Inflammation markers in relation to cognition in a healthy aging population

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Abstract

The relation between serum inflammatory protein levels and cognitive performance was investigated in a healthy population. Individuals were tested during 6 years of follow-up. Serum concentrations of 10 inflammatory proteins were correlated to cognitive speed (Letter–Digit Coding Test, LDCT), attention and information processing (Stroop) and memory (Word Learning).

Haptoglobin levels at baseline correlated negatively with cognitive performance on the Stroop and Word Learning Recall test over the 6 years follow-up period. C-reactive protein (CRP) levels at baseline correlated negatively with performance on the Word Learning tests over the 6 years follow-up period.

Thus, relatively high concentrations of haptoglobin and C-reactive protein may be indicative for impaired cognitive performance.

1. Introduction

A decline in cognitive functions is an inevitable feature of normal aging, but the degree of deterioration varies within the healthy older population. Some older persons experience a larger decline in certain cognitive functions than others and the patterns of cognitive functions affected may vary among older people. The processes affecting cognitive deterioration, however, are not understood. Known factors influencing, or possibly conferring a risk for, decline in cognitive performance are among others, age, level of education (Elwood et al., 1999; Anstey and Christensen, 2000), aerobic fitness (van Boxtel et al., 1997), gender and hypertension (Meyer et al., 2000). Insight into biochemical correlates of normal cognitive aging in individuals may be helpful to understand normal and pathological cognitive aging. Biochemical correlates may exist for the differentiation between people who age successfully and people who are characterized by a pathological process. While the use of cerebrospinal fluid (CSF) to investigate biochemical correlates in body fluids in normal healthy individuals is restricted due to ethical considerations, the use of peripheral blood samples provides a more readily available tool to assess possible biochemical correlates of cognitive aging.

Recently, we observed that concentrations of the cholesterol precursors lathosterol and lanosterol in serum were related to cognitive performance in a healthy aging population (Teunissen et al., in press). Nevertheless, despite considerable effort (see below) there is still little known concerning single markers of which serum levels parallel the cognitive aging process. Several factors may have obstructed the search for such biological markers. First, the brain is separated from the blood by the blood–brain...
barrier (BBB), which allows only limited exchange of compounds between the blood and the brain. Nevertheless, the brain is directly linked to peripheral systems, such as the immune system or the endocrine system via the hypothalamus–pituitary axis. Secondly, even for the severe forms of cognitive deterioration, dementia, no conclusive serum marker has been established (Working-Group, 1998).

It is known that the plaques of patients with Alzheimer’s disease are associated with activated microglia and increased expression and protein concentration of inflammatory proteins, e.g. IL-1, IL-6, C-reactive protein (CRP), α1-antichymotrypsin or tumor necrosis factor-α (McGeer et al., 1987; Iwamoto et al., 1994; Griffin et al., 1995). Studies on blood proteins related to inflammation in patients with Alzheimer’s disease have reported that some serum or plasma constituents may be increased in at least a subpopulation of patients with dementia of the Alzheimer type (see for a recent review: Teunissen et al., 2003). For example, altered concentrations of pro-inflammatory cytokines, such as interleukin-6 (IL-6), or acute phase reactants, e.g. α1-antichymotrypsin, have been observed in serum of patients with Alzheimer’s disease, though the results on each of these markers varied considerably among the studies (van Duijn et al., 1990; Blum-Degen et al., 1995; Kalman et al., 1997; Licastro et al., 2000b). This variation may be due to the low number of patients included, sometimes only 11 (Blum-Degen et al., 1995), or the sensitivities of the tests used. Thus, IL-6 concentrations in normal aging population as well as in patients with Alzheimer’s disease are often below the detection level of the ELISA-tests used (Angelis et al., 1998; Licastro et al., 2000b). Recent studies therefore have concentrated on the IL-6 receptor, which shows less variation in blood than IL-6 and is more easily detectable (Angelis et al., 1998).

