Apolipoprotein E, B Vitamins, and Cognitive Function in Older Adults

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Recognition of dated and contemporary famous faces, short-term memory, and visuospatial abilities were investigated in adults aged 75 years and older as a function of apolipoprotein E (APOE) genotype, ε4 or not ε4, and whether participants recorded normal or low levels of B vitamins. No associations between B vitamins and APOE were identified in respect to short-term memory or visuospatial skills, or for contemporary famous faces. However, in respect to the recognition of dated famous faces, deficits in persons carrying the ε4 allele who also recorded low vitamin B12 values were found. The results suggest that the neurological structures and processes supporting face recognition may be vulnerable to the combined influence of the APOE ε4 allele and low levels of vitamin B12. This finding was unrelated to incipient dementia up to 6 years following testing. The results are discussed with reference to the neuroanatomical reserves that ε4 carriers may possess.

The association between the apolipoprotein E (APOE) ε4 allele and dementia is well established (see Farrer et al., 1997), and there is accumulating evidence that the allele is related to cognitive deficits in old age (e.g., Anstey & Christensen, 2000). Currently, however, there is little research investigating APOE and so-called environmental factors in respect to cognitive performance in older adults. Environment in this sense is broadly defined and includes anything nongenetic. For instance, lifestyle variables such as physical activity may interact with genetic factors to predict cognitive function. Nutritional status is another potential influence, and two variables relevant to cognitive performance in older adults are vitamin B12 and folate. Our first objective in the present study was to examine APOE genotype and those B vitamins in respect to cognitive performance in older adults. Environment in this sense is broadly defined and includes anything nongenetic. For instance, lifestyle variables such as physical activity may interact with genetic factors to predict cognitive function. Nutritional status is another potential influence, and two variables relevant to cognitive performance in older adults are vitamin B12 and folate. Our first objective in the present study was to examine APOE genotype and those B vitamins in respect to cognitive performance in a population-based sample of adults aged 75 years and older. Specifically, we asked if the combined influence of the ε4 allele and low levels of B vitamins on cognitive function was greater than either factor alone. Given the association between APOE and dementia, our second objective in this study was to determine the extent to which relations identified among the ε4 allele, B vitamins, and cognition were independent of incident dementia up to 6 years following testing.

There is evidence suggesting that APOE is associated with neuroanatomical and functional variation in the brain. For instance, structural imaging studies indicate that nondemented APOE ε4 carriers have smaller hippocampi (Plassman et al., 1997) and suffer greater hippocampal atrophy over time (Cohen, Small, Lalonde, Friz, & Sunderland, 2001; Moffat, Szekely, Zonderman, Kabani, & Resnick, 2000). In addition, in nondemented APOE ε4 homozygotes, the prefrontal cortex displays reduced rates of glucose metabolism (Reiman et al., 1996), and the magnitude and extent of brain activation is greater among APOE ε4 carriers in the prefrontal cortex, hippocampus, and parietal cortex during a challenging memory task (Bookheimer et al., 2000). This latter finding has been interpreted as compensatory processing among ε4 carriers during episodic memory encoding in demanding conditions (Burggren, Small, Sabb, & Bookheimer, 2002).

