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Utilization of Cognitive Support in Episodic Free Recall as a Function of Apolipoprotein E and Vitamin B_{12} or Folate Among Adults Aged 75 Years and Older

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Apolipoprotein E (*APOE*), vitamin B_{12}, and folate were examined in relation to free recall among 167 community-based older adults. Cognitive support at encoding and retrieval was also taken into account. Participants were classified as *APOE* $\epsilon 4$ or non-$\epsilon 4$ allele carriers and as either low or normal vitamin B_{12} or folate status. A significant association was identified between low vitamin B_{12} and the $\epsilon 4$ genotype in respect to free recall, but only in circumstances of low cognitive support. This result remained after removing dementia cases that occurred up to 6 years after testing. A similar, but nonsignificant, trend was evident in relation to folate. The research is discussed with reference to vulnerability models and genetic influences on brain reserves.

Psychosocial models of life stress propose that the vulnerability of an individual faced with a stressful life event to adverse health consequences is moderated by the combined influence of preexisting personal dispositions and prevailing social conditions (e.g., Dohrenwend & Dohrenwend, 1981). Individuals of a particular disposition may be more vulnerable to negative outcomes if detrimental social conditions exist. A parallel exists between these vulnerability models and research investigating genetic associations with cognitive function in old age and the extent to which nongenetic factors may influence such associations. It is possible that individuals of a particular genetic disposition are more vulnerable to cognitive deficits in later life given certain environmental conditions. Here, we test this vulnerability hypothesis in nondemented adults aged 75 years and older. Specifically, we investigated episodic memory performance in relation to apolipoprotein E (*APOE*) and two nutritional variables, vitamin B_{12} and folate. We evaluated how far *APOE* genotype and low B vitamin levels rendered individuals vulnerable to episodic memory deficits in old age. Also, task demands were taken into account by varying the level of cognitive support at the encoding and retrieval phases of the episodic memory task. Manipulation of such support is of interest because earlier work (Bunce, 2001a, 2001b) suggests the tasks most sensitive to underlying physiological mechanisms in older adults are those that place the greatest demands on cognitive processes (i.e., low cognitive support). Here, we question whether vitamin B_{12} or folate and *APOE* genotype interact to influence episodic memory in very old age and whether cognitive support affects that relationship.

APOE is involved in cholesterol transportation and is determined by the combination of three alleles: $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$. There is clear evidence that possession of the $\epsilon 4$ allele is a risk factor for dementia (for a review, see Farrer et al., 1997). However, work investigating whether the presence of the $\epsilon 4$ allele confers a greater vulnerability to cognitive impairment among nondemented older adults is more equivocal. For example, individuals possessing the $\epsilon 4$ allele exhibited more precipitous decline 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). By contrast, studies suggest no association between the $\epsilon 4$ allele and decline in fluid intelligence (Pendleton et al., 2002), composite visuospatial and language factors (Mayeux et al., 2001), and proxy measures of IQ (Deary et al., 2003). Cross-sectional work found no association between *APOE* and episodic, semantic, or working memory, perceptual speed, or visuospatial ability after controlling for dementia (Bennett et al., 2003). Regarding measures of global cognitive performance, some studies suggest decline to be greater in $\epsilon 4$ carriers (e.g., Bretsky, Guralnik, Launer, Albert, & Seeman, 2003; Fillenbaum et al., 2001; Jonker et al., 1998), whereas others show no such differentials (Winnock et al., 2002). Given those equivocal

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findings, research that has investigated *APOE* in the presence of another deleterious physiological factor suggests that ε4 carriers are indeed more vulnerable to cognitive deficits. For instance, cognitive impairment was greater in ε4-carrying older adults suffering peripheral vascular disease and atherosclerosis (Haan, Shemanski, Jagust, Manolio, & Kuller, 1999), olfactory dysfunction (Borenstein Graves, et al., 1999), and low estrogen use (Yaffe, Haan, Byers, Tangen, & Kuller, 2000).