The possibility that mild cognitive deterioration may develop into the pathological state of dementia (Howieson et al., 1997; Kawas et al., 2000) led us to hypothesize that inflammation markers as assessed in serum of the aging population might correlate with cognitive performance. The aim of the present study was to investigate whether serum inflammation markers could be related to cognitive performance in healthy aging individuals, or even form a risk factor for cognitive decline. Therefore, in this study we determined serum levels of the inflammation markers IL-6, IL-6 receptor, Clara cell protein 16 (CC16, which is a 15.8-kDa homodimeric anti-inflammatory protein and secreted in large amounts in airways by the non-ciliated bronchiolar Clara cells; Broeckaert and Bernard, 2000), CRP, albumin, protein fractions, haptoglobin and haptoglobin phenotype. The baseline serum levels of these proteins were compared with the individual cognitive performance during 6 years of follow-up in a healthy older population. The study was performed in the course of the Maastricht Aging Study (MAAS), a large longitudinal study involving individuals aged 25–85 in the Netherlands.

2. Materials and methods

2.1. Study population

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) (Jolles et al., 1995; van Boxtel et al., 1998). Individuals (n = 1823) for the MAAS were randomly drawn from the Registration Network Family Practices, a research database that contains basic health information of patients in primary care facilities (Metsemakers et al., 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 (Jolles et al., 1995). 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. All samples were analyzed simultaneously.

Cognitive data and a complete set of serum samples were available from a randomly drawn group of 144 individuals. After assessing the serum samples and cognitive testing, data were available from 92 individuals at baseline and 116 individuals after 6 years of follow-up. A complete set of serum data as well as cognitive performance data at both time-points was available from 65 individuals. The incompleteness of the data set of the remaining individuals was due to logistical problems.

The mean age of the 92 individuals at baseline was 57 (SD 11). The sex distribution was 55 man to 37 women. The age of the 65 individuals of whom a complete set of serum data was present was slightly lower (mean 54 years, SD 10) compared to the remaining 27 individuals with an incomplete data set (mean 65, SD 11, Mann–Whitney U-test: Z = −4.2, P < 0.001). The sex distribution was not statistically different (37 man to 28 women in the 65 individuals, 18 man to 9 woman in the remaining 27 individuals)(Chi-square, df = 1: 0.75, P < 0.38). The performance on the cognitive performance tests was not statistically different between the subgroups (P > 0.05).

Five persons used anti-inflammatory drugs on a daily basis. The serum inflammation marker concentrations of these individuals were within the S.E.M. of the mean results as shown in Table 1, though the CRP concentration was elevated in three out of five of these individuals.

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.
Table 1
Concentrations of serum markers at baseline and after 6 years of follow-up

