. Total, LDL, and HDL cholesterol decrease with age in older men and women. The Rancho Bernardo Study 1984-1994. Circulation 96, 37-43.

Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. Mini-Mental State. Practical method for grading cognitive state of patients for clinician. J. Psychiatr. Res. 12, 189-198.

Fricke, C.B., Schrøder, M., Poulsen, M., von Bergmann, K., Wester, I., Knudsen, I., Mortensen, A., Lütjohann, D., 2007. Increased plant sterol and stanol levels in brain of Watanabe rabbits fed rapeseed oil derived plant sterol or stanol esters. Br. J. Nutr. 98, 890-899.

Grundy, S.M., 1991. Multifactorial etiology of hypercholesterolemia. Implications for prevention of coronary heart disease. Arterioscler. Thromb. 11, 1619-1635.

Gylling, H., Strandberg, T., Tilvis, R., Miettinen, T.A., 1994. Regulation of serum cholesterol level in middle-aged and elderly men. Relation of cholesterol absorption and synthesis to lipoprotein metabolism. Arterioscler. Thromb. Vasc. Biol. 14, 694-700.

Hascalovici, J.R., Vaya, J., Khatib, S., Holcroft, C.A., Zukor, H., Song, W., Arvanitakis, Z., Bennett, D.A., Schipper, H.M., 2009. Brain sterol dysregulation in sporadic AD and MCI: relationship to heme oxygenase-1. J. Neurochem. 110, 1241-1253.

Havekes, L.M., de Knijff, P., Beisiegel, U., Havinga, J., Smit, M., Klasen, E., 1987. A rapid micromethod for apolipoprotein E phenotyping directly in serum. J. Lipid Res. 28, 455-463.

Helzner, E.P., Luchsinger, J.A., Scarmeas, N., Cosentino, S., Brickman, A.M., Glymour, M.M., Stern, Y., 2009. Contribution of vascular risk factors to the progression in Alzheimer disease. Arch. Neurol. 66, 343-348.

Heverin, M., Bogdanovic, N., Lütjohann, D., Bayer, T., Pikuleva, I., Bretillon, L., Diczfalusy, U., Winblad, B., Björkhem, I., 2004. Changes in the levels of cerebral and extracerebral sterols in the brain of patients with Alzheimer's disease. J. Lipid Res. 45, 186-193.

Jansen, P.J., Lütjohann, D., Abildayeva, K., Vanmierlo, T., Plösch, T., Plat, J., von Bergmann, K., Groen, A.K., Ramaekers, F.C.S., Kuipers, F., Mulder, M., 2006. Dietary plant sterols accumulate in the brain. Biochim. Biophys Acta 1761, 445-453.

Jolles, J., Verhey, F.R.J., Riedel, W.J., Houx, P.J., 1995. Cognitive impairment in elderly people. Predisposing factors and implications for experimental drug studies. Drugs Aging 7, 459-479.

Kölsch, H., Heun, R., Kerksiek, A., von Bergmann, K., Maier, W., Lütjohann, D., 2004. Altered levels of plasma 24S-and 27-hydroxycholesterol in demented patients. Neurosci. Lett. 368, 303-308.

Kriegsman, D.M.W, Penninx, B.W.J.H., Eijk Van, J., Boeke, A.J.P., Deeg, D.J.H., 1996. Self-reports and general practitioner information on the presence of chronic diseases in community dwelling elderly. A study on the accuracy of patients' self-reports and on determinations of inaccuracy. J. Clin. Epidemiol. 49, 1407-1417.

Law, M., 2000. Plant sterol and stanol margarines and health. BMJ. 320, 861-864.

Lütjohann, D., Brzezinka, A., Barth, E., Abramowski, D., Staufenbiel, M., von Bergmann, K., Beyreuther, K., Multhaup, G., Bayer, T.A., 2002. Profile of cholesterol-related sterols in aged amyloid precursor protein transgenic mouse brain. J. Lipid Res. 43, 1078-1085.

Miettinen, T.A., Tilvis, R.S., Kesäniemi, Y.A., 1990. Serum plant sterols and cholesterol precursors reflect cholesterol absorption and synthesis in volunteers of a randomly selected male population. Am. J. Epidemiol. 131, 20-31.

Moller, J.T., Cluitmans, P., Rasmussen, L.S., Houx, P., Rasmussen, H., Canet, J., Rabbitt, P., Jolles, J., Larsen, K., Hanning, C.D., Langeron, O., Johnson, T., Lauven, P.M., Kristensen, P.A., Biedler, A., van Beem, H., Fraidakis, O., Silverstein, J.H., Beneken, J.E.W., Gravenstein, J.S., 1998. Long-term postoperative cognitive dysfunction in the elderly: ISPOCD1 study. ISPOCD investigators. International Study of Post-Operative Cognitive Dysfunction. Lancet. 351, 857-861.

Nissinen, M.J., Gylling, H., Miettinen, T.A., 2008. Effects of dietary cholesterol and fat on serum non-cholesterol sterols according to different apolipoprotein E subgroups among healthy men. Br. J. Nutr. 100, 373-379.

Piccinin, A.M., Rabbitt, P.M., 1999. Contribution of cognitive abilities to performance and improvement on a substitution coding task. Psychol. Aging 14, 539-551.

