Serum cholesterol, precursors and metabolites and cognitive performance in an aging population

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Abstract
The present study investigated if a causal relation exists between serum concentrations of precursors and metabolites of cholesterol and cognitive performance in a healthy aging population.

Cognitive function addressing four domains of 144 individuals (30–80 years) was tested at baseline and after 6 years of follow-up. Serum concentrations of different sterols related to cholesterol were measured.

Serum levels of lathosterol and lanosterol correlated negatively with cognitive performance on the Word Learning tests for verbal learning and memory. This was observed at baseline and follow-up and was independent of age, sex and educational level. Furthermore, the levels of lathosterol and lanosterol at baseline correlated with performance on the Stroop test and Word Learning tests over the 6-year follow-up period. Serum levels of 27-hydroxycholesterol and 24S-hydroxycholesterol showed inconsistent correlations, while cholesterol, desmosterol, sitosterol and campesterol were not related to cognitive performance.

Thus, relative high serum ratios of the cholesterol precursors lanosterol and lathosterol, indicative for a high rate of endogenous cholesterol synthesis, are associated with relatively low memory performance in this aging population.

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1. Introduction
Cholesterol is an important lipid constituent of all cellular membranes and myelin. The brain contains about one quarter of the total unesterified cholesterol in human subjects, while this organ constitutes only 2% of the total body mass. Brain cholesterol is mainly synthesized locally and only negligible amounts (<1%) of circulatory cholesterol takes part in brain cholesterol metabolism as its transfer is restricted by the blood–brain barrier (BBB) [16]. Within recent years the interest in the description of the cholesterol metabolism and homeostasis in the human brain has increased [9].

Research on serum cholesterol levels in pathological cognitive aging, such as Alzheimer’s disease (AD), did not yield consistent results. In some studies decreased serum high-density lipoprotein (HDL)–cholesterol concentration in patients with AD were observed compared to controls, whereas others observed increased total and LDL–cholesterol in AD patients [6,20,25,30]. Very recently, it was reported that people with high serum cholesterol concentrations had a significant higher risk for development of mild cognitive impairment, as defined by criteria from the Mayo Clinic Alzheimer’s Disease Research Center [34], in late life [18]. There are several other indications for an involvement of altered cholesterol homeostasis in cognitive deterioration, e.g. Alzheimer’s or vascular dementia. (1) Carriers of at least one of the apolipoprotein E (ApoE)4 allele are at increased risk for the incidence of AD at an early age [23,36]. ApoE is involved in intracellular cholesterol transport in the human body and probably also in the brain [9]. The presence of at least one ApoE4 allele is associated with higher circulatory cholesterol concentrations compared to E2 or E3 alleles. Thus, a hypothetical relationship between ApoE-dependent cholesterol homeostasis and AD seems plausible. (2) Several studies showed an association of the ApoE4 allele with...
lower cognitive performance in the normal aging population [2,32,35]. (3) Lower plasma concentration of the oxidized cholesterol 24S-hydroxycholesterol (24S-OH-Chol) has been found in the late stage of AD patients and patients with severe head trauma [8,28]. Plasma 24S-OH-Chol in humans originates exclusively from the brain [5,24]. It has been shown that hydroxylation by a specific 24S-hydroxylase may be an important mechanism for cholesterol removal out of the brain [4], in addition to ApoE-mediated mechanisms [9]. (4) Recent epidemiological retrospective studies indicate that treatment of individuals with drugs that lower cholesterol synthesis in the whole body, mainly in the liver, (3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors or statins) was associated with decreased prevalence of AD [41]. Similarly, individuals of 50 years and older who were prescribed statins had a substantially lower risk of developing dementia, independent of the presence or absence of untreated hyperlipidemia [14]. Finally, there is increasing evidence indicating an important link between cholesterol, β-amyloid (Aβ) and AD [10,17,19,29,31].