<table>
<thead>
<tr>
<th></th>
<th>All individuals</th>
<th></th>
<th>Individuals ≥ 50 years old</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Follow-up</td>
<td>Baseline</td>
<td>Follow-up</td>
</tr>
<tr>
<td>Haptoglobin (g/l)</td>
<td>1.21 ± 0.48</td>
<td>1.22 ± 0.06</td>
<td>1.26 ± 0.48</td>
<td>1.26 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>(0.08 – 3.41)</td>
<td>(0.34 – 2.84)</td>
<td>(0.08 – 3.41)</td>
<td>(0.43 – 2.39)</td>
</tr>
<tr>
<td>Total protein (g/l)</td>
<td>66.00 ± 7.10</td>
<td>70.74 ± 0.97</td>
<td>66.50 ± 6.82</td>
<td>70.89 ± 1.06</td>
</tr>
<tr>
<td></td>
<td>(43.00 – 80.00)</td>
<td>(49.00 – 91.00)</td>
<td>(43.00 – 80.00)</td>
<td>(51.00 – 83.00)</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>38.10 ± 4.16</td>
<td>42.05 ± 0.60</td>
<td>38.11 ± 4.31</td>
<td>41.92 ± 0.70</td>
</tr>
<tr>
<td></td>
<td>(26.90 – 47.20)</td>
<td>(29.70 – 53.00)</td>
<td>(26.90 – 47.20)</td>
<td>(30.30 – 52.00)</td>
</tr>
<tr>
<td>α₁-Fraction (g/l)</td>
<td>3.67 ± 0.51</td>
<td>4.52 ± 0.12</td>
<td>3.72 ± 0.48</td>
<td>4.57 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>(2.20 – 4.80)</td>
<td>(2.30 – 7.40)</td>
<td>(2.70 – 4.70)</td>
<td>(3.20 – 7.40)</td>
</tr>
<tr>
<td>α₂-Fraction (g/l)</td>
<td>6.39 ± 1.55</td>
<td>6.01 ± 0.16</td>
<td>6.56 ± 1.48</td>
<td>6.15 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>(3.60 – 11.30)</td>
<td>(3.40 – 8.60)</td>
<td>(3.60 – 11.30)</td>
<td>(3.50 – 8.60)</td>
</tr>
<tr>
<td>γ-Fraction (g/l)</td>
<td>9.27 ± 2.33</td>
<td>9.80 ± 0.29</td>
<td>9.33 ± 2.34</td>
<td>9.75 ± 0.35</td>
</tr>
<tr>
<td></td>
<td>(4.60 – 18.00)</td>
<td>(5.50 – 17.00)</td>
<td>(4.60 – 18.00)</td>
<td>(5.50 – 17.00)</td>
</tr>
<tr>
<td>IL-6 receptor (μg/l)</td>
<td>158.3 ± 42.1</td>
<td>176.7 ± 59.0</td>
<td>157.4 ± 41.9</td>
<td>170.0 ± 66.0</td>
</tr>
<tr>
<td></td>
<td>(61.9 – 276.4)</td>
<td>(100.1 – 290.4)</td>
<td>(61.9 – 276.4)</td>
<td>(100.1 – 287.6)</td>
</tr>
<tr>
<td>CC16 (μg/l)</td>
<td>31.75 ± 12.69</td>
<td>25.61 ± 1.29</td>
<td>32.58 ± 12.60</td>
<td>26.92 ± 1.61</td>
</tr>
<tr>
<td></td>
<td>(3.45 – 82.00)</td>
<td>(3.69 – 67.14)</td>
<td>(7.52 – 82.00)</td>
<td>(10.96 – 67.14)</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>5.73 ± 6.74</td>
<td>7.55 ± 1.57</td>
<td>6.68 ± 7.09</td>
<td>6.93 ± 1.25</td>
</tr>
<tr>
<td></td>
<td>(0.50 – 27.88)</td>
<td>(0.50 – 83.54)</td>
<td>(0.50 – 27.88)</td>
<td>(0.50 – 30.93)</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.E.M. (range).

2.2. Cognitive assessment

Major aspects of cognitive function were tested according to the MAAS-protocol described in detail elsewhere (Jolles et al., 1995; van Boxtel et al., 1998). The focus was upon processes involving memory, attention and different aspects of cognitive processing speed.

2.2.1. Word Learning Task (WLT)

This test is based upon the Auditory Verbal Learning Test (Brand and Jolles, 1985) 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 (Jolles et al., 1995). A higher score on this test reflects better cognitive performance.

2.2.2. Letter–Digit Coding Test (LDCT)

This paper-and-pencil test is a modified version of the Symbol Digit Modalities Test (Smith, 1968) 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 90 s is recorded as test outcome. A relatively better cognitive 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 of 10 columns or colored spots. The test examines the speed at which color names are read (subtask I) and the speed at which color spots are 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, in contrast with the higher scores linked to better cognitive performance on the other three memory tasks in this paper. This test shows robust effects of chronological age (Houx et al., 1993). Only the data of subtask III are used in the current study and are referred to as ‘Stroop’.