Pitas, R.E., Boyles, J.K., Lee, S.H., Hui, D., Weisgraber, K.H., 1987. Lipoproteins and their receptors in the central nervous system. Characterization of the Lipoproteins in cerebrospinal fluid and identification of Apolipoprotein B,E(LDL) receptors in the brain. J. Biol. Chem. 262, 14352-14360.

Plat, J., Mensink, R.P., 2001. Effects of plant sterols and stanols on lipid metabolism and cardiovascular risk. Nutr. Metab. Cardiovasc. Dis. 11, 31-40.

Poirier, J., 1996. Apolipoprotein E in the brain and its role in Alzheimer's disease. J. Psychiatry Neurosci. 21, 128-134.

Radloff, L.S., 1977. The CES-D scale: a self-report depression scale for research in the general population. Appl. Psychol. Meas 1, 385-401.

Reinhard, O., Rood-Bakker, D., 1998. Alcoholgebruik in beeld. Standaardmeetlat. Nederlands Economisch Instituut, Rotterdam (in Dutch).

Rey, A., 1964. L'examen clinique en psychologie. Presses Universitaire de France, Paris, France.

Schiepers, O.J.G., de Groot, R.H.M., van Boxtel, M.P.J., Jolles, J., de Jong, A., Lütjohann, D., Plat, J., Mensink, R.P., 2009. Consuming functional foods enriched with plant sterol or stanol esters for 85 weeks does not affect neurocognitive functioning or mood in statin-treated hypercholesterolemic individuals. J. Nutr. 139, 1368-1373.

Silbernagel, G., Fauler, G., Renner, W., Landl, E.M., Hoffmann, M.M., Winkelmann, B.R., Boehm, B.O., März, W., 2009. The relationships of cholesterol metabolism and plasma plant sterols with the severity of coronary artery disease. J. Lipid Res. 50, 334-341.

Solomon, A., Kåreholt, I., Ngandu, T., Winblad, B., Nissinen, A., Tuomilehto, J., Soininen, H., Kivipelto, M., 2007. Serum cholesterol changes after midlife and late-life cognition: twenty-one-year follow-up study. Neurology 68, 751-756.

Solomon, A., Leoni, V., Kivipelto, M., Besga, A., Oksengård, A.R., Julin, P., Svensson, L., Wahlund, L.O., Andreasen, N., Winblad, B., Soininen, H., Björkhem, I., 2009. Plasma levels of 24S-hydroxycholesterol reflect brain volumes in patients without objective cognitive impairment but not in those with Alzheimer's disease. Neurosc. Lett. 462, 89-93.

Stampfer, M.J. 2006. Cardiovascular disease and Alzheimer's disease: common links. J. Intern. Med. 260, 211-223.

Sudhop, T., Lütjohann, D., Kodal, A., Igel, M., Tribble, D.L., Shah, S., Perevozskaya, I., von Bergmann, K., 2002. Inhibition of intestinal cholesterol absorption by ezetimibe in humans. Circulation 106, 1943-1948.

Teunissen, C.E., de Vente, J., von Bergmann, K., Bosma, H., van Boxtel, M.P., de Bruijn, C., Jolles, J., Steinbusch, H.W., Lütjohann, D., 2003. Serum cholesterol, precursors and metabolites and cognitive performance in an aging population. Neurobiol. Aging 24, 147-155.

Thelen, K.M., Falkai, P., Bayer, T.A., Lütjohann, D., 2006. Cholesterol synthesis rate in human hippocampus declines with aging. Neurosci. Lett. 403, 15-19.

Van de Rest, O., Spiro, A., Krall-Kaye, E., Geleijnse, J.M., de Groot, L.C.P.G., Tucker, K.L., 2009. Intakes of (n-3) fatty acids and fatty fish are not associated with cognitive performance and 6-year cognitive change in men participating in the Veterans Affairs Normative Aging Study. J. Nutr. 139, 2329-2336.

Van den Heuvel N, Smits C.H.M., Deeg, D.J.H., Beekman, A.T.F., 1996. Personality: a moderator of the relation between cognitive functioning and depression in adults aged 55-85? J. Affect Disord. 41, 229-240.

Van den Kommer, T.N., Comijs, H.C., Dik, M.G., Jonker, C., Deeg, D.J.H., 2008. Development of classification models for early identification of persons at risk for persistent cognitive decline. J. Neurol. 255, 1486-1494.

Van den Kommer, T.N., Dik, M.G., Comijs, H.C., Fassbender, K., Lütjohann, D., Jonker, C., 2009. Total cholesterol and oxysterols: early markers for cognitive decline in elderly? Neurobiol. Aging 30, 534-545.

Van Gelder, B.M., Tijhuis, M., Kalmijn, S., Kromhout, D., 2007. Fish consumption, n-3 fatty acids, and subsequent 5-y cognitive decline in elderly men: the Zutphen Elderly Study. Am. J. Clin. Nutr. 85, 1142-1147.

Weingärtner, O, Lütjohann, D., Böhm M, Laufs U., 2010. Relationship between cholesterol synthesis and intestinal absorption is associated with cardiovascular risk. Atherosclerosis 210, 362-365.

Wilund, K.R., Yu, L.Q., Xu, F., Vega, G.L., Grundy, S.M., Cohen, J.C., Hobbs, H.H., 2004. No association between plasma levels of plant sterols and atherosclerosis in mice and men. Arterioscler. Thromb. Vasc. Biol. 24, 2326-2332.