These observations lead to the hypothesis that high concentrations of cholesterol are associated with cognitive deterioration. The aim of the present study was to investigate if a possible causal relationship exists between serum markers for whole body cholesterol metabolism and cognitive performance in a healthy aging population. Cholesterol homeostasis is a balance between absorption, de novo synthesis, metabolism, and excretion [13]. Therefore, we investigated the serum levels of cholesterol, the cholesterol precursors lanosterol, lanosterol and desmosterol, the oxidized cholesterol metabolites 24S-OH-Chol and 27-OH-Chol (Fig. 1), and the plant sterols campesterol and sitosterol in a healthy aging population. Cognitive performance was investigated addressing domains as information processing speed and memory. The individual serum levels were related to each individual cognitive performance at baseline, after 6 years of follow-up. Finally, baseline sterol levels were correlated to cognitive performance over the whole 6 years follow-up period to investigate if sterol levels may be related to cognitive decline in time.

2. Methods

2.1. Individuals

Participants in this study were recruited from a larger research program investigating determinants of cognitive aging in the healthy population: The Maastricht Aging Study (MAAS) [15,39]. Individuals (n = 1823) for this study were randomly drawn from the Registration Network Family Practices, a research database that contains basic health information of patients in primary care facilities (Metsemakers, Hoppener, Knottnerus, Kocken & Limonard, 1992). Exclusion criteria were clinical evidence of past or present morbidity that can compromise cognitive performance including cerebrovascular disease, chronic neurological pathology, mental retardation or chronic psychotropic drug use. The sample was stratified for age (12 discontinuous groups; 30 ± 1, 35 ± 1, . . . , 80 ± 1 years), sex and two levels of occupational achievement as an indicator of intellectual ability [15]. Individuals aged >50 years at the beginning of the study (baseline) were re-examined every third year over a period of 6 years. Individuals aged between 30 and 50 years at baseline were re-examined after 6 years. A serum sample was drawn at the beginning and the end of the study and stored at −80 °C until analysis.

For this study, data of three cognitive performance measurements over 6 years of follow-up were obtained from a random group of 144 individuals. A complete set of serum
samples and cognitive data was available from 92 individuals at baseline and 116 individuals after 6 years of follow-up, while of a subgroup of 65 individuals these data were available at both time points. An incomplete data set was available from the other individuals due to logistic problems.

The mean age of the 92 individuals at baseline was 57 (S.D. = 11). The sex distribution was 55 men to 37 women. The age of the 65 individuals of whom a complete set of serum data was present was lower (mean = 54 years, S.D. = 10) compared to the remaining 27 individuals (mean = 65 years, S.D. = 11, Mann–Whitney U-test: \( Z = -4.2, P < 0.001 \)). The sex distribution was not statistically different (37 men to 28 women in the 65 individuals, 18 men to 9 women in the remaining 27 individuals) (Chi-square, \( d.f. = 1, 0.75 < P < 1 \)).

Eight individuals used cholesterol-lowering drugs and the analyses were also redone without these individuals (see Section 3).

The study was in accordance with the principles of the declaration of Helsinki and the local medical ethics committee approved the study protocol. Written informed consent was obtained from all participants.

2.2. Cognitive assessment

Cognitive function was tested according to the MAAS-protocol described in detail elsewhere [15,39]. In the present study, we focussed on processes involving memory, attention and different aspects of information processing speed.

2.2.1. Word learning task (WLT)

This test is based upon the Auditory Verbal Learning Test [7] and evaluates the ability to acquire and retain new verbal information. Fifteen frequently used monosyllabic words are presented and the subject is instructed to memorize the words. The trial ends with a free recall of the words. This procedure is repeated five times, using the same word set in fixed order. The total number of correctly reproduced words on the five immediate recall trials is recorded (WLTTOT). After 20 min the subject is asked to reproduce the set of words (Delayed Recall). The total number of correctly reproduced words after 20 min is recorded [15]. A higher score on this test reflects better performance.

2.2.2. Letter digit coding task (LDCT)

This paper-and-pencil test is a modified version of the Symbol Digit Modalities Test [33] and measures information processing speed. The subject is requested to copy numbers in cells that are indexed by a letter. The letter refers to nine letter-number combinations at the top of the form. The total number of correctly copied corresponding numbers in 60 s is recorded as test outcome. Thus, better performance is associated with a higher score on this test.

2.2.3. Stroop color–word test (Stroop)

This perceptual interference test consists of three subtasks. Each subtask consists of a test sheet containing four rows and 10 columns of color names or colored spots. The test examines the speed at which color names are read (subtask I) and the speed at which the color of spots is named (subtask II). Subtask III involves color names but the printing ink is different from the color name. The time needed to name the color of the printing ink of the words is recorded.