Such structural neuroanatomical and functional differences in ε4 and non-ε4 carriers suggest that APOE-related deficits would be apparent in behavioral measures of cognition, and indeed this is the case. For instance, carriers of the ε4 allele exhibit deficits in face and word recognition (Small, Basun, & Bäckman, 1998), delayed word recall (Hyman et al., 1996), factor scores for episodic memory and processing speed (Hofer et al., 2002), memory and nonmemory composite measures (Jonker, Schmand, Lindeboom, Havekes, & Launer, 1998; Mayeux, Small, Tang, Tycko, & Stern, 2001), digit symbol and visuospatial skills (Mortensen & Hogh, 2001), and verbal and nonverbal reasoning (Deary et al., 2002). However, there is also research investigating fluid intelligence (Pendleton et al., 2002), composite visuospatial and language factors (Mayeux et al., 2001), and proxy measures of IQ (Deary et al., 2003) in which no association with the ε4 allele has been found. In respect to global measures of cognitive performance, some investigations suggest decline to be greater in ε4 carriers (e.g., Bretsky, Guralnik, Launer, Albert, & Seeman, 2003; Fillenbaum et al., 2001; Jonker et al., 1998), whereas others show no differential decline (Winnock et al., 2002). In addition, there is evidence that cognitive function is lower in individuals possessing the APOE ε4 allele in combination with a second factor that may be related to brain pathology. For example, greater cognitive deficits have been observed in ε4-carrying older adults who also suffered from peripheral vascular disease, atherosclerosis (Haan, Shemanski, Jagust, Manolio, & Kuller, 1999), olfactory dysfunction (Borenstein Graves et al., 1999), and low estrogen (Yaffe, Haan, Byers, Tangen, & Kuller, 2000) and vitamin B12 levels (Bunce, Kivipelto, & Wahlin, 2004). In the foregoing studies, although future dementia is often considered, the possible presence of putative transitional conditions such as mild cognitive impairment (MCI) is seldom taken into account. This is likely due to current conceptual uncertainties in differentiating the condition.
With regard to the influence of vitamin B₁₂ and folate on cognitive performance in older adults, both nutrients have been associated cross-sectionally with episodic memory (e.g., Hassing, Wahlin, Winblad, & Bäckman, 1999; Wahlin, Hill, Winblad, & Bäckman, 1996), spatial and working memory ability and verbal fluency (Robins Wahlin, Wahlin, Winblad, & Bäckman, 2001), and spatial copying ability (Riggs, Spiro, Tucker, & Rush, 1996). Intervention studies (e.g., Martin, Francis, Protetch, & Huff, 1992; Meadows, Kaplan, & Bromfield, 1994) have demonstrated cognitive benefits in demented or cognitively impaired individuals, and low levels of those nutrients have been identified as a risk factor for Alzheimer’s disease (e.g., Wang et al., 2001).

Vitamin B₁₂ is obtained mainly from foods of animal origin, and folate from fresh leafy vegetables, fruits, yeast, and liver. They have close biochemical linkages, and two hypotheses have emerged (Calvaresi & Bryan, 2001) to explain the association with cognition. The hypomethylation hypothesis suggests low levels of B₁₂ and folate influence methylation throughout the central nervous system. This may detrimentally affect cognitive function, because, among other effects, this inhibits the metabolism of the neurotransmitters dopamine, norepinephrine, and serotonin. The homocysteine hypothesis proposes that impaired neurocognitive function is a consequence of elevated levels of homocysteine arising from low vitamin B levels, and related cerebrovascular changes. For our present purposes, it is important to note that both hypotheses suggest physiological mechanisms by which neurological processes are either impaired or damaged. Given the neuroanatomical and functional differences associated with APOE ε4 noted earlier, it is possible that the deleterious influence on neural structures and processes of ε4 in combination with low B vitamin levels will be greater than either factor alone. Moreover, this is likely to be particularly apparent in cognitive functions that rely heavily on brain structures affected by those factors such as the hippocampus.

In the present study, we extend earlier research (Bunce, Kivipelto, et al., 2004) investigating APOE and B vitamins in respect to free recall of words in a population-based sample of adults aged 75 years and older. That study focused specifically on encoding processes, finding that associations were strongest in the most demanding study conditions. Here we build on that research by investigating retrieval processes in face recognition. Specifically, we take into account the influence of cognitive demands on performance by investigating the recognition of dated famous faces relative to contemporary famous faces. Previous research suggests that older persons have more knowledge about dated than contemporary faces (Lipinska, Bäckman, & Herlitz, 1992). Such prior knowledge is likely to facilitate the recognition of dated relative to contemporary famous faces. In addition, to assess how selective the APOE–B vitamin effect is, we investigate associations in two further cognitive domains, namely, short-term memory and spatial abilities. Finally, a consideration of some importance is how far associations between APOE ε4 and cognitive deficits are independent of impending dementia. Although studies have eliminated demented individuals at baseline (e.g., Small et al., 1998) or have statistically controlled for dementia (Hofer et al., 2002) and have found that associations remain significant, it is often unclear whether participants are in the preclinical phase of dementia at the time of testing. Indeed, research has found that APOE-related cognitive effects are minimized when future dementia is taken into account (Bäckman et al., in press; Bondi, Salmon, Galasko, Thomas, & Thal, 1999).

