LOWER BLOOD LEVELS OF VITAMIN B12 ARE RELATED TO DECREASED PERFORMANCE OF
HEALTHY SUBJECTS IN THE STROOP COLOR-WORD TEST

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SUMMARY
The effects of age and sex on the serum levels of vitamin B12 were determined after an overnight fast in 80 adult ambulatory, disease-free persons who had undergone rigorous health screening and neuropsychological testing. No significant age or sex differences were found. When adjusted for the effects of age and education, it appeared that individual vitamin B12 levels influenced cognitive functioning. In that subjects with lower vitamin B12 levels performed the Stroop test significantly less well than persons with higher levels of the vitamin, especially on a modified subtask of increased complexity.

KEY WORDS: Aging, Cognitive functions, Stroop test, vitamin B12

INTRODUCTION
The mammalian brain requires several nutrients from the blood for proper functioning (1). Disturbances in mental function ranging from mild memory loss to severe dementia may be caused by a deficiency in any one of several vitamins, such as vitamin B12 (2,3). In a study involving healthy elderly subjects, memory and abstract thinking were poorest in those subjects with the lowest levels of B12 (4). Psychometric tests may be useful tools in detecting subtle degrees of impairment related to biochemical indices that are relevant to brain function in disease-free subjects.

We investigated the combined effects of aging and individual vitamin B12 levels in relation to performance of the Stroop Color-Word Interference test. The hypothesis was that lower levels of vitamin B12 in the blood were related to decreased cognitive performance.
SUBJECTS AND METHODS

Subjects

The subjects were selected on the basis of rigorous health-related eligibility criteria. Specific details of the study design, population description, disease ascertainment, and methodology are reported elsewhere (5), but certain relevant points are summarized here. Subjects were divided into four age groups: 17-23, 37-43, 57-63 & 76-85 years (10 men and 10 women per age group). There were no dietary restrictions. The study was approved by the Medical Ethical Council of the University Hospital and all subjects gave their informed consent.

Procedure

Venous blood samples were collected after an overnight fast between 8.00 and 9.00 am, with the subject in a half-sitting position (5). Serum vitamin B12 levels were determined by radioimmunoassay, using a commercially available kit (Biorad). The intra- and interassay coefficients of variation were both 4%. After the collection of blood, the subjects underwent neuropsychological testing. Relationships with other blood parameters are currently being assessed in further analyses.

Test Material

Stroop Test. The Stroop Color-Word Interference Test used in the present study is a version commercially available in the Netherlands (Swets & Zeitlinger). The test consists of three parts. Ten lines are printed in each subtask with 10 items per line. The test examines the speed at which a 100 color names (yellow, green, red and blue) are read (subtask I) and the speed at which 100 colored spots are named (subtask II). Subtask III again involves 100 color names, but the color of the printing ink is different from the color the word describes. The naming of the printing ink of the words is taken as the test variable. The Color-Word Interference score is obtained by subtraction of the time needed for subtask II from that of subtask III.

New subtask IV. We recently developed a new subtask of the Stroop test (6). Twenty items from subtask III were randomly selected, and small rectangles were drawn around these words (see figure 1). The modified subtask (subtask IV) was further the same as subtask III, except that the subject was requested to read the printed word within the rectangles. The modified interference measure is calculated as a weighted measure according to the following formula: Int-IV= tIV-(0.8^*tI + 0.2^*tII; t=time in seconds per subtask).

Statistical analysis

Levels of vitamin B12 were analyzed by multiple regression analysis. Results on the two interference measures were analyzed by ANOVA with a median split grouping factor of vitamin B12.

RESULTS

Regression analysis indicated that there were no age (t=0.44, ns) or sex (t=1.95, ns) differences in the levels of vitamin B12, nor was there a significant interaction between sex and age (t=0.25, ns).

With respect to cognitive functioning, it was found that there was a clear effect of age on the interference measures tested (see table I). In addition, when adjusted for the effects of aging and education (7) there was a significant effect of vitamin B12 on the Stroop interference measure of the modified subtask. There was a nearly significant effect of vitamin B12 and performance on the original measure of interference (table I). Subjects with lower levels of vitamin B12 in the blood performed less well than subjects with higher levels.
Table I
Results of the Stroop Color-Word Interference Test.
ANOVA F(1,76)-values with significance levels are presented for the regression coefficients. Int-III is the original measure of color word interference, whereas Int-IV is the modified measure.

<table>
<thead>
<tr>
<th></th>
<th>F value</th>
<th>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Int-III</td>
<td>12.1 ***</td>
<td>F=2.9 P&lt;0.1</td>
</tr>
<tr>
<td>Int-IV</td>
<td>6.9 *</td>
<td>F=5.4 *</td>
</tr>
</tbody>
</table>

*: P<0.05
***: P<0.001
Two-tailed significance levels.

As can be seen from table II, the scores for the modified interference scores (Int-IV) for the different age groups categorized by the median split grouping factor of vitamin B12 indicate that subjects with lower levels of vitamin B12 in the blood generally have higher interference scores in comparison with subjects with higher levels of vitamin B12. It should be noted that these mean values are unadjusted for the effects of age and education. This may explain the absence of a relevant difference in the third age subgroup.

Table II
Mean performance (±SEM) in the modified Stroop interference test (Int-IV). Data are presented for the four age groups subdivided on the basis of the median split grouping factor of vitamin B12.

<table>
<thead>
<tr>
<th>Age Group (yr)</th>
<th>17-23</th>
<th>37-43</th>
<th>57-63</th>
<th>76-85</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low levels of vitamin B12</td>
<td>63.74 (±8.23)</td>
<td>62.66 (±7.23)</td>
<td>58.53 (±5.39)</td>
<td>87.35 (±8.18)</td>
</tr>
<tr>
<td>n=11</td>
<td>n=7</td>
<td>n=9</td>
<td>n=14</td>
<td></td>
</tr>
<tr>
<td>High levels of vitamin B12</td>
<td>46.25 (±3.76)</td>
<td>52.27 (±3.70)</td>
<td>61.74 (±5.82)</td>
<td>70.85 (±6.15)</td>
</tr>
<tr>
<td>n=9</td>
<td>n=13</td>
<td>n=11</td>
<td>n=6</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

The present study confirms the findings of Hitzhusen et al. (8) in that there was no significant decline in vitamin B12 levels in healthy elderly individuals, nor a difference between men and women.

With respect to the scores for the Stroop Color-Word test, the present results confirm the expected decline in the scores for the Stroop Color-Word Interference test with increasing age. In addition, there was a significant effect of vitamin B12 as well. The results indicate that besides the effects of age and educational level, individuals with lower levels of vitamin B12 could have a decreased cognitive performance, especially at a cognitively more demanding subtask.

The benefit of nutritional intervention for subjects with subclinical malnutrition is not well documented. Controlled intervention studies with vitamin B12 supplementation are needed to further establish the implications of the present findings.

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REFERENCES


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