To date, little research has investigated nutritional factors and *APOE* ε4 in respect to cognitive performance in older adults. Earlier work suggests two B vitamins, B_{12} and folate, may provide a particularly worthwhile avenue for exploration. For instance, among older adults, vitamin B_{12} and folate 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 (Lindeman et al., 2000; Robins Wahlin, Wahlin, Winblad, & Bäckman, 2001), and also with spatial copying skills (Riggs, Spiro, Tucker, & Rush, 1996). Intervention studies (e.g., Martin, Francis, Protetch, & Huff, 1992; Meadows, Kaplan, & Bromfield, 1994) have established a link with improved cognition in demented or cognitively impaired individuals, and low levels of those nutrients have also been associated with an increased risk of Alzheimer's disease (e.g., Wang et al., 2001). More broadly, there is evidence suggesting that subclinical differences in those B vitamins may influence cognitive performance (see Calvaresi & Bryan, 2001).

Together, those findings raise the possibility that possession of the ε4 allele and low B vitamin levels will increase vulnerability to cognitive impairment because of their combined deleterious effect on neural structures and processes. However, no empirical research has tested this possibility. Here, we address this shortfall in a population-based sample of dementia- and depression-free adults 75 years of age and older. As noted earlier, there is evidence that demanding task conditions are more sensitive to underlying physiological mechanisms than those that are less demanding. Therefore, we manipulated the level of task demands by varying cognitive support at both the encoding and retrieval phases of an episodic free-recall task. Finally, given the possibility that cerebro- and cardiovascular diseases are related to both *APOE* and vitamin B_{12} and folate levels, we took those influences into account in our investigation.

Method

Participants

The sample was drawn from inhabitants of the Kungsholmen parish in Stockholm 75 years of age and older, participating in a multidisciplinary project involving medical examination, social and family interviews, laboratory blood analysis, and cognitive testing (see Fratiglioni, Viitanen, Bäckman, Sandman, & Winblad, 1992, for a detailed description). Data were available for 528 individuals. Of this number, 130 were diagnosed according to the *Diagnostic and Statistical Manual of Mental Disorders III–R* criteria (American Psychiatric Association, 1987) as suffering dementia, and a further 33 were diagnosed with depression. Because inclusion of those individuals would affect interpretation of our results, they were removed from the statistical analyses. A further 37 were removed because of incomplete vitamin B_{12} or folate data. Sixteen participants who were taking vitamin B_{12} or folate supplements were also excluded. Inspection of the remaining data revealed 32 individuals to have abnormally high folate levels. Because such high values may be indicative of undetected disease, those persons also were removed from the sample. Finally, *APOE* data were unavailable for 113 persons. The final sample size included 167 participants (mean age = 82.81 years, *SD* = 5.68); 134 (80.24%) were female, and the sample had a mean of 8.85 years of education (*SD* = 2.98). Table 1 provides descriptive data for persons included in, and eliminated from, the sample. For a minority of cases in the statistical analyses, missing data were replaced by imputing the group mean for that variable.

Episodic Memory Measures

Free recall of semantically unrelated words. Two lists of 12 semantically unrelated concrete nouns were randomly selected from a pool of 48 nouns, equivalent in respect to visual and tactile imagery, meaningfulness, and frequency (Molander, 1984). The two lists were presented bimodally to participants at either rapid (2 s per word) or slow (5 s per word) rates, counterbalanced across participants. The interim interval was 1 s. Participants were told to remember as many words as possible for a subsequent recall test. Immediately after presentation, 2 min were allowed for oral free recall.

Free and cued recall of organizable words. A further word list of 12 nouns belonging to four taxonomic categories was administered (i.e., clothes, furniture, professions, musical instruments) bimodally at a rate of 5 s per word. Participants were not informed of the taxonomic categories beforehand. In free recall, participants were given 2 min to recall as many of the words as possible. A cued-recall condition followed in which the taxonomic categories served as cues. For each category, 30 s was allowed for recall.