2.3. Biochemical measurements

For the determination of IL-6, IL-6 receptor, CC16 and CRP in serum commercial ELISA kits were used (Eurogenetics, Tessenderlo, Belgium). Detection limit of IL-6 was 10 pg/ml, the detection limit for IL-6 receptor was 1 ng/ml, for CC16 the detection limit was 0.1 ng/ml and for CRP the detection limit was 0.25 μg/ml. The intra-assay coefficients of variance (C.V.) of all assays were below 8%. Serum haptoglobin concentration was measured by fixed-time immunonephelometry on a BN II analyzer (Dade Behring, Marburg, Germany) and the assay was calibrated against the international CRM 470 reference material (Dati et al., 1996). Haptoglobin phenotype was determined using starch gel electrophoresis of hemoglobin-supplemented serum, followed by peroxidase staining (Smithies, 1955). The
distribution of the phenotypes (1-1: 12.9%; 2-1: 50.5%; 2-2: 36.6%) was in accordance with the distribution in the northwestern European population (Langlois and Delanghe, 1996). Serum protein electrophoresis was performed by capillary electrophoresis using the automated Beckman Paragon CZE 2000 (Analis, Namur, Belgium). The intra-assay CV values for the serum protein fractions are as follows: albumin 1.2%; α1 1.6%; α2 1.6% and γ 4.9%. Considering the long storage period of the samples (up to 6 years), stability of the acute phase proteins IL-6, IL-6 receptor and CC16 was confirmed under different storage conditions. In addition, stability of these proteins after repeated freezing thawing was confirmed (Kenis et al., 2002).

2.4. Statistical analysis

Normality of the distributions was tested using the Kolmogorov–Smirnov test. Zero order bivariate correlation analysis was performed using Pearson’s correlation coefficient. Because the distribution of CRP was skewed, the ranks of the values of this protein were calculated for the correlation analysis.

The further analysis was performed in two phases. First, ordinary least squares multiple regression analysis was performed for the four cognitive tests at baseline with each separate serum protein as potential predictor, and age, gender and level of education as covariates as in the following expression:

\[
\text{cognitive test outcome} = b \times \text{protein} + (b_2 \times \text{age} + b_3 \times \text{gender} + b_4 \times \text{education})
\]

Secondly, the markers showing a significant relation (\(P<0.05\)) with at least two of the cognitive tests, adjusted for age, gender and educational level, multi-level regression analysis was additionally performed. Multi-level repeated measurement analysis was performed to analyze the association between the predictors and cognitive function during the whole follow-up. This method uses all observations, including the observations of persons with only one or two observations (Albandar and Goldstein, 1992; Goldstein, 2000). Persons who were over 50 years of age were included at all three measurement phases. Persons younger than 50 years were included for the first and third phase only. To increase homogeneity regarding age across the different phases, the analyses were re-done for persons older than 50 years of age only. The influence of influential cases and outliers was examined using the studentized residuals and Cook’s distances in the normal hierarchical regression analysis. As four cognitive tests were used as outcomes, a Bonferroni adjustment was considered. However, as this study is primarily exploratory in nature, results with significance level of \(P<0.05\) are also indicated. Data are expressed as regression coefficient (\(b\)) with 95% confidence intervals. All analyses were performed with SPSS statistical software.

3. Results

3.1. Biochemical measurements

The mean values of the serum proteins at baseline are presented in Table 1. The mean haptoglobin concentration was comparable with reference ranges reported (Langlois and Delanghe, 1996). The mean concentrations of total protein, albumin, and protein fractions were in agreement with laboratory reference ranges (total protein range 60–78; albumin range 36.0–51.0; α1 range 2.7–5.2; α2 range 3.5–8.2; γ range 6.2–14.4). The mean and median of the CRP concentrations were higher compared to proposed reference ranges of 0.98 (0.34–2.85) mg/l for healthy individuals of 27–75 years old (Chenillot et al., 2000). The mean and range for the IL-6 receptor concentration as measured in our study were about three times higher than values reported by the only other study investigating this marker in serum, using a different ELISA, for elderly controls (47.6 ± 1.3 μg/l; range 29.0–61.3 μg/l; Angelis et al., 1998). The mean and range of CC16 concentrations were higher then reported values of 13.3 μg/l (range 5.2–34.5 μg/l) for individuals from 18 to 67 years by Hermans et al. (1998). The variation in standard error between baseline and follow-up concentrations for part of the cytokines might be due to differences in numbers of individuals (\(n=65\)) included at both time-points. Inter-assay variation is not a likely cause, since all samples were analyzed simultaneously, neither is variation due to storage conditions, as investigated in our previous study (Kenis et al., in press).