Thus, a better cognitive performance is associated with a lower score on the Stroop test. Only the data of subtask III are used in the current study and are referred to as ‘Stroop’.

2.3. Sterol analysis by gas chromatography flame-ionization and mass selective detection

Sterols and oxysterols were extracted from serum by cyclohexane after saponification. Fifty microgram 5α-cholestan (Sigma), 1 μg epicoprostanol (Sigma) and 200 ng racemic [23,23,24,25-2H4]24-cholesterol and [2H2]25S-27-OH-Chol were added as internal standards. The solvents were evaporated and the hydroxy groups of the sterols and oxysterols were trimethylsilylated. The trimethylsilyl (TMSi)-ether of cholesterol was separated on an HP1 (Methyl Siloxane, crosslinked) fused silica capillary column (10 m × 0.1 mm i.d., 0.4 μm phase thickness, Hewlett-Packard (HP), Böblingen, Germany). Gas chromatography flame ionization was performed with a HP6890 gas-chromatograph. The oven temperature was initially kept at 150 °C for 1.5 min, then increased at 59 °C/min to a final temperature of 290 °C for 8 min. An aliquot of 1.0 μl was injected by an automated injector (HP) in a pulsed splitless mode at 280 °C. Hydrogen was used as carrier gas with an initial flow of 1.0 ml/min and flame ionization detection was performed at 280 °C with a constant column and make-up flow mode. The concentration of cholesterol was calculated from the ratio of the peak area of cholesterol to the area of 5α-cholestan multiplied by the amount of internal standard (50μg) added to a defined serum volume. Gas chromatography–mass spectrometry–selected ion-monitoring (GC–MS–SIM) analysis for quantification of epicoprostanol, lathosterol, desmosterol, lanosterol, campesterol, stigmasterol, 24S-OH-Chol, and 27-OH-Chol and the deuterated internal oxysterol standards was performed on a DB-XLB column (30 m × 0.25 mm i.d. × 0.25 μm film thickness, J&W Scientific; Alltech) using an HP5890 Series.
II plus gas-chromatograph combined with an HP5972 mass selective detector. An aliquot of 1.0μl was injected by automated injection in a splitless mode at an injection temperature of 280°C. Helium was used as carrier gas with a column gas-flow of 1.0 ml/min. The initial oven temperature was kept at 150°C for 1 min, thereafter increased at a rate of 30°C/min to 290°C and kept for 20.33 min. TMS-ether of epicoeprostanol was measured at m/z 370 (M+—OTMSi), lathosterol at m/z 458 (M+), desmosterol at m/z 456 (M+), lanosterol m/z 393 (M+—CH3—OTMSi), campesterol at m/z 472 (M+), sitosterol at m/z 488 (M+), authentic and deuterated 24S-OH-Chol were measured at m/z 413 (M+—OTMSi—CH2CH3) and m/z 416 (M+—OTMSi—CH(CD3)2), respectively, and authentic and deuterated 27-OH-Chol at m/z 456 (M+—OTMSi—CH2CH3) and 461 (M+—OTMSi), respectively, after electron impact ionization at 70eV. The temperature of the transfer line was kept at 280°C.

2.4. Statistical analysis

Zero-order bivariate correlation analysis was performed using Spearman’s correlation coefficient. Normality of the distribution was tested using Kolmogorov–Smirnov test. Our further analysis was performed in two phases. First, ordinary least squares multiple regression analysis was performed for the four cognitive tests at baseline and each separate serum sterol as potential predictor, with age, sex and level of education as covariates. All variables were treated as continuous variables, except sex (categorical). Second, for the markers showing a significant relation (P < 0.05) with at least two of the cognitive tests, adjusted for age, sex and educational level, an additional multi-level regression analysis was performed. Multi-level repeated measurement analysis was carried out to analyze the association between the steroids and cognitive function during the whole follow-up period, i.e. to investigate an eventual risk modulating role of serum sterol concentrations. This method uses all observations, including the observations of persons with only one observation [1,12]. To increase homogeneity regarding age across the different phases, the analyses were re-done for persons older than 50 years of age only. As this study evaluates results from four cognitive tests, a Bonferroni adjustment of the significance to P < 0.013 was considered. However, as this study was primary exploratory in nature, data with a significance level of P < 0.05 are also shown.