The episodic memory tasks can be conceived of as lying along a continuum of cognitive support. The lowest level of cognitive support was available in the condition allowing 2 s encoding time for semantically unrelated words. Cognitive support was increased in each condition, respectively, by extending encoding time for semantically unrelated words to 5 s, providing organizable taxonomic categories at encoding, and finally, offering cued recall of those taxonomic categories.

Physiological Variables

Cardio- and cerebrovascular factors. Because cardio- and cerebrovascular factors may be independently associated with *APOE,* vitamin B_{12},

Table 1
Means and Standard Deviations for Persons Included in and Excluded From the Sample

Variable	Included	Excluded	*p*
N	167	361	
Age			< .01
M	82.81	85.05	
SD	5.68	5.05	
Women (%)	80.24	80.90	*ns*
Education (years)			*ns*
M	8.85	8.44	
SD	2.98	2.28	
No. diseases			< .05
M	26	98	
SD	15.57%	27.15%	
B_{12} (pmol/L)			< .01
M	279.77	370	
SD	139.35	342.38	
Folate (nmol/L)			< .01
M	18.19	23.17	
SD	8.71	16.10	

Note. Chi-square tests were used for women and diseases, and *t* tests were used otherwise.

and folate, and also cognitive performance in older adults, it was desirable to take those variables into account. Therefore, computerized hospital inpatient admission records were examined for the entire sample for the 5-year period before cognitive testing. Admissions for any of the following complaints were recorded: diabetes, cerebrovascular diseases, stroke (hemorrhage, ischemic, or nonspecific), transient ischemic attack, ischemic heart disease, heart failure, myocardial infarction, angina, arrhythmia, and arterial fibrillation.

Vitamin B_{12}, folate, and APOE. Analyses of serum vitamin B_{12} and folate were conducted in the same laboratory using the radioimmunoassay method (see Chen, Silberstein, Maxon, Volle, & Sohnlein, 1982). *APOE* genotyping was conducted, without knowledge of clinical information, on DNA extracted from peripheral white blood cells. A microsequencing method involving polymerase chain reaction was used to determine the *APOE* genotype (see Small et al., 1998, for further details).

Procedure

The ethical committee of the Karolinska Institute, Stockholm, approved the project.

A battery of cognitive measures, including the memory measures reported here, was administered. Blood samples for analyses of vitamin B_{12} and folate levels were collected on the morning of the day of cognitive testing.

Participants were classified as either *APOE* ϵ4 (ϵ2/ϵ4, ϵ3/ϵ4, ϵ4/ϵ4), or non-ϵ4 (ϵ2/ϵ2, ϵ2/ϵ3, ϵ3/ϵ3). Within those groups, vitamin B_{12} and folate were stratified as follows: low $B_{12} < 251$ pmol/L; low folate < 13 nmol/L. Participants with values greater than those thresholds were designated as normal.

Results

Vitamin B_{12}, APOE, and Episodic Memory

Descriptive data relating to the four *APOE*-vitamin B_{12} groups for age, gender, years of education, and vitamin levels are presented in Table 2. Before the statistical analyses of primary interest were undertaken, several preliminary procedures were performed. First, between-groups differences in the biographical variables listed in Table 2 were subjected to a series of 2×2 analyses of variance (ANOVAs) in which vitamin B_{12} (low–normal) and *APOE* genotype (ϵ4–non-ϵ4) formed between-subjects factors. For chronological age, the ANOVA revealed significant main effects for *APOE*, $F(1, 163) = 7.74$, $\eta^2 = .045$, $p = .01$, and vitamin B_{12}, $F(1, 163) = 19.22$, $\eta^2 = .105$, $p < .01$, but the *APOE* $\times$ B_{12} interaction was nonsignificant ($p > .46$). Members of the non-ϵ4 groups were older (83.61 vs. 81.11 years), as were those in the low-B_{12} groups (84.33 vs. 80.39 years). Regarding years of education, the main effect for *APOE* and the *APOE* $\times$ B_{12} interaction were statistically unreliable ($ps > .71$). However, persons with normal vitamin B_{12} levels had significantly more years of education (9.42 vs. 8.21 years), $F(1, 163) = 5.77$, $\eta^2 = .034$, $p = .017$. Given those significant differences, both age and years of education were entered as covariates in the analyses reported next. Although a greater proportion of women comprised the sample, gender was also entered as a covariate.