Our second objective in the present study, therefore, is to directly address this issue by conducting our statistical analyses in the full sample and, then again, having removed those who become demented up to 6 years following testing. Finally, we control for the potentially confounding influence of cerebrovascular and cardiovascular factors in our statistical analyses, as those factors are known to be related to APOE, and also vitamin B₁₂, folate, and associated homocysteine levels, and indeed, cognitive performance (Barclay, Weiss, Mattis, Bond, & Blass, 1988; Ebly, Schaefer, Campbell, & Hogan, 1998).

**METHODS**

**Participants**

The sample for this and the earlier study (Bunce, Kivipelto, et al., 2004) was drawn from individuals aged 75 years or older who participated in the Kungsholmen Project. This is a multidisciplinary project located in Stockholm involving medical examination, social and family interviews, laboratory blood analysis, and cognitive testing (see Fratiglioni, Viitanen, Bäckman, Sandman, & Winblad, 1992, for further details). The ethical committee of the Karolinska Institute, Stockholm, approved the project. In total, data for 528 persons were available. Of that number, 130 individuals were diagnosed as demented according to criteria in the Diagnostic and Statistical Manual of Mental Disorders, third edition, revised (American Psychiatric Association, 1987; DSM-III-R), and a further 33 were diagnosed as suffering depression. As it is possible that inclusion of those persons would confound interpretation of our results, we excluded them from the sample. We removed 37 individuals as a result of incomplete vitamin B₁₂ or folate data, and we also excluded persons taking vitamin B₁₂ or folate supplements (n = 16). In addition, we removed 32 participants with abnormally high folate levels, because such high values may be indicative of undetected disease. Finally, APOE data were unavailable for 113 persons. The final sample of 167 persons had a mean age of 82.81 years, was 80.24% female, and recorded a mean of 8.85 years of education. The corresponding values for those persons removed from the sample were 85.05, 80.90%, and 8.44, respectively. We calculated group means for missing data in a minority of cases.

**Cognitive Variables**

**Face recognition measures.**—Black and white photographs (12.6 cm × 17.6 cm) of 20 dated famous faces from the period 1930–1950 (e.g., Greta Garbo) and 20 contemporary famous faces from the 1980s (e.g., Stefan Edberg) were presented for 5 s each at study. The faces were drawn from the domains of politics, literature, music, film, and sport, and they were equal with respect to the age when the photo was taken. At test, which immediately followed the study phase, those faces were presented randomly with an equal number of distracter faces, half of which were dated, and half contemporary. Participants indicated whether they recognized the face from the earlier
presentation. We report hits, false alarms, and adjusted hits (i.e., hits – false alarms) for dated and contemporary famous faces in the paragraphs that follow.

**Short-term memory.**—We used the forward and backward digit span tasks (FDS and BDS tasks, respectively) of the Wechsler Adult Intelligence Scale–Revised (WAIS-R: Wechsler, 1981) to measure short-term memory, and we administered them according to standard procedures.

**Visuospatial ability.**—Three variables related to this cognitive domain. First, we administered the time-restricted version of the block design test from the WAIS-R battery following standard procedures. In addition, we also recorded clock setting and clock-reading ability. In the former task, participants were required to draw the hands on a series of five blank clock faces to given times spoken by the experimenter. The latter task involved reading aloud the times indicated by the hands on a series of five printed clock faces. Both tasks draw on visuospatial skills; clock setting involves visuoconstructual skills, whereas clock reading calls on visuoperceptual abilities.

**Physiological Variables**

**Cardiovascular and cerebrovascular diseases.**—We examined computer records of all participant inpatient hospital admissions for the 5-year period prior to cognitive testing. We recorded diagnoses of any of the following diseases: diabetes, cerebrovascular diseases, stroke (hemorrhage, ischemic, or nonspecific), transient ischemic attack, ischemic heart disease, heart failure, myocardial infarction, angina, arrhythmia, and arterial fibrillation.

**Vitamin B₁₂ and folate, and APOE.**—Assistants collected blood serum samples for analyses of vitamin B₁₂ and folate levels on the morning of the day of cognitive testing. We conducted analyses of both vitamins in the same laboratory by using the radio immunoassay method (see Chen, Silberstein, Maxon, Volle, & Sohlein, 1982). We used a microsequencing method involving polymerase chain reaction to determine the APOE genotype, using DNA extracted from peripheral white blood cells (see Small et al., 1998, for further details). This procedure was conducted blind to clinical information.