3. Results

The absolute sterol concentrations and the sterol to cholesterol ratios (RSterol) in our aging population at baseline and after 6 years of follow-up are summarized in Tables 1 and 2. The values of the cholesterol levels were comparable to levels in the normal population [38]. The 24S-OH-Chol concentration (range: 68–74 ng/ml) in all individuals was lower than reported previously (range: 77–105 ng/ml) [8]. The 27-OH-Chol concentration was similar to the concentration observed in previous data obtained in the laboratory in Bonn (mean = 146 ± 43 ng/ml; range: 73–358 ng/ml) for a group of 50 men and 50 females aged 30–79 years.

3.1. Cross-sectional correlation analysis at baseline and after 6 years of follow-up

The results from the cross-sectional correlation analysis between the cholesterol precursors, metabolites and plant sterols and cognitive test outcomes both at baseline and at follow-up are shown in Table 3. The data in Table 3 show that the cholesterol concentration did not correlate with any of the cognitive tests.

3.1.1. Cholesterol precursors

The RSterol showed a negative correlation with the NLTTOT and Delayed Recall test for the whole group at baseline, while a relation with the Delayed Recall only was observed at follow-up (Table 3). No significant relation was present between the RLathosterol and the cognitive outcomes on the Stroop of DCT. The same negative correlation between lathosterol and the NLTTOT and Delayed Recall test was observed when the individuals older than 50 were

Table 1 Absolute sterol concentrations and sterol to cholesterol ratios in serum, all individuals

<table>
<thead>
<tr>
<th>Unit Absolute values</th>
<th>Unit Sterol to cholesterol ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline, 1993</td>
</tr>
<tr>
<td>Cholesterol mg/dl</td>
<td>155 (122, 185)</td>
</tr>
<tr>
<td>Lathosterol mg/dl</td>
<td>0.18 (0.14, 0.25)</td>
</tr>
<tr>
<td>Desmosterol μg/ml</td>
<td>101 (71, 126)</td>
</tr>
<tr>
<td>Lanosterol μg/ml</td>
<td>11.9 (10.1, 14.4)</td>
</tr>
<tr>
<td>24S-OH-Chol ng/ml</td>
<td>69.5 (52.3, 88.8)</td>
</tr>
<tr>
<td>27-OH-Chol ng/ml</td>
<td>161 (133, 203)</td>
</tr>
<tr>
<td>Campesterol μg/ml</td>
<td>0.32 (0.22, 0.46)</td>
</tr>
<tr>
<td>Sitosterol μg/ml</td>
<td>0.27 (0.21, 0.40)</td>
</tr>
</tbody>
</table>

Data are expressed as median (25 and 75% percentile). Mean age was 57.4 and S.D. 11.6 (range: 29.9–81.3).
Table 2
Median (25, 75% percentile) for sterol concentrations and sterol to cholesterol ratio in serum at baseline and at follow-up, individuals over 50 years only

<table>
<thead>
<tr>
<th>Sterol</th>
<th>Unit</th>
<th>Baseline, 1993 (n = 77)</th>
<th>Follow-up, 1999 (n = 101)</th>
<th>Sterol to cholesterol ratio (mg/dl)</th>
<th>Baseline, 1993 (n = 77)</th>
<th>Follow-up, 1999 (n = 101)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesteryl</td>
<td>mg/dl</td>
<td>169 (135, 196)</td>
<td>215 (176, 239)</td>
<td>Lanosterol (mg/mg)</td>
<td>108 (73, 134)</td>
<td>95 (77, 124)</td>
</tr>
<tr>
<td>Lathosterol</td>
<td>pg/ml</td>
<td>198 (134, 253)</td>
<td>138 (95, 196)</td>
<td>27-OH-Chol (ng/ml)</td>
<td>72.7 (65.2, 87.1)</td>
<td>68.6 (52.5, 79.1)</td>
</tr>
<tr>
<td>Sitosterol</td>
<td>mg/dl</td>
<td>0.27 (0.20, 0.40)</td>
<td>0.31 (0.22, 0.42)</td>
<td>24-S-Hydroxy-Chol (ng/ml)</td>
<td>38.3 (32.8, 47.1)</td>
<td>35.0 (27.2, 41.5)</td>
</tr>
<tr>
<td>Campesterol</td>
<td>mg/dl</td>
<td>1.08 (0.44, 1.54)</td>
<td>1.04 (0.28, 1.51)</td>
<td>27-OH-Chol (ng/ml)</td>
<td>108 (73, 134)</td>
<td>95 (77, 124)</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>mg/dl</td>
<td>1.92 (1.30, 2.85)</td>
<td>1.73 (1.21, 2.53)</td>
<td>24-S-Hydroxy-Chol (ng/ml)</td>
<td>1.54 (1.19, 2.46)</td>
<td>1.49 (1.06, 2.06)</td>
</tr>
</tbody>
</table>