As noted earlier, it was desirable to take cardio- and cerebrovascular diseases into account. Initially, those variables were subjected to principal-component analysis with varimax rotation for several reasons. First, the procedure provides a means by which to reduce highly intercorrelated variables to meaningful clusters, thereby increasing reliability. Also, it addresses the statistical problems associated with the use of dichotomous variables (i.e., participants either received a diagnosis for a disease, scored 1, or they did not, scored 0), and overlap of diagnoses in a particular episode (i.e., participants may receive several diagnoses in a specific episode of illness, underpinned by a common cause). Four factors resulted from this procedure, accounting for 78.1% of the explained variance. The diagnoses groupings related to stroke (hemorrhage, ischemic, and nonspecific), coronary heart disease (ischemic, angina, myocardial infarction), other heart diseases (heart failure, arterial fibrillation, arrhythmia) and diabetes, and other cerebrovascular diseases (transient ischemic attack, cerebrovascular diseases). However, bivariate correlations did not suggest that the factor scores arising from each of those four factors were correlated significantly with any of the cognitive variables. Neither did univariate ANOVA (*APOE* and either vitamin B_{12} or folate formed the between-subjects factors) on each of those factors reveal any variation as a function of *APOE*, vitamin B_{12}, or folate. Consideration of Table 2 suggests that the lack of association with cognitive, vitamin, or genetic variables may be due to the low prevalence of those diseases in this sample. Given that lack of association, cerebrovascular and cardiovascular diseases were not considered further in our analyses.

Turning to the main statistical analyses, episodic memory variables were subjected to a series of $2 \times 2 \times 2$ analyses of

Table 2
Biographical and Memory Variables as a Function of APOE and Vitamin B_{12}

Variable	Non-ϵ4		ϵ4	
	Low B_{12}	Normal	Low B_{12}	Normal
n	54	64	28	21
Demographic data				
Age (years)				
M	85.91	81.31	82.75	79.48
SD	5.87	5.32	5.00	2.98
Women (%)	75.93	79.69	92.86	76.19
Education (years)				
M	8.38	9.41	8.04	9.43
SD	2.58	3.46	1.71	3.39
Diseases (*n*)	7	10	4	5
Vitamin				
B_{12} (pmol/L)				
M	170.82	381.59	177.18	386.38
SD	57.27	113.80	53.81	126.02
Folate (nmol/L)				
M	15.83	20.55	15.93	20.05
SD	5.89	9.90	7.94	10.02
Memory				
unrelated				
2-s encoding				
M	4.78	5.32	3.68	6.48
SD	1.72	1.62	1.42	2.18
5-s encoding				
M	4.77	5.71	4.68	6.38
SD	1.90	1.64	2.13	2.56
Organizable				
Free recall				
M	5.92	6.91	5.93	7.14
SD	2.24	2.24	2.36	1.68
Cued recall				
M	7.81	8.78	8.11	8.81
SD	2.32	2.10	2.67	1.66

covariance (ANCOVAs), in which vitamin B_{12} level (low–normal) and *APOE* genotype (ϵ4–non-ϵ4) formed the between-subjects factors and cognitive support was the within-subjects factor (see later discussion for specific details). In all analyses, age, years of education, and gender were entered as covariates. Descriptive data for episodic memory variables as a function of *APOE* and B_{12} group are also provided in Table 2.

Free Recall of Semantically Unrelated Words After 2 s or 5 s Encoding Time

The cognitive support within-subjects factor in this ANCOVA was the amount of time allowed for encoding: 2 s or 5 s. Statistics suggested recall performance of low-B_{12}–ϵ4 carriers was less than that of the other groups, but only in the faster, 2-s encoding condition; this group exhibited more marked improvement relative to other groups, when encoding time was increased to 5 s.