### Table 1. Biographical Variables as a Function of APOE and Vitamin B₁₂

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-ε4</th>
<th>ε4</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Low B₁₂</td>
<td>Normal</td>
</tr>
<tr>
<td>Biographical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>85.91 (5.87)</td>
<td>81.31 (5.32)</td>
</tr>
<tr>
<td>Women (%)</td>
<td>75.93</td>
<td>79.69</td>
</tr>
<tr>
<td>Education (years)</td>
<td>8.38 (2.58)</td>
<td>9.41 (3.46)</td>
</tr>
<tr>
<td>Vitamin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B₁₂ (pmol/L)</td>
<td>170.82 (57.27)</td>
<td>381.59 (113.80)</td>
</tr>
<tr>
<td>Folate (nmol/L)</td>
<td>15.83 (5.89)</td>
<td>20.55 (9.90)</td>
</tr>
</tbody>
</table>

*Notes: The B₁₂ cutoff was <251 pmol/L. APOE = apolipoprotein E. Standard deviations are given in parentheses.*

**Procedure**

We administered a battery of cognitive measures, including those reported here, and we took blood samples. For statistical purposes, we classified participants as either APOE ε4 (ε2/ε4, ε3/ε4, ε4/ε4), or non-ε4 (ε2/ε2, ε2/ε3, ε3/ε3). Within those groups, we stratified vitamin B₁₂ and folate as follows: low B₁₂ ≤ 250 pmol/L; low folate ≤ 12 nmol/L. We assigned participants with values above those thresholds to a “normal” group. Prior research (Bunce et al., 2004; Hassing et al., 1999) has shown these cutoffs to be sensitive to B vitamin–cognitive effects in older adults.

**RESULTS**

For analyses with B₁₂ as the between-subjects factor, we present descriptive data in Table 1. We subjected group means for the biographical variables to a series of 2 × 2 analyses of variance (ANOVAs) in which vitamin status (low or normal) and APOE genotype (ε4 or non-ε4) served as between-subjects factors. For chronological age, we identified significant main effects for APOE, F(1, 163) = 7.74, p = .006, η² = .045, and B₁₂, F(1, 163) = 19.92, p = .001, η² = .105. The non-ε4 groups were older, as were those in the low B₁₂ groups. The APOE × B₁₂ interaction was nonsignificant. For years of education, the main effect for APOE and the APOE × B₁₂ interaction were statistically nonsignificant. However, persons recording normal B₁₂ values had significantly more years of education, F(1, 163) = 5.77, p = .017, η² = .034. Because of these significant between-group differences, we entered age and years of education as covariates into all of the analyses reported in the paragraphs that follow. Even though women made up the majority of the sample, we also entered gender as a covariate into the analyses.

The principal statistical procedure involved a 2 × 2 analysis of covariance (ANCOVA), in which APOE genotype (ε4 vs non-ε4) and vitamin level (low vs normal) served as between-subjects factors. In the case of face recognition measures, dated versus contemporary famous faces formed a within-subjects factor.

**Vitamin B₁₂, APOE, and Recognition of Contemporary and Dated Famous Faces**

APOE and vitamin B₁₂ group means for the face recognition variables are provided in Table 2.

**Hits.**—The main effect for APOE was nonsignificant, as were the two-way interactions for APOE × B₁₂, APOE × Face type,
Table 2. Cognitive Variables as a Function of APOE and Vitamin B₁₂