3.2. Zero order correlation analysis at baseline

The results from the correlation analysis of the serum proteins with age and cognitive tests at baseline for all individuals are shown in Table 2.

As expected, the cognitive tests scores on the Stroop test correlated positively with age, while the LDCT, WLTTOT and Delayed Recall correlated negatively with age (Jolles et al., 1998). The albumin level correlated negatively with age, while the CC16 and CRP levels correlated positively with age (Tietz et al., 1992; Bernard et al., 1994).

Haptoglobin levels showed a negative correlation with the LDCT, and showed a positive relation with outcomes on the Stroop test for the whole group as well as for individuals older than 50 years. These results indicate a relatively decreased cognitive performance when haptoglobin concentrations are higher.

A negative correlation between albumin concentration and scores obtained in the Stroop test was observed in the whole group and in the older individuals. A positive correlation between albumin levels and Delayed Recall was observed in the group of older individuals.
A positive correlation was observed between CRP concentration and the Stroop test and a negative correlation between CRP and the WLTTOT in the total group. Thus, higher concentration of CRP may indicate a less adequate cognitive performance. IL-6 levels were detectable in only 12 of the individuals (mean 31.44, SD 55.04 (range 10.01–205.05) and therefore those data were not included for analysis. All other serum protein concentrations did not correlate with cognitive outcome measures or age.

### 3.3. Correlation between cognitive performance and serum markers at baseline adjusted for age, gender and education

The correlation between the serum markers and the scores on the cognitive tests at baseline after adjustment for age, gender and education was investigated using multiple regression analysis. The data of markers showing significant results are presented in Table 3.

A positive correlation between haptoglobin concentration and the Stroop test was observed in the total population, indicating decreased cognitive performance in individuals with higher haptoglobin levels. A similar tendency was obtained in the subset of individuals over 50 years old. In accordance with these results, a negative correlation between haptoglobin concentration and Delayed Recall was observed for all individuals and a tendency for the subset of older individuals. Haptoglobin concentrations are dependent on phenotype, with lowest concentration in 2-2 phenotypes (0.38–1.50 g/l), then 2-1 (0.44–1.83 g/l) and highest concentration in 1-1 phenotype (range 0.57–2.27 g/l) (Langlois and Delanghe, 1996). When haptoglobin concentration was additionally adjusted for phenotype, the

### Table 3

Unstandardized regression coefficients (95% confidence interval) of serum markers and in models for four cognitive outcome measures adjusted for baseline age, gender, and educational level