Specifically, although the main effect for *APOE* was not statistically reliable ($p > .87$), that for vitamin B_{12} was significant, $F(1, 160) = 16.86$, $\eta^2 = .095$, $p = .01$; Table 2 suggests that persons with normal B_{12} levels recalled a greater number of words. The main effect for time support was also statistically reliable, $F(1, 163) = 4.17$, $\eta^2 = .025$, $p = .04$; greater time at encoding was associated with superior recall. With the exception of that involving *APOE* and B_{12}, $F(1, 160) = 8.70$, $\eta^2 = .052$, $p = .01$, all two-way interactions were nonsignificant (ps $> .27$). However, that significant two-way interaction was modified by a statistically reliable *APOE* $\times$ B_{12} $\times$ Time Support interaction, $F(1, 163) = 5.72$, $\eta^2 = .034$, $p = .018$. Table 2 suggests a relatively greater benefit was incurred in the ϵ4–low-vitamin group from 2-s to 5-s encoding conditions. Simple effects tests confirmed this impression. In the first tests, *APOE* group (ϵ4–non-ϵ4) was assessed within each level of vitamin group. That test for individuals of normal B_{12} levels was nonsignificant ($p > .48$). The equivalent test within the low-vitamin group, however, reached significance, $F(1, 164) = 4.81$, $\eta^2 = .028$, $p = .03$. Further simple effects tests were performed within the low-vitamin group for both levels of *APOE*. That test for the non-ϵ4 group was not significant ($p > .95$). Most importantly, though, the test for the ϵ4 group reached significance, $F(1, 165) = 8.30$, $\eta^2 = .048$, $p = .01$; the recall performance of ϵ4 carriers with low B_{12} levels significantly benefited when encoding time was increased from 2 s to 5 s.

Free Recall of Semantically Unrelated Versus Organizational Words

The within-subjects factor in this ANCOVA was the degree of support intrinsic to the word lists at study; lists were either semantically unrelated (low support) or organizable into four taxonomic categories (higher support). Main effects were significant for vitamin group, $F(1, 160) = 6.66$, $\eta^2 = .040$, $p = .01$, and level of support available, $F(1, 163) = 38.06$, $\eta^2 = .189$, $p = .00$; free-recall means were higher for the normal B_{12} group and for the word list with taxonomic categories embedded within it. The *APOE* main effect and all interactions were nonsignificant (ps $> .38$). In sum, increasing the level of support beyond provision of 5 s for encoding resulted in parallel performance gains irrespective of *APOE*–vitamin group.

Free Versus Cued Recall of Semantically Organizable Words

In this ANCOVA, the level of cognitive support (the within-subjects factor) was manipulated through providing cued recall, relative to free recall, of taxonomic categories. The main effect for cognitive support was significant, $F(1, 163) = 261.33$, $\eta^2 = .616$, $p = .00$; cued recall produced higher scores than free recall. However, all other statistics were nonsignificant (ps $> .12$).

In sum, the prior analyses suggest that the detrimental effect of ϵ4 in combination with low B_{12} levels was manifest in the most demanding condition of the current experiment (i.e., free recall after 2 s encoding time for semantically unrelated words; see Table 2). The magnitude of the increase in recall performance from 2 s to 5 s encoding is greater in the ϵ4–low-B_{12} group relative to the other groups. This between-group differential reduces as the level of cognitive support increases.

In considering these data, it is important to take into account two factors that may have influenced our findings. First, dementia has a long preclinical phase, and it is possible that persons in our ϵ4–low-B_{12} group were in the early stages of the disease. Data relating to incident dementia and mortality were available for participants 3 and 6 years after cognitive testing. To eliminate the possibility that our findings reflected the preclinical phase of the disease, all ANCOVAs were repeated; incident dementia cases were removed from the analyses. Regarding the second factor, investigators have used various cutoffs to define low vitamin B_{12} levels. To confirm that our results were not an artifact of the cutoff we adopted, statistical analyses were also rerun but with low B_{12} status defined as less than 201 pmol/L. Analyses were repeated with incident dementia cases 3 and 6 years after testing removed.