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-ε4 Low B₁₂</th>
<th>Normal</th>
<th>ε4 Low B₁₂</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recognition</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hits Contemp</td>
<td>13.43 (5.10)</td>
<td>15.57 (3.80)</td>
<td>13.89 (4.67)</td>
<td>17.00 (1.92)</td>
</tr>
<tr>
<td></td>
<td>15.34 (4.13)</td>
<td>16.91 (2.89)</td>
<td>14.44 (4.26)</td>
<td>18.57 (1.43)</td>
</tr>
<tr>
<td>Dated</td>
<td>1.96 (1.76)</td>
<td>1.49 (1.97)</td>
<td>2.04 (2.50)</td>
<td>2.00 (2.05)</td>
</tr>
<tr>
<td></td>
<td>1.86 (2.49)</td>
<td>0.91 (1.19)</td>
<td>2.19 (2.89)</td>
<td>0.71 (1.27)</td>
</tr>
<tr>
<td>FAs Contemp</td>
<td>11.47 (5.27)</td>
<td>14.08 (4.23)</td>
<td>11.85 (5.64)</td>
<td>15.00 (3.02)</td>
</tr>
<tr>
<td></td>
<td>13.48 (4.96)</td>
<td>16.00 (3.40)</td>
<td>12.26 (5.60)</td>
<td>17.86 (2.35)</td>
</tr>
<tr>
<td></td>
<td>0.390 (0.473)</td>
<td>0.308 (0.430)</td>
<td>0.400 (0.441)</td>
<td>0.133 (0.374)</td>
</tr>
<tr>
<td></td>
<td>0.276 (0.405)</td>
<td>0.253 (0.324)</td>
<td>0.354 (0.401)</td>
<td>0.139 (0.253)</td>
</tr>
<tr>
<td>Short-term memory</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FDS</td>
<td>5.26 (1.09)</td>
<td>5.83 (1.15)</td>
<td>5.36 (0.91)</td>
<td>5.52 (1.29)</td>
</tr>
<tr>
<td>BDS</td>
<td>4.00 (1.06)</td>
<td>4.17 (1.11)</td>
<td>4.07 (0.94)</td>
<td>4.19 (1.03)</td>
</tr>
<tr>
<td>Vissuospatial</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Block design</td>
<td>11.33 (5.72)</td>
<td>14.95 (5.55)</td>
<td>12.32 (4.30)</td>
<td>13.95 (4.53)</td>
</tr>
<tr>
<td>Clock reading</td>
<td>4.53 (0.86)</td>
<td>4.58 (1.02)</td>
<td>4.57 (0.79)</td>
<td>4.95 (0.22)</td>
</tr>
<tr>
<td>Clock drawing</td>
<td>3.37 (1.18)</td>
<td>3.86 (1.11)</td>
<td>3.61 (0.99)</td>
<td>3.95 (0.97)</td>
</tr>
</tbody>
</table>

Notes: The B₁₂ cutoff was < 251 pmol/L; APOE = apolipoprotein E; contemp = contemporary; FAs = false alarms; Adj. hits = hits – FAs; BDS = forward digit span; BDS = backward digit span; C = response bias. Standard deviations are given in parentheses.

and B₁₂ × Face type. Main effects were statistically significant for B₁₂, \( F(1, 160) = 7.15, p = .008, \eta^2 = .043 \), and face type, \( F(1, 163) = 34.74, p < .001, \eta^2 = .176 \); low B₁₂ groups recorded poorer face recognition scores, and participants recognized a greater number of dated famous faces. The APOE × B₁₂ × Face type interaction approached statistical significance at conventional levels (\( p = .05 \)), \( F(1, 163) = 3.03, p = .083, \eta^2 = .018 \). Table 2 suggests that the recognition performance of ε4-carrying persons in the low vitamin group did not benefit from prior knowledge of dated famous faces to the same degree as other groups.

False alarms.—With two exceptions, all statistics involving false alarms (FAs) were nonsignificant. Fewer FAs were produced in the dated face condition, \( F(1, 163) = 7.38, p = .007, \eta^2 = .043 \), although this was modified by a significant B₁₂ × Face type interaction, \( F(1, 163) = 8.15, p = .005, \eta^2 = .048 \); participants recording normal B₁₂ values produced fewer FAs for dated faces.