Data are expressed as median (25, 75% percentile). Mean age was 60.7 ± 9.7 (range: 49.6–81.3).

Table 3
Zero-order correlation of sterol to cholesterol ratio (R_s) with cognitive performance in all individuals

<table>
<thead>
<tr>
<th>Sterol</th>
<th>LDCT</th>
<th>WLTOT</th>
<th>Delayed Recall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± S.D. (range)</td>
<td>47.3 ± 10.4 (42.6 ± 9.3)</td>
<td>8.9 ± 3.0 (6.9 ± 3.0)</td>
<td></td>
</tr>
<tr>
<td>Cholesteryl (mg/dl)</td>
<td>0.15</td>
<td>−0.08</td>
<td>0.05</td>
</tr>
<tr>
<td>Lathosterol (mg/dl)</td>
<td>0.20</td>
<td>0.14</td>
<td>−0.23</td>
</tr>
<tr>
<td>Sitosterol (mg/dl)</td>
<td>0.11</td>
<td>0.03</td>
<td>−0.13</td>
</tr>
<tr>
<td>Campesterol (mg/dl)</td>
<td>0.16</td>
<td>−0.21</td>
<td>−0.29</td>
</tr>
<tr>
<td>Stigmasterol (mg/dl)</td>
<td>0.16</td>
<td>−0.20</td>
<td>−0.35</td>
</tr>
<tr>
<td>24-S-Hydroxy-Chol (mg/ml)</td>
<td>0.01</td>
<td>−0.01</td>
<td>−0.08</td>
</tr>
<tr>
<td>27-OH-Chol (mg/ml)</td>
<td>0.29</td>
<td>−0.20</td>
<td>−0.07</td>
</tr>
<tr>
<td>24-S-Hydroxy-Chol (ng/ml)</td>
<td>0.03</td>
<td>−0.13</td>
<td>−0.16</td>
</tr>
<tr>
<td>27-OH-Chol (ng/ml)</td>
<td>0.20</td>
<td>−0.20</td>
<td>−0.07</td>
</tr>
<tr>
<td>24-S-Hydroxy-Chol (ng/ml)</td>
<td>0.03</td>
<td>−0.13</td>
<td>−0.16</td>
</tr>
<tr>
<td>27-OH-Chol (ng/ml)</td>
<td>0.03</td>
<td>−0.13</td>
<td>−0.16</td>
</tr>
<tr>
<td>24-S-Hydroxy-Chol (ng/ml)</td>
<td>0.03</td>
<td>−0.13</td>
<td>−0.16</td>
</tr>
<tr>
<td>27-OH-Chol (ng/ml)</td>
<td>0.03</td>
<td>−0.13</td>
<td>−0.16</td>
</tr>
</tbody>
</table>

Data are expressed as Spearman correlation coefficients. Data are obtained from 92 individuals at baseline and 116 individuals at follow-up, of whom 65 individuals were included in both measurements.

Similar results were obtained for the subgroup of individuals over 50 years old (data not shown).

The R_Lanosterol correlated negatively with the WLTOT and Delayed Recall tests both at baseline and follow-up, while the relation between the R_Lanosterol and LDCT was significant only at follow-up (Table 3). Similar results were obtained when the individuals older than 50 years were selected only (data not shown). These results indicate a relatively better performance on these aspects of cognition when lanosterol concentrations are low, unadjusted for age, sex or education.