The results of those additional analyses are summarized in Table 3. Overall, the pattern of results from the reanalyses were remarkably consistent with those from the original regardless of cutoff used to define low-B_{12} status or whether incident dementia cases were excluded from the analyses. Specifically, for analyses in which low B_{12} was defined as less than 251 pmol/L, when data 3 years after testing were taken into account, 20 demented and 20 deceased participants were removed from the sample. An additional 7 individuals declined participation at follow-up. Of the final sample of 120, 95 (79.2%) were women. Participants had a mean age of 82.28 years ($SD = 5.08$) years and an average of 9.22 ($SD = 3.29$) years of education. Because age varied significantly according to B_{12} group ($p < .01$), and years of education was marginal to conventional levels of statistical significance ($p = .05$), both variables, together with gender, were again entered as covariates into the analyses.

According to Table 3, the results virtually replicated our earlier analyses. The ANCOVA comparing semantically unrelated words with 2-s versus 5-s encoding time produced a significant main effect for cognitive support ($p = .02$), whereas main effects for *APOE* and B_{12} were both nonsignificant (ps $> .15$). As in the earlier analyses, greater time support was associated with superior aggregate recall performance. The *APOE* $\times$ B_{12} interaction was statistically reliable ($p = .03$), and the other two-way interactions were nonsignificant ($p > .08$). However, the *APOE* $\times$ B_{12} $\times$ Time Support interaction again reached significance, this time with an increased effect size, $F(1, 116) = 6.77$, $\eta^2 = .055$, $p = .01$. Inspection of group means and simple effects tests confirmed the

Table 3
Significance Levels for Statistical Analyses With B_{12} Cutoffs at ≤ 250 or ≤ 200 pmol/L

	Cutoff ≤ 250			Cutoff ≤ 200		
	Original	3-year rem	6-year rem	Original	3-year rem	6-year rem
	p	*p*	*p*	*p*	*p*	*p*
2s vs. 5s						
$N_{1–6}$	167	120	86	136	102	74
APOE						
B_{12}	.01			.01		
APOE × B_{12}	.01	.03	.01	.01	.03	.01
CS	.04	.02	.02			.04
APOE × CS						.04
B_{12} × CS						
APOE × B_{12} × CS	.02	.01	.05	.05	.03	.04
5s vs. org						
APOE						
B_{12}	.01					
APOE × B_{12}						
CS	.00	.00	.00	.00	.00	.00
APOE × CS						
B_{12} × CS						
APOE × B_{12} × CS						
Org vs. cued						
APOE						
B_{12}						
APOE × B_{12}						
CS	.00	.00	.00	.00	.00	.00
APOE × CS						
B_{12} × CS						
APOE × B_{12} × CS						

Note. Empty cell denotes statistic was nonsignificant. 3-year rem = demented up to 3 years posttest removed; 6-year rem = demented up to 6 years posttest removed; CS = cognitive support; 2 s = 2-s encoding time for semantically unrelated words; 5 s = 5-s encoding time for semantically unrelated words; org = free recall of semantically organizable words; cued = cued recall of semantically organizable words; $N_{1–6}$ = Sample size for the respective analyses.

source of the interaction to be the ϵ4–low-B_{12} group in conditions of low cognitive support. Therefore, the removal of participants who were in the preclinical phase of dementia, were deceased, or who refused participation 3 years after testing did not affect our original results.