<table>
<thead>
<tr>
<th>Haptoglobin concentration</th>
<th>Number of subjects</th>
<th>Stroop</th>
<th>LDCT</th>
<th>WLTTOT</th>
<th>Delayed Recall</th>
</tr>
</thead>
<tbody>
<tr>
<td>All observations</td>
<td>92</td>
<td>11.19 (0.75, 21.64)**</td>
<td>-1.73 (-5.53, 2.06)</td>
<td>-1.51 (-5.28, 2.25)</td>
<td>-1.29 (-2.52, -0.58)**</td>
</tr>
<tr>
<td>≥ 50 years</td>
<td>71</td>
<td>10.87 (-1.94, 23.68)*</td>
<td>-0.42 (-4.85, 4.01)</td>
<td>-0.18 (-5.81, 3.45)</td>
<td>-1.27 (-2.78, 0.24)*</td>
</tr>
<tr>
<td>Haptoglobin phenotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All observations</td>
<td>92</td>
<td>8.16 (0.93, 15.38)**</td>
<td>-1.14 (-3.72, 1.45)</td>
<td>0.33 (-2.90, 2.24)</td>
<td>-0.56 (-1.41, 0.29)</td>
</tr>
<tr>
<td>≥ 50 years</td>
<td>71</td>
<td>8.42 (-0.17, 17.01)*</td>
<td>-1.08 (-4.06, 1.90)</td>
<td>-0.96 (-4.08, 2.17)</td>
<td>-0.77 (-1.79, 0.26)</td>
</tr>
<tr>
<td>Haptoglobin*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All observations</td>
<td>92</td>
<td>13.97 (3.73, 24.22)***</td>
<td>-2.08 (-5.93, 1.77)</td>
<td>-1.65 (-5.48, 2.19)</td>
<td>-1.47 (-2.71, -0.24)**</td>
</tr>
<tr>
<td>≥ 50 years</td>
<td>71</td>
<td>12.89 (0.34, 25.44)***</td>
<td>-0.66 (-5.15, 3.83)</td>
<td>-1.41 (-6.10, 3.29)</td>
<td>-1.46 (-2.97, 0.04)*</td>
</tr>
<tr>
<td>CRP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All observations</td>
<td>88</td>
<td>0.76 (0.02, 1.49)**</td>
<td>-0.06 (-0.33, 0.21)</td>
<td>-0.11 (-0.38, 0.15)</td>
<td>-0.10 (-0.18, -0.01)**</td>
</tr>
<tr>
<td>≥ 50 years</td>
<td>70</td>
<td>0.74 (-0.08, 1.56)*</td>
<td>0.01 (-0.28, 0.29)</td>
<td>-0.11 (-0.41, 0.19)</td>
<td>-0.10 (-0.20, -0.00)**</td>
</tr>
</tbody>
</table>

* Additionally adjusted for haptoglobin phenotype.

** P<0.10.

*** P<0.05.

* P<0.01.
regression coefficients of the correlation with scores on the Stroop or Delayed Recall test increased in magnitude and significance (Table 3).

A significant positive correlation between CRP concentration and the Stroop test at baseline was observed in the total population, and a tendency for the subgroup of individuals over 50 years. Thus, high CRP concentrations are observed in persons with a less adequate cognitive performance. In agreement with this, a negative correlation between CRP and the Delayed Recall test at baseline was observed in the total population as well in the individuals over 50 years only.

A positive correlation between CC16 and the WLTTOT test was observed in the total population only ($b = 0.13$, $P < 0.01$). No correlation between the concentration of total protein, albumin, protein fractions, IL-6 receptor and cognitive performance on the current tests was observed (data not shown).

3.4. Correlation between cognitive performance and serum markers adjusted for age, gender and education during the whole 6 years follow-up period

We next questioned whether serum inflammatory marker concentrations might have a risk modulating effect on cognitive performance. Therefore, the relation between serum marker concentrations at baseline and cognitive performance over the whole 6 years follow-up was examined. This was performed for the serum inflammation markers showing cross-sectional correlation with at least two cognitive outcomes in the hierarchical regression analysis, haptoglobin and CRP, using multi-level repeated measurement analysis (Table 4). Multi-level repeated measurement analysis allows the simultaneous examination of several observations per person (this increases the power of the study). Furthermore, multi-level analysis allows different numbers of observations between persons (Albandar and Goldstein, 1992; Goldstein, 2000).

A tendency towards a negative correlation (positive $b$) between haptoglobin levels at baseline and scores on the Stroop test over the whole 6 years of follow-up was observed for the total population. A significant negative correlation between haptoglobin concentration at baseline and Delayed Recall during 6 years of follow-up was observed for the total population as well as a tendency towards a negative correlation for the older individuals only. Both these relations became stronger, i.e. $P < 0.05$ for both groups and increased in magnitude of coefficients, after additional adjustment for haptoglobin phenotype, indicating a significant relation between haptoglobin concentration and cognitive performance that was independent of phenotype (Table 4).

Since a serum sample at both baseline and follow-up was available from 65 individuals, we could explore the relation between haptoglobin and cognitive performance during the follow-up period further for these individuals. No significant relation between haptoglobin concentration and cognitive performance was observed for these subjects at follow-up, either for the crude correlation or when adjusted for age, gender and education. In fact, when the small number of 65 persons alone was examined at baseline, no significant relations were observed (data not shown).

