Serum 24S-hydroxycholesterol and hippocampal size in middle-aged normal individuals

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Received 16 August 2007; received in revised form 5 October 2007; accepted 18 October 2007
Available online 3 December 2007

Abstract

The present study assessed the association between serum 24S-hydroxycholesterol (24S-OH-Chol) and 27-hydroxycholesterol (27-OH-Chol) and hippocampal volumes in 69 middle-aged cognitively normal individuals. Results showed that subjects with high levels of oxysterols had significantly larger hippocampal volumes than subjects with low levels of oxysterols. Multiple regression analysis revealed that 24S-OH-Chol, but not 27-OH-Chol or cholesterol, was able to significantly predict hippocampal size. Future studies should elucidate whether high brain cholesterol metabolism in the middle age is protective against hippocampal atrophy and cognitive decline.

Keywords: 24S-hydroxycholesterol; Cholesterol; Hippocampus; Aging

1. Introduction

Increasing evidence suggests that elevated cholesterol may be implicated in the pathophysiology of Alzheimer’s disease (AD). Epidemiologic studies have shown that elevated serum cholesterol may be a potential risk factor for clinically probable AD (Kivipelto et al., 2001; Notkola et al., 1998). Since almost all circulating 24S-hydroxycholesterol (24S-OH-Chol) originates from the brain (Björkhem et al., 1998), it has been hypothesized that peripheral levels of 24S-OH-Chol may reflect cholesterol turnover in the central nervous system (Lütjohann and von Bergmann, 2003). Accordingly, significantly higher cerebrospinal fluid (CSF) concentrations of 24S-OH-Chol were found in the early stages of AD compared with later stages (Papassotriopoulos et al., 2002), possibly reflecting increased release of membrane cholesterol during neurodegeneration.

Conversion of cholesterol to 24S-OH-Chol is mediated by cholesterol 24S-hydroxylase (CYP46), and is the major pathway for the elimination of brain cholesterol and the maintenance of brain cholesterol homeostasis (Björkhem et al., 1997; Lund et al., 1999; Lütjohann et al., 1996). 27-hydroxycholesterol (27-OH-Chol) is another important cholesterol degradation product on its way to bile acids and is formed in most cells. However, in contrast to 24S-OH-Chol, there is no net flux from the brain across the blood–brain barrier (Lütjohann et al., 1996). Recent research gives evidence that the absolute levels of 24S-OH-Chol and 27-OH-Chol in plasma and/or CSF, may be used as markers both for neurodegenerative diseases and for disturbances in the blood–brain barrier (Leoni et al., 2004).

Presently, the effect of brain cholesterol metabolism on hippocampal structure has received only limited systematic investigation. Hippocampal size reduction is a feature of AD (Gosche et al., 2002) and mild cognitive impairment (MCI) (Wolf et al., 2003), and was shown to predict AD in cognitively intact elderly people (Den Heijer et al., 2006). Low levels of high-density lipoprotein cholesterol (HDL-C) have been recently associated with reduced hippocampal size in non-demented elderly individuals (Wolf et al., 2004). A trend towards reduced 24S-OH-Chol in hippocampal specimens...
was found in elderly persons when compared with young persons (Thelen et al., 2006). In the present study, we set out to investigate whether brain cholesterol metabolism is associated with hippocampal volumes in cognitively normal middle-aged individuals.

### 2. Methods

#### 2.1. Subjects

Participants of the study (n = 69) were recruited by an advertisement in a local newspaper and leaflets distributed in the hospital of the University of Göttingen and in town (Table 1). Only subjects without a history of any neurological or any psychiatric (as assessed with the Structured Clinical Interview for DSM-IV) (Wittchen et al., 1997) disorder were studied. Subjects with MCI or neuropsychological test results or any psychiatric (as assessed with the Structured Clinical Interview for DSM-IV) (Wittchen et al., 1997) disorder were excluded. After a complete description of the study was given to the subjects, written informed consent was obtained. The study design was approved by the Ethical Committee of the Medical Faculty of the University of Göttingen.

#### 2.2. Sterol analysis

Serum concentrations of oxidized cholesterol metabolites (24S-OH-Chol and 27-OH-Chol) were analyzed in all subjects using an isotope dilution method and quantified by selected ion monitoring gas chromatography–mass spectrometry (Teunissen et al., 2003). We measured 25R (27)-OH-Chol. For the determination of authentic 24S-OH-Chol we used racemic 24R, 5-OH-Chol for the preparation of standard curves and racemic 24R, [2H4]-OH-Chol as internal standard.