3.1.2. Oxysterols
A positive correlation was observed between the R_24S-OH-Chol and performance on the Stroop test at follow-up (Table 3). A negative correlation was present between the R_24S-OH-Chol and performance on the LDCT at follow-up. In the group of individuals older than 50 years similar relations were observed (data not shown).

The R_27-OH-Chol showed a negative correlation with the Delayed Recall at baseline only, indicating better cognitive performance when serum 27-OH-Chol levels are lower.

3.1.3. Plant sterols
A positive correlation was observed between serum R_Sitosterol and performance on the WLTOT and Delayed Recall test at baseline only (Table 3).

Similar, though weaker, results were obtained for the group of older individuals for both plant sterols (data not shown).

3.2. Cross-sectional correlation between cognitive performance and serum markers at baseline and follow-up adjusted for age, sex and education

To investigate whether the correlations observed in Table 3 were independent of age, sex and level of education, multiple regression analysis of the data obtained at baseline and at follow-up was performed (Table 4). Due to the similarity of
the results for the subgroup of people over 50 years old as compared with the whole population, these results are not discussed in further detail.

The serum cholesterol concentrations did not correlate with any of the cognitive test outcomes, adjusted for age, sex and education.

The R_Lathosterol correlated negatively with the WLT-TOT and the Delayed Recall test at baseline as well as at follow-up (Table 4). These results indicate a relatively better cognitive performance when lathosterol levels are lower, independent of age, sex and education.

No correlation between the R_Desmosterol at baseline and any cognitive test was observed.

A significant positive correlation between the R_Lanosterol and the Stroop test was observed at baseline only. The R_Lanosterol showed a negative correlation with the WLT-TOT and Delayed Recall test, adjusted for age, sex and education, at baseline and after 6 years of follow-up (Table 4). These results indicate a relatively better cognitive performance when lanosterol levels are lower.

The R_245-OH-Chol correlated positively with the Stroop test at baseline only. The R_27-OH-Chol showed a significant (negative) correlation with the WLT-TOT at baseline. A negative correlation was observed between R_27-OH-Chol and performance on the Delayed Recall test and on the LDCT at follow-up.

No significant relation between the plant sterols R_Campesterol and R_Sitosterol with any of the cognitive outcomes was observed after adjustment to age, sex and education.

The observed relations in this regression analysis remained unaffected after exclusion of the eight individuals that turned out to be influential cases and outliers, based on the studentized residuals and Cook’s distances. Eight other individuals were daily users of cholesterol-lowering drugs. The relations remained also largely unaffected after additional exclusion of the eight individuals who used cholesterol-lowering drugs at any time during this study.

3.3. Longitudinal correlation between cognitive performance and serum markers adjusted for age, sex and education over the whole 6-year follow-up period

We next investigated if a causal relation exists between serum sterol concentrations and cognitive decline in time. For this purpose, multi-level repeated measurement analysis was used [11]. Multi-level repeated measurement analysis
Lathosterol level at baseline correlated positively with cognitive performance and lanosterol levels at baseline correlated positively with the performance on the WLTTOT and Delayed Recall test over the whole follow-up period. These results indicate a better cognitive performance over 6 years when lanosterol levels are lower at baseline.

The relations between the levels of the R_{L}lathosterol and R_{L}lanosterol and the performance on the WLTTOT and Delayed Recall test over the whole follow-up period were related to all four test for cognitive functioning in the normal aging population. We investigated the steroid ratios to cholesterol of the cholesterol precursors lathosterol, lanosterol and desmosterol, the cholesterol metabolites 24S-OH-Chol and 27-OH-Chol and the plant sterols sitosterol and campesterol. First, the steroid ratios to cholesterol at baseline and after 6 years of follow-up were correlated with the individual cognitive performance at those time-points. As a final step, we studied the relation between the steroid levels at baseline and cognitive performance during the whole follow-up period, using multi-level regression analysis [11], to investigate whether the concentrations at baseline are indicative for cognitive performance over a longer period, or even for cognitive decline.