The relation between CRP and cognitive performance over the whole follow-up period was restricted to the word learning tasks. A negative correlation between CRP concentration at baseline and scores on the Delayed Recall test was

| Table 4 | Unstandardized regression coefficients (95% confidence interval) of three biomarkers in models for four cognitive outcome measures, adjusted for baseline age, sex, educational level, and longitudinal time (multi-level model with observations at level 1 and individuals at level 2)** |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | Number of observations (individuals) | Stroop | LDCT | WLTTOT | Delayed Recall |
| **Haptoglobin** |                               |       |     |       |                 |
| All observations | 92 (252) | 8.62 (−0.88, 18.13)** | −2.08 (−5.68, 1.52) | −2.10 (−5.24, 1.04) | −1.17 (−2.09, −0.24)** |
| ≥ 50 years      | 79 (226) | 8.78 (−1.70, 19.26) | −1.27 (−5.16, 2.62) | −2.25 (−5.81, 1.31) | −1.06 (−2.18, 0.08)** |
| **Haptoglobin** |                               |       |     |       |                 |
| All observations | 92 (252) | 10.93 (1.68, 20.18)** | −2.43 (−6.06, 1.20) | −2.29 (−5.47, 0.89) | −1.10 (−2.09, −0.12)** |
| ≥ 50 years      | 79 (226) | 11.02 (0.85, 21.19)** | −1.62 (−5.54, 2.29) | −2.50 (−6.10, 1.09) | −1.18 (−2.31, −0.04)** |
| **CRP**         |                               |       |     |       |                 |
| All observations | 240 (88) | 0.39 (−0.31, 1.08) | −0.14 (−0.39, 0.12) | −0.18 (−0.40, 0.04) | −0.09 (−0.15, −0.04)** |
| ≥ 50 years      | 217 (76) | 0.33 (−0.35, 1.00) | −0.10 (−0.35, 0.15) | −0.27 (−0.45, −0.08)** | −0.08 (−0.15, −0.00)** |

* All persons participated in at least two of the three measurement phases during the 6 years follow-up. Inter-individual random variation in the intercepts of the association between longitudinal time and cognitive function was significant in all analyses. Inter-individual random variation in the slopes of this association was, however, never statistically significant. This indicates that persons differed in cognitive function at baseline (first phase), but there were no significant differences between persons in the course of cognitive function during follow-up. Interactions between longitudinal time and the serum inflammation markers, i.e. modeling a differential longitudinal course of cognitive function related to the biomarkers, were therefore not investigated further.

** $P < 0.05$.
observed for the total population as well as for the subset of individuals over 50 years old. A significant negative correlation between the CRP concentration at baseline and scores on the WLTTOT during 6 years of follow-up was observed in the subset of individuals older than 50 years. There were no significant differences in cognitive function between persons during the follow-up period, as indicated in the legend of Table 4. Therefore, interactions between longitudinal time and the serum inflammation markers, i.e., eventual risk modulating effects of serum marker concentrations at baseline on cognitive performance at follow-up, were not investigated further. In line with these observations, no correlations were observed between the change in haptoglobin concentration between baseline and follow-up and the change in cognitive performance outcomes (data not shown). This was also observed for all other markers examined, in both the whole group (n = 65) and the individuals over 50 years old (n = 45) (data not shown), except for a correlation between change in protein α2 and change in Stroop score (whole group: r = 0.275, P < 0.05; >50 years: r = 0.319, P < 0.05) and between change in protein α1 concentration and change in WLTTOT (r = −0.249, P < 0.05) in the whole group.

Finally, the influence of the outliers and influential cases on the results was examined (see Section 2.4). In this way, three individuals were excluded from the Stroop, LDCT and WLTTOT and two individuals from the Delayed Recall test. The significant relation between haptoglobin concentration and the performance on the Stroop test and the Delayed Recall test remained in both the ordinary least squares multiple regression analysis and the multi-level regression analysis. This was observed for the haptoglobin concentration as well as after correction for haptoglobin phenotype. The relation between CRP and the cognitive test as observed by regression analysis and multi-level repeated measurement analysis disappeared after exclusion of the outliers and influential cases.