We then repeated those analyses having removed individuals diagnosed as demented 6 years after testing (see Table 3). The consequent sample was 86. With this reduced sample ($n = 12$ for both low-vitamin *APOE* groups), the pattern of results was virtually the same, and the *APOE* × B_{12} × Time Support interaction attained significance, $F(1, 82) = 3.96$, $\eta^2 = .046$, $p = .05$. Although of reduced effect size, this again suggests that our findings were uninfluenced by individuals in the preclinical phase of dementia during the 6 years after testing. We repeated this analysis on persons who were removed from the sample because of incident dementia in the 6 years after testing. The *t* tests revealed this group were significantly older (83.81 vs. 81.91 years, $p < .05$) and had significantly fewer years of education (8.10 vs. 9.52, $p < .01$) than the nondemented group. In respect to *APOE* genotype, gender, B_{12}, and folate levels, the two groups did not significantly differ. Notably, however, the *APOE* × B_{12} × Time Support interaction became nonsignificant ($p > .07$) in the demented group, suggesting further that our main finding was unrelated to the preclinical phase of dementia. Finally, ANCOVAs comparing free recall of unrelated words with those grouped into taxonomic categories, and free and cued recall of organizational words, with the exception of main effects for cognitive support ($p < .001$), found that all other main effects and interactions were nonsignificant. This was the case when incident dementia cases 3 years and also 6 years after testing were removed from the sample.

Turning to statistical analyses in which the B_{12} cutoff was lowered to less than 201 pmol/L (as in the earlier analyses the normal group was defined as B_{12} >250 pmol/L), the sample was reduced to 136 individuals. Because statistically significant between-group differences existed in age and years of education, those variables were again entered, with gender, as covariates into analyses. Table 3 shows that the analyses, with one exception, replicated the results obtained using the higher cutoff. The single statistic that differed was in the analysis of free recall after 2-s or 5-s encoding; the main effect for cognitive support that was significant in the original analysis became nonsignificant. Removing participants who were demented ($n = 11$), deceased ($n = 16$), or declined further involvement in the study ($n = 7$) 3 years later (consequent $N = 102$) rendered the main effects for B_{12} and time support nonsignificant for free recall after 2- or 5-s encoding. All other statistics were as before. Although removal of individuals who were demented 6 years after testing reduced the sample considerably ($n = 74$; ϵ4–low-B_{12} group = 8 individuals), again, the key interactions obtained in the original analyses remained statistically reliable. Together, the reanalyses do not suggest that our findings were related to either the preclinical phase of dementia or the cutoff used to define low vitamin B_{12}.

Folate, APOE, and Episodic Memory

The foregoing statistical analyses were repeated, but with folate values determining the normal and low vitamin groups. Specifically, individuals with values less than 13 nmol/L were designated as the low-folate group, and those with values greater than 12 were designated as normal. Age, years of education, and gender were entered as covariates into the analyses.

For the ANCOVA comparing 2- and 5-s encoding time, with the exception of a statistically significant main effect for cognitive support, $F(1, 163) = 6.79$, $\eta^2 = .040$, $p = .01$, none of the other main effects or interactions were statistically reliable (*p*s > .12). However, there was a nonsignificant trend in the data suggesting that low-folate–ϵ4 carriers benefit from time support (2 s to 5 s) at encoding to a greater extent than other groups (Table 4). We elected to explore this trend further through hierarchical multiple regression in which the *APOE* × Folate cross-product interaction term was entered into the regression at the third step after age, education, and gender (Step 1) and *APOE* and folate (Step 2). For 2-s encoding time for words, that interaction term added a 1.6% increment to the variance explained ($p = .08$), whereas for all other conditions that interaction was nonsignificant (*p*s > .21). Therefore, although unreliable at conventional levels of statistical