Adjusted hits.—We found it desirable to calculate hit scores adjusted for the number of FAs (i.e., hits – FAs), as high scores for hits may come at the expense of high scores for FAs. The main effect for APOE and the two-way interactions involving APOE and B₁₂ and APOE and face type were all statistically nonsignificant. Main effects for B₁₂, \( F(1, 160) = 9.39, p = .003, \eta^2 = .055 \), and face type, \( F(1, 163) = 57.47, p < .001, \eta^2 = .261 \), and the B₁₂ × Face type interaction, \( F(1, 163) = 6.23, p = .014, \eta^2 = .037 \), were all significant; those statistics suggested that fewer adjusted hits were produced by the low B₁₂ group in respect to contemporary faces. However, the foregoing was modified by a significant APOE × B₁₂ × Face type interaction, \( F(1, 163) = 7.13, p = .008, \eta^2 = .042 \). As with unadjusted hits, Table 2 suggests that ε4-carrying low vitamin persons benefited least from prior knowledge of dated faces. We confirmed this impression by a series of simple tests. Although the B₁₂ × Face type interaction was nonsignificant in the non-ε4 group, that interaction was significant among ε4 carriers, \( F(1, 164) = 10.11, p = .002, \eta^2 = .058 \). A further simple test within the ε4 group showed prior knowledge of dated faces to benefit recognition performance among persons with normal B₁₂ levels, \( F(1, 165) = 16.63, p < .001, \eta^2 = .092 \). However, the simple test did not attain significance in the ε4–low B₁₂ group. Therefore, it appears that carrying the ε4 allele in combination with low levels of vitamin B₁₂ is associated with impaired retrieval of prior knowledge relative to groups possessing either the ε4 allele alone or low B₁₂ levels alone.

To eliminate the possibility that our main finding in the aforementioned analyses was related to the preclinical phase of as yet undetected dementia, we repeated the ANCOVAs on adjusted hits, having removed individuals diagnosed as demented according to DSM-III-R criteria up to 6 years following testing. We also omitted from the analysis those who died or declined further participation in that period (consequent \( n = 86 \)). Consistent with the original analyses, the APOE × B₁₂ × Face type interaction for adjusted hits remained significant, \( F(1, 82) = 10.48, p = .002, \eta^2 = .113 \), with simple tests confirming that ε4-carrying low B₁₂ persons did not utilize prior knowledge as effectively as other groups.

Vitamin B₁₂, APOE, Short-Term Memory, and Vissuospatial Skills

Table 2 also includes means for FDS, BDS, clock reading and drawing, and block design. We entered age, years of education, and gender as covariates in ANCOVAs for each variable. In these analyses, neither main effects nor interactions were statistically significant for any of the variables. Removing incident dementia cases up to 6 years following testing did not affect these nonsignificant results.

Folate, APOE, and Recognition of Contemporary and Dated Famous Faces

We present biographical data partialed according to APOE and folate group in Table 3, and face recognition data in Table 4. We repeated the series of ANCOVAs for cognitive variables with folate as the second between-subjects factor, and we included age, years of education, and gender as covariates.

Hits.—All statistics were nonsignificant with the exception of the main effects for folate, \( F(1, 160) = 6.58, p = .011, \eta^2 = .039 \), and face type, \( F(1, 163) = 25.26, p < .001, \eta^2 = .134 \). Higher recognition scores were produced in persons recording normal folate values, and in the dated face condition.

FAs.—All main effects and interactions were nonsignificant.

Adjusted hits.—Statistics for the APOE main effect, and all two-way interactions, were not significant. Main effects for folate, \( F(1, 160) = 6.93, p = .009, \eta^2 = .042 \), and face type,
A range of cognitive variables, and it took into account cardiovascular and cerebrovascular diseases and the possibility that participants were in the preclinical phase of dementia. We found an association between the APOE e4 allele and low B vitamin values in respect to face recognition, but not short-term memory or visuospatial skills. However, the effect for face recognition arose as e4-carrying persons with low B vitamin values appeared less able to utilize prior knowledge of dated famous faces to enhance recognition performance, relative to participants who possessed either the e4 allele alone or low B vitamin values alone. It appears, therefore, that possession of the e4 allele in combination with low B vitamin levels deleteriously affected retrieval processes in more demanding face recognition conditions. The findings suggest that this effect was independent of future dementia and was not related to cerebrovascular and cardiovascular diseases.

### Table 3. Biographical Variables as a Function of APOE and Folate

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-e4</th>
<th>e4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low Folate</td>
<td>Control</td>
</tr>
<tr>
<td>N</td>
<td>30</td>
<td>88</td>
</tr>
<tr>
<td>Biographical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>84.97 (6.01)</td>
<td>82.89 (5.96)</td>
</tr>
<tr>
<td>Women (%)</td>
<td>70.00</td>
<td>80.68</td>
</tr>
<tr>
<td>Education (years)</td>
<td>9.67 (4.11)</td>
<td>8.69 (2.68)</td>
</tr>
<tr>
<td>Vitamin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folate (nmol/L)</td>
<td>10.27 (1.44)</td>
<td>21.16 (8.27)</td>
</tr>
<tr>
<td>B_{12} (pmol/L)</td>
<td>259.48 (142.90)</td>
<td>293.89 (138.64)</td>
</tr>
</tbody>
</table>

Notes: The folate cutoff was < 13 nmol/L. APOE = apolipoprotein E. Standard deviations are given in parentheses.