4. Discussion

The aim of the present study was to investigate whether cognitive performance in the healthy aging population could be reflected by serum inflammation marker concentrations. The strongest result of the present study was the finding of a negative correlation of the serum inflammation marker haptoglobin with cognitive performance on the Stroop test and the Delayed Recall test, at baseline as well as over the whole follow-up period of 6 years. In addition, CRP concentration correlated with performance on the Delayed Recall and WLTTOT at baseline as well as over the 6 years follow-up period, at least in the subgroup of older individuals. These effects were independent of age, gender or level of education of the individuals.

There are several indications that the observed correlations for haptoglobin and CRP serum levels might be rather specific than just a reflection of a general state of enhanced inflammation as no relation between the concentration of albumin, total protein or α1, α2 and γ protein fractions and cognitive performance was observed. In addition, no detectable increase in IL-6 concentration, a general marker for inflammation, was observed in the majority of the individuals in our study. Finally, our findings show a correlation of haptoglobin and CRP concentrations with certain aspects of memory rather than with general cognitive performance, since no relation with the LDCT test for processing speed was observed.

Haptoglobin may function as an acute phase protein and as an antioxidant. This latter function suggests that high haptoglobin levels may exert a protective effect against oxidative stress, one of the putative mechanisms involved in aging and neurodegeneration (Beal, 1995). Haptoglobin plays a role in clearing hemoglobin and its highly reactive iron group from the circulation by forming a complex with hemoglobin, which interacts with the CD163 receptor for uptake of this complex into macrophages. Haptoglobin and the CD163 receptor are upregulated by acute phase reactants such as IL-6 (Kristiansen et al., 2001).

The studies on CRP concentration during aging and in demented patients are relatively scarce and contradictory (Gioiello et al., 1988; Bruunsgaard et al., 1999; Licastro et al., 2000a). The CRP levels in the present study were relatively high compared to reference ranges (Section 3.1), likely due to the use of a different assay. It is not plausible that these high levels are caused to a diseased state of the subjects, since almost all had undetectable IL-6 levels, as mentioned in Section 3.2. Nevertheless, although the ‘true’ concentrations may be biased by some systematical error, this would account for all CRP levels and thus would not affect the observed correlations between CRP and cognitive performance. CRP is a so-called “first class” acute phase reactant because it is one of the most sensitive plasma proteins indicating inflammatory activity (Yamada, 1999). Haptoglobin has a longer half-life of 5.4 days (Moretti et al., 1963) compared to 6–8 h for CRP (Cambau, 1988) in humans. For CRP, triplicate sampling is recommended to assess an individual serum level due to a relatively high contribution of intra-individual variation to the total variance (de Maat et al., 1996). Haptoglobin levels give a better reflection of baseline inflammation level than CRP and therefore haptoglobin may be more valuable.

The correlation between serum CRP and WLTTOT reached significance in the older individuals only. This interesting result is probably due to differences in cognitive performance between the young and old, as the relative number of individuals with decreased cognitive functioning increases above age 60 (Houx et al., 1991; Kaye et al., 1994). The relation between haptoglobin concentration and cognitive performance was not significant in the cross-sectional analysis at follow-up (Section 3.4). This may be explained by several factors including loss of power due to the lower number of individuals of whom a second serum
sample was available \((n = 65)\), variability due to the differences in age as described in Section 2, or other unknown factors. At least, the results warrant cautious interpretation and further research.

In conclusion, several inflammatory molecules were unrelated to cognitive performance in the current study and the low number, short follow-up time and the lack of correlation on the processing speed task warrant cautious interpretation. Nevertheless, our results show correlations between cognitive performance and specific serum markers for inflammation in the healthy aging population, which were independent for age, sex and level of education. Further follow-up of this population will indicate whether individuals with relatively lower cognitive performance and higher levels of haptoglobin develop dementia in time.

References