Two groups of subjects with high or low levels of 24S-OH-Chol and 27-OH-Chol were formed. The values of 24S-OH-Chol and 27-OH-Chol were subjected to a median split, respectively. Subjects ranging in the upper quartiles of both metabolites formed the group with high OH-Chol (n = 22), and subjects ranging in the lower quartiles of both metabolites formed the group with low OH-Chol (n = 22) (cf. Table 1).

#### 2.3. MRI acquisition and analysis

All subjects received an MRI scan using a 1.5-T Philips Gyroscan machine using previously described techniques (Koschack and Irle, 2005). Volumetric analysis was done on the basis of 3D-MRIs. The images were transferred to a computer workstation and processed using the CURRY® software (version 4.5; Neurosoft, Inc., El Paso, Tex.). Images were reformatted into continuous 1 mm-thick slices.

Intracranial volume, total brain volume and ventricular volume were calculated with automated multistep algorithms and 3D region growing methods that are limited by gray value thresholds. The hippocampus was disarticulated from surrounding tissue on coronal slices by means of manual tracing according to a standardized protocol (Pruessner et al., 2000). Simultaneous three-dimensional visualization of brain structures and manual tracing allowed a precise identification and delineation of the borders of the hippocampus.

For defining the intrarater and interrater reliability (the raters were blind to OH-Chol groups), each one hemisphere of 10 randomly chosen cases were reassessed, respectively. The intraclass correlation coefficients for this procedure were $r = 0.95$ (intrarater) and $r = 0.96$ (interrater) for the hippocampus.

### Table 1

Demographic and clinical characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total sample (n = 69)</th>
<th>Subjects with low OH-Chol (n = 22)</th>
<th>Subjects with high OH-Chol (n = 22)</th>
<th>Difference between-subjects with low or high OH-Chol</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50 ± 13</td>
<td>51 ± 14</td>
<td>53 ± 11</td>
<td>$n(42) = -0.35$</td>
<td>0.728</td>
</tr>
<tr>
<td>Education (years)</td>
<td>14 ± 3</td>
<td>13 ± 2</td>
<td>13 ± 3</td>
<td>$n(42) = -0.31$</td>
<td>0.760</td>
</tr>
<tr>
<td>Sex (female: male)</td>
<td>37: 32</td>
<td>14: 8</td>
<td>10: 12</td>
<td>$\chi^2(1) = 1.47$</td>
<td>0.226</td>
</tr>
<tr>
<td>WAIS-R (Full scale IQ)</td>
<td>124 ± 15</td>
<td>122 ± 15</td>
<td>126 ± 13</td>
<td>$n(42) = -1.11$</td>
<td>0.274</td>
</tr>
<tr>
<td>WMS-R (General memory)</td>
<td>114 ± 14</td>
<td>115 ± 12</td>
<td>115 ± 12</td>
<td>$n(42) = -0.06$</td>
<td>0.956</td>
</tr>
<tr>
<td>24S-OH-Chol (ng/ml)</td>
<td>62 ± 19</td>
<td>47 ± 9</td>
<td>81 ± 20</td>
<td>$n(42) = -7.18$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>27-OH-Chol (ng/ml)</td>
<td>214 ± 58</td>
<td>169 ± 28</td>
<td>260 ± 53</td>
<td>$n(42) = -7.24$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>236 ± 39</td>
<td>215 ± 30</td>
<td>263 ± 34</td>
<td>$n(42) = -5.01$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index</td>
<td>26 ± 5</td>
<td>26 ± 5</td>
<td>27 ± 4</td>
<td>$n(42) = -0.59$</td>
<td>0.561</td>
</tr>
<tr>
<td>Arterial hypertension (yes:noa)</td>
<td>14: 55</td>
<td>7: 15</td>
<td>4: 18</td>
<td>$\chi^2(1) = 1.09$</td>
<td>0.296</td>
</tr>
<tr>
<td>Hyperlipidemia (yes:noa)</td>
<td>7: 62</td>
<td>4: 18</td>
<td>1: 21</td>
<td>$\chi^2(1) = 2.03$</td>
<td>0.154</td>
</tr>
<tr>
<td>Diabetes (yes:noa)</td>
<td>2: 67</td>
<td>2: 20</td>
<td>0: 22</td>
<td>$\chi^2(1) = 2.09$</td>
<td>0.148</td>
</tr>
<tr>
<td>ApoE e4 carrier state (yes:no)</td>
<td>18: 51</td>
<td>7: 15</td>
<td>3: 19</td>
<td>$\chi^2(1) = 2.07$</td>
<td>0.150</td>
</tr>
<tr>
<td>Family history of AD (yes:no)</td>
<td>6: 63</td>
<td>3: 19</td>
<td>1: 21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table values are given as mean ± S.D. unless indicated otherwise.