Finally, it is possible that either cardiovascular or cerebrovascular diseases were associated with APOE, B vitamins, or the cognitive variables and were, therefore, influential in our analyses. To control for this possible confound, we initially subjected those diseases to principal component analysis with varimax rotation. This procedure reduces highly intercorrelated variables to meaningful clusters, thereby increasing reliability. Four factors with loadings greater than 0.5 emerged from this procedure, accounting for 78.09% of the explained variance. The diagnoses groupings related to stroke (hemorrhage, ischemic, and nonspecific), coronary heart disease (ischemic, angina, and myocardial infarction), other heart diseases (heart failure, arterial fibrillation, and arrhythmia) and diabetes, and other cerebrovascular diseases (transient ischemic attack or cerebrovascular diseases). We repeated all the ANCOVAs for both B_{12} and folate, with the factor scores for each component identified by the principal component analysis entered as additional covariates. This procedure did not affect the results of our original analyses in any way, suggesting that our findings were unrelated to the influence of cardiovascular and cerebrovascular diseases.
The results are consistent with earlier research that demonstrates an association between B vitamins and episodic memory in old age (e.g., Hassing et al., 1999; Wahlin et al., 1996), and work showing an association between APOE and cognitive function (e.g., Deary et al., 2002; Hofer et al., 2002; Hyman et al., 1996; Jonker et al., 1998; Mayeux et al., 2001; Mortensen & Hogh, 2001). However, the present study extends this work by demonstrating that the observed APOE-related effects in respect to face recognition varied according to B vitamin status and the cognitive demands of the task. This finding was evident in respect to both vitamin B12 and folate, and, together with evidence of close biochemical linkage between the two vitamins elsewhere (Calvaresi & Bryan, 2001), suggests that the mechanisms mediating the relationship with APOE are similar for both types of B vitamins. It is also apparent that the effect was selective as associations with visuospatial skills. This finding is in line with work elsewhere (Haan et al., 1999) that also failed to find an interaction between APOE and health-related factors in respect to digit-symbol substitution, which is another task calling on short-term memory. More broadly, the findings suggest that, in ε4 carriers, some brain structures and processes are more vulnerable than others to the deleterious influence of an additional risk factor such as low B vitamin levels. Indeed, as we noted earlier, structural imaging work indicates that the hippocampus is compromised in ε4 carriers relative to noncarriers (Cohen et al., 2001; Moffat et al., 2000; Plassman et al., 1997), and functional imaging studies suggest more diffuse brain activation during a cognitive task in persons possessing the ε4 allele (Buckheimer et al., 2000). Moreover, both the hypomethylation and homocysteine hypotheses hold that low B vitamin deficits compromise neurological structures and processes. The consequence is likely to be deficits in cognitive domains supported by those vulnerable neuroanatomical structures and processes. That we found deficits in face recognition but not short-term memory or visuospatial skills suggests that the structures and processes underpinning that cognitive domain are vulnerable to the deleterious effects of possession of the ε4 allele in combination with low B vitamin levels.

The foregoing suggests that the biochemical processes described by the hypomethylation and homocysteine hypotheses may render ε4 carriers more vulnerable to brain insult. Further, ε4 carriers may not possess the same level of neural reserves to buffer against the deleterious neurological consequences of additional factors such as low B vitamin levels. This idea is consistent with the brain or cognitive reserve hypotheses (e.g., Cummings, Vinters, Cole, & Khachaturian, 1998; Katzman, 1993; Mortimer, 1988; Satz, 1993; Skoog, 2000; Stern, 2002), in which reserves are commonly held to account for the lower incidence of dementia or reduced cognitive impairment in more highly educated individuals. A high level of education and a subsequent intellectually stimulating lifestyle are said to enrich neural interconnectivity, and this acts as a buffer against the progression of neurodegenerative disease (e.g., Katzman, 1993). This “active” version of the reserve hypothesis (Stern, 2002) is directly related to the degree of intellectual stimulation an individual is exposed to. In contrast, however, a “passive” version holds that reserves are determined by the integrity of neuroanatomical structures and the extent to which they buffer the pathological progression of disease, or consequences of brain insult. Our results are consistent with this latter version of the hypothesis because the detrimental influence of low B vitamin levels was greater in ε4 carriers relative to non-ε4 carriers, presumably because of their heightened vulnerability to brain injury.