The results showed a negative correlation between the R_{L}lanosterol and R_{L}lathosterol and cognitive performance as measured with the WLTTOT and Delayed Recall at both time points. In addition, a relation was observed between the levels of these precursors at baseline and cognitive performance on these tests and the Stroop test over the whole follow-up period. These results indicate a better cognitive performance when serum levels of cholesterol precursors are low. No correlation between serum cholesterol concentrations and the performance of the cognitive tests was observed in our study. The 24S-OH-Chol and 27-OH-Chol levels showed variable correlations. These results do not support a relation between these oxidized cholesterol metabolites and cognitive performance in the normal aging population. The influence on the analysis of the individual with the most extreme precursor-concentrations was minimal, as shown by the regression coefficients in the multivariate regression analysis and multi-level regression analysis after exclusion of these cases. Since the precursors were related to all four tests for cognitive functioning in the multi-level regression analysis, we may conclude that these sterols are related to global cognitive performance. Interestingly, exclusion of users of cholesterol synthesis lowering drugs did not alter the relations observed. This unique study combines biological markers and behavioral data from a

### Table 5

<table>
<thead>
<tr>
<th>Individuals/observations</th>
<th>Cognitive outcome measurement</th>
<th>Stroop</th>
<th>LDCT</th>
<th>WLTTOT</th>
<th>Delayed Recall</th>
</tr>
</thead>
<tbody>
<tr>
<td>R_{L}lathosterol (mg/mg)</td>
<td>92/252</td>
<td>9184 (2351, 16017)</td>
<td>−2781 (−5578, −184)</td>
<td>−2655 (−5310, −79)</td>
<td>−981 (−1710, −252)</td>
</tr>
<tr>
<td>R_{L}lanosterol (mg/mg)</td>
<td>92/252</td>
<td>169 (52, 286)</td>
<td>−35 (−8046, 10)</td>
<td>−61 (−99, −22)</td>
<td>−19 (−31, −6)</td>
</tr>
</tbody>
</table>

Data are expressed as unstandardized regression coefficients (95% confidence interval) and adjusted for baseline age, sex, educational level, and longitudinal variations in interval of follow-up and increases in the power of the analysis (see second column of Table 5).

This relation was investigated for the markers that correlated consistently in time with at least two cognitive tests. The R_{L}lathosterol level at baseline correlated positively with the performance on the Stroop test and negatively with performance on the LDCT, WLTTOT and Delayed Recall test over the whole follow-up period. These results indicate a relatively better cognitive performance over a 6-year period when the R_{L}lathosterol level is lower at baseline.

The R_{L}lanosterol levels at baseline correlated positively with the Stroop test and negatively with performance on the WLTTOT and Delayed Recall test over 6 years of follow-up. These results indicate a relatively better cognitive performance over 6 years when lanosterol levels are lower at baseline.

The relations between the levels of the R_{L}lathosterol and R_{L}lanosterol and the performance on the WLTTOT and Delayed Recall remained unaffected after repeating these analysis with exclusion of the individuals which turned out to be influential cases and outliers. The relation between the precursor levels and the Stroop test observed with the multi-level analyses was absent after exclusion of the outliers, thus obtaining results in line with the data of Table 4. The relations remained also essentially unaffected after exclusion of the eight individuals using cholesterol lowering drugs. The results were similar in the group of all individuals as well as in the group of individuals older than 50 years (data not shown).

Due to the lack of inter-individual differences in cognitive decline over the follow-up period (see Table 5), the relation between serum data and differences in cognitive performance, for example the examination of risk modulating effects, could not be investigated further.
levels, as a possible indication for low cholesterol synthesis, 
tive performance associated with low cholesterol precursor 
of Alzheimer’s dementia [41]. In contrast to better cogni-
thesis, by statins was associated with decreased prevalence 
of lathosterol, the rate limiting enzyme of cholesterol syn-
it was shown that inhibition of 3-hydroxymethyl-3-glutaryl 
cursors may be a risk-factor for cognitive deterioration, as 
may speculate that higher serum levels of cholesterol pre-
conclusions addressing causality from our present data. We 
that inhibition of 3-hydroxymethyl-3-glutaryl 
deterioration or even dementia. Unfortunately, our data 
did not provide a clear association of cholesterol with 
mental tests.

To our knowledge, no research has been done so far on 
cholesterol precursors and cognition.

In conclusion, even though the low number and relatively 
short follow-up time warrant caution in interpretation, a cor-
relation between the cholesterol precursors lanosterol and 
lathosterol and cognitive performance was observed in this 
normal aging population. The results of this study encour-
greater investigations into a causal relation between 
cholesterol precursors and cognition.

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