WAIS-R: Wechsler adult intelligence scale-revised. IQ estimates were derived from information, similarities, picture completion and block design scores; WMS-R: Wechsler memory scale-revised.

a Yes: currently on drugs; no: no drug treatment needed according to medical examination.

b Fisher’s exact test.
2.4. Statistical analysis

Differences between groups on demographic, clinical and cognitive variables and on total brain volume and intracranial volume were compared with t-tests or χ²-tests. Hippocampal volumes were compared by an overall 2 × 2 analysis of covariance (ANCOVA) with the between-subjects factor group (subjects with low OH-Chol, subjects with high OH-Chol) and the within-subjects factor hemisphere (left, right) adjusting for total brain volume. This model was followed up by separate one-way ANCOVAs for the left and right hemisphere, respectively. Relationship between hippocampal volumes and clinical variables was analyzed using multiple regression analysis. All analyses were two-tailed and the alpha was defined at 0.05. Statistical comparisons were performed using the Statistical Package for Social Sciences (SPSS for Windows, version 11.5).

3. Results

3.1. General

Subjects with low OH-Chol did not differ from subjects with high OH-Chol with respect to demographic, cognitive, or clinical variables, except cholesterol measures (for detailed results see Table 1). Intracranial volume, total brain volume and ventricular volume did not differ as well (see Table 2).

3.2. Hippocampal volume: group comparison

A 2 × 2 analysis of covariance (ANCOVA) with the between-subjects factor group (subjects with high OH-Chol, subjects with low OH-Chol), the within-subjects factor hemisphere (left, right) and adjusting for total brain volume revealed a significant effect of group (p = 0.018), indicating smaller hippocampal volumes in subjects with low OH-Chol when compared with subjects with high OH-Chol. One-way ANCOVAs confirmed significantly smaller right hippocampal volumes (p = 0.011) and revealed a trend towards smaller left hippocampal volumes (p = 0.057) in subjects with low OH-Chol (see Table 2 for detailed results).

To assess the influence of total body cholesterol on hippocampal volume, one-way ANCOVAs adjusting for total brain volume and total cholesterol were performed. Results confirmed significantly smaller right hippocampal volumes in subjects with low OH-Chol when compared with subjects with high OH-Chol (F(1) = 6.12; p = 0.018). The analysis concerning left hippocampal volumes was not significant.

There were twice as many apolipoprotein E ε4 (ApoE ε4) carriers in the low OH-Chol group (n = 7) when compared with the high OH-Chol group (n = 3) (cf. Table 1). However, comparison of ApoE ε4 positive subjects (n = 10) with ApoE ε4 negative subjects (n = 34) revealed similar size of the left and right hippocampus, respectively (ApoE ε4 positive subjects: left, 3.2 ± 0.4, right, 3.3 ± 0.4; ApoE ε4 negative subjects: left, 3.3 ± 0.5, right, 3.3 ± 0.5). T-tests revealed no significant differences between ApoE ε4 positive and ApoE ε4 negative subjects (left hippocampus: t(42) = 0.21, p = 0.831; right hippocampus: t(42) = 0.011, p = 0.992).

3.3. Regression analysis

Relationship between 24S-OH-Chol, 27-OH-Chol, cholesterol and hippocampal volume was further analyzed using multiple regression analysis (significance level for selecting variables: α = 0.05) in the total study sample (n = 69). Age, total brain volume and ApoE ε4 carrier state were entered as further predictors as these variables have been repeatedly shown to be related to hippocampal size as well. The overall regression model was found to be significant (R² = 0.300; p = 0.000). Looking at the single regression coefficients, 24S-OH-Chol (β = 0.262, p = 0.040) (see Fig. 1) and total brain volume (β = 0.458, p = 0.000) were able to significantly predict hippocampal volume. All other variables did not meet the inclusion criterion of α = 0.05 (p-values > 0.26). A further multiple regression analysis using the above-mentioned predictors and additionally body mass index, arterial hypertension, hyperlipidemia and diabetes showed also significant results.

![Table 2 Brain volume measures](image)