It is also of note that, for face recognition, the association between APOE and B vitamins was characterized by ε4-carrying low B vitamin persons’ inability to utilize prior knowledge of dated famous faces. This finding is consistent with provisional data suggesting persons in possession of the ε4 allele were less able to engage cognitive support (e.g., physical enactment of to-be-remembered materials during learning, and retrieval cues at recall) to aid the mental operations required in episodic memory (Nilsson, Nyberg, & Bäckman, 2002). In contrast, though, there are instances where ε4 carriers with low B vitamin values are able to benefit from experimental manipulations designed to enhance episodic memory performance. For example, our earlier study investigating free recall of words in the present sample (Bunce, Kivipelto, et al., 2004) found that performance in ε4 carriers with low B vitamin values was poorer in a speeded relative to a slower encoding condition (2 s vs 5 s per word). In other words, the additional encoding time appears to have helped the mental operations required for conferring the information to memory. One explanation for that finding relates to processing speed accounts of cognitive aging, and older adults’ reduced capacity for processing information rapidly. However, another possibility is that the vulnerability of encoding and retrieval operations varies according to the domain of episodic memory under investigation. The exact mechanisms by which this may occur are unclear, but the present findings together with those of the related study suggest that it is encoding operations in free recall, and retrieval operations in face recognition, that are vulnerable. It should be noted that, in both studies, it was the more demanding task conditions in which the APOE–B vitamin effect was found. Further research is required across a range of cognitive tasks that systematically vary cognitive demands.

An important finding was that the main results remained, even after we controlled for future dementia up to 6 years following testing. Although previous work has found ε4-related effects on cognitive performance to disappear when impending dementia was taken into account (e.g., Bäckman et al., in press; Bondi et al., 1999), other studies suggest that cognitive decline is unrelated to APOE in the preclinical phase of dementia (Bunce, Fratiglioni, Small, Winblad, & Bäckman, 2004; Lange et al., 2002). Although it is plausible that the influence of APOE on cognitive deficits is purely related to impending dementia, those null findings and the present data suggest that this may not always be the case. Indeed, it is possible that there are complex circumstances in which APOE influences cognition in old age irrespective of future dementia status. The present findings suggest that additional deleterious influences such as low B vitamin levels and cognitive task demands may be influential in this respect. Again, it is important that researchers explore this possibility further.

The present study possesses some limitations that should be acknowledged. First, data relating to homocysteine levels were not available, and inclusion of such information would have added to our conclusions. Second, stratifying for vitamin level
meant that group sizes were too small for us to examine the e4 dose effect, and that we were unable to control for future dementia in analyses involving folate. Third, although we took hospital admissions for cerebrovascular and cardiovascular diseases into account, those data obviously omit cases that did not require hospitalization. Therefore, we may have underestimated the occurrence of those diseases. Fourth, although we controlled for future dementia up to 6 years following testing in respect to vitamin B₁₂, we cannot dismiss the possibility that this period was insufficiently long to capture all of the participants who eventually became demented. Therefore, it is possible that our findings were related to a preclinical phase of dementia stretching beyond the 6-year study period. Finally, we should note that, as a result of the current conceptual debate surrounding mild cognitive impairment, and evidence of low conversion rates to dementia (e.g., Ritchie, Artero, & Touchon, 2001), we did not take this condition into account in our analyses.

To conclude, our findings suggest that APOE e4 carriers with low vitamin B₁₂ levels may be vulnerable to cognitive impairment in demanding task conditions. Importantly, our analyses indicate that such associations are unrelated to incident dementia up to 6 years following testing. The results are consistent with theoretical accounts suggesting that individual differences exist in the neurological reserves available to buffer against the detrimental influence of disease and environmental factors. The present findings suggest that there may be a genetic basis to such reserves, and that future research should investigate APOE and cognitive functioning in older persons while taking into account additional environmental factors that potentially damage neurological structures and processes.

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