<table>
<thead>
<tr>
<th>Volume (mL)</th>
<th>Total sample (n = 69)</th>
<th>Subjects with low OH-Chol (n = 22)</th>
<th>Subjects with high OH-Chol (n = 22)</th>
<th>Difference between-subjects with low or high OH-Chol</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracranial</td>
<td>1491 ± 150</td>
<td>1446 ± 146</td>
<td>1501 ± 156</td>
<td>t(42) = -1.21</td>
<td>0.232</td>
</tr>
<tr>
<td>Total brain</td>
<td>1191 ± 130</td>
<td>1156 ± 134</td>
<td>1189 ± 130</td>
<td>t(42) = -0.84</td>
<td>0.404</td>
</tr>
<tr>
<td>Lateral ventricles</td>
<td>19 ± 10</td>
<td>21 ± 12</td>
<td>20 ± 9</td>
<td>t(42) = -0.26</td>
<td>0.793</td>
</tr>
<tr>
<td>Hippocampus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totalb</td>
<td>6.6 ± 0.9</td>
<td>6.2 ± 0.7</td>
<td>6.9 ± 1.1</td>
<td>F(1) = 6.04</td>
<td>0.018</td>
</tr>
<tr>
<td>Leftb</td>
<td>3.3 ± 0.5</td>
<td>3.1 ± 0.4</td>
<td>3.4 ± 0.6</td>
<td>F(1) = 3.84</td>
<td>0.057</td>
</tr>
<tr>
<td>Rightb</td>
<td>3.3 ± 0.5</td>
<td>3.1 ± 0.4</td>
<td>3.5 ± 0.5</td>
<td>F(1) = 7.05</td>
<td>0.011</td>
</tr>
</tbody>
</table>

Table values are given as mean ± S.D.

- The group (subjects with low vs. high OH-Chol) × hemisphere (left, right) (2 × 2) ANCOVA adjusting for total brain volume had the following F values: group, F(1) = 6.04, p = 0.018; hemisphere, F(1) = 0.06, p = 0.804; group × hemisphere, F(1) = 0.59, p = 0.447.
- One-way ANCOVA adjusting for total brain volume.
for 24S-OH-Chol and total brain volume. All other variables were not significant.

4. Discussion

Our results demonstrate smaller hippocampal size in subjects with low OH-Chol when compared with subjects with high OH-Chol. The amount of hippocampal size reduction in subjects with low OH-Chol (10%) parallels that found in individuals with MCI who later developed clinically probable AD (Wolf et al., 2003). Subjects with low OH-Chol did not differ from subjects with high OH-Chol on demographic, clinical or cognitive variables, nor on intracranial volume, total brain volume and ventricular volume. A multiple regression analysis using all subjects of the study sample confirmed the results of the group comparison and underlined the important role of 24S-OH-Chol as a clinically relevant marker of brain cholesterol metabolism in the aging brain (Lütjohann et al., 2000; Lütjohann and von Bergmann, 2003; Papassotiropoulos et al., 2002; Teunissen et al., 2003; Thelen et al., 2006). Since an inverse causality (hippocampal size influences serum 24S-OH-Chol) seems to be unlikely, it may be concluded that serum 24S-OH-Chol moderately predicts hippocampal size in middle-aged cognitively normal individuals.

Our results confirm and extend those of Wolf and colleagues (Wolf et al., 2003), reporting a positive relationship between HDL-C and hippocampal volume in elderly people. However, this (Wolf et al., 2005) and another study (Den Heijer et al., 2005) failed to demonstrate this relationship when subjects with possible mild AD were excluded. Our results were obtained in middle-aged subjects (mean: 50 years) in whom MCI or AD and other mental disorders had been definitely ruled out, and may have profited from the analysis of 24S-OH-Chol, which seems to be a more specific indicator of brain cholesterol than HDL-C.

The question arises whether high brain cholesterol metabolism may be a trait-like factor and may be protective against hippocampal atrophy and AD (Wolf et al., 2004). Present evidence does not allow drawing firm conclusions whether too high or too low brain cholesterol or both disrupt neuronal structure and function (Michikawa, 2003; Wood et al., 1999). Future studies have to clarify whether elderly cognitively normal individuals with low levels of 24S-OH-Chol are more likely to develop MCI or AD than subjects with high levels of 24S-OH-Chol. Since the early stages of AD were shown to be associated with elevated levels of 24S-OH-Chol (Lütjohann et al., 2000; Papassotiropoulos et al., 2002) (possibly reflecting ongoing neurodegeneration), it seems valuable to have a marker of brain cholesterol metabolism at a time point definitely preceding the onset of AD and MCI.

A further point for future research seems to be the elucidation of regional specificity of brain cholesterol metabolism. Studies are currently done in our department to resolve whether the sizes of specific cortical areas are related to 24S-OH-Chol as well. Neuropathological studies of AD suggest that AD-related degenerative changes start in allocortical areas and later spread in a predictable manner across the isocortex (Braak and Braak, 1991). It seems possible that there are regions of the brain being more susceptible to changes in cholesterol homeostasis possibly induced by AD than others (Wood et al., 1999).

Disclosure statement

All authors declare that they have no conflict of interest related to this work (financial of otherwise).

Acknowledgements

We express our appreciation to the subjects who participated in this study. The authors further wish to thank N. van Ahsen for ApoE genotyping. Research was supported by grant IR 15/6-2 from the Deutsche Forschungsgemeinschaft and a grant to J. Koschack from the Medical Faculty of the University of Göttingen.

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