Table 4
Biographical and Memory Variables as a Function of APOE and Folate

Variable	Non-ε4		ε4	
	Low folate	Normal	Low folate	Normal
n	30	88	15	34
Demographic data				
Age (years)				
M	84.97	82.89	81.33	81.35
SD	6.01	5.96	4.22	4.71
Women (%)	70.00	80.68	93.33	82.35
Education (years)				
M	9.67	8.69	8.00	8.91
SD	4.11	2.68	1.65	2.94
Diseases (*n*)	8	9	2	7
Vitamin				
Folate (nmol/L)				
M	10.27	21.16	10.67	20.79
SD	1.44	8.27	1.18	9.26
B_{12} (pmol/L)				
M	259.48	293.89	212.67	290.74
SD	142.90	138.64	105.26	145.90
Memory				
unrelated				
2-s encoding				
M	4.68	5.22	4.27	5.15
SD	1.73	1.65	1.67	2.44
5-s encoding				
M	5.21	5.32	5.00	5.59
SD	2.04	1.74	2.24	2.55
Organizable				
Free recall				
M	6.23	6.55	5.67	6.79
SD	2.19	2.32	2.09	2.13
Cued recall				
M	8.00	8.46	7.87	8.65
SD	2.07	2.30	1.92	2.07

significance, the trend in the data suggests that had the sample size and consequent statistical power been greater the findings in respect to folate would have replicated those for vitamin B_{12}.

Returning to the ANCOVAs comparing recall at higher levels of cognitive support, as was predominantly the case in analyses involving vitamin B_{12}, only the cognitive support main effect was statistically reliable ($p < .01$). All other main effects and interactions were nonsignificant. As in the earlier analyses, providing cognitive support improved free-recall performance. Because the ε4–low-folate group included only 15 persons, removing those who were demented or deceased or who refused participation 3 years or more after testing rendered that cell too small for meaningful analyses. A similar problem was encountered when, following investigators elsewhere, a cutoff of less than 11 nmol/L was adopted to define the low-folate groups. Therefore, although the trend in the data was similar to that for vitamin B_{12}, we are unable to draw any firm conclusions concerning low folate levels and *APOE* in respect to episodic memory in the current sample.

Discussion

In this study, we have produced evidence that low vitamin B levels in combination with possession of the *APOE* ε4 allele is associated with an increased vulnerability to free-recall deficits in old age. This association was found in respect to vitamin B_{12} but only in circumstances of high task demands, in which cognitive support was low. Our findings are consistent with the vulnerability hypothesis and are important for several reasons. First, they demonstrate the complexity of associations between genetic and nongenetic influences on episodic free recall in a population-based sample of older adults. Second, this is one of the first empirical demonstrations that nutritional factors interact multiplicatively with *APOE* genotype and the demands of a cognitive task to influence episodic memory in the very old; the benefits of providing cognitive support in demanding task conditions was greater in the ε4–low-B_{12} group relative to other groups. Third, the results qualify suggestions that associations between *APOE* genotype and cognitive performance are related to the preclinical phase of dementia. Our findings raise the possibility that there may be complex circumstances in which *APOE* exerts an influence on cognitive performance in older adults independently of future dementia. When individuals who became demented up to 6 years after testing were removed from the analyses, the findings were unaffected. Additionally, we took into account cerebro- and cardiovascular diseases, age, gender, and education. Furthermore, the results were not an artifact of the cutoffs used to define low B_{12} levels because they remained after cutoff was lowered. Those factors can, therefore, be eliminated as potential confounds to our findings.

Because no previous research has assessed either vitamin B_{12} or folate and *APOE* in respect to episodic memory, we elected to evaluate the two B vitamins separately. Although the variables are related, it is not yet known whether any associations with *APOE* are mediated by the same or differing biochemical mechanisms. Our data suggested a dissociation because a significant interaction was identified in relation to *APOE* and vitamin B_{12} but not folate. Before too much weight is attached to this finding, however, several factors should be considered. First, in the statistical analyses involving ANCOVA, although the *APOE* × Folate × Cognitive Support (2-s to 5-s) interaction was nonsignificant, the data trend was similar to that involving vitamin B_{12}, suggesting the ε4–low-folate group benefited more from additional time at encoding than the other groups. Second, after stratification according to *APOE* genotype, there were only 15 participants in the ε4–low-folate group. Therefore, statistical power was limited in confirming differences when they existed. Additionally, as McClelland and Judd (1993) note, there are notorious difficulties associated with detecting statistically significant interactions in field studies such as this. Third, when hierarchical multiple regression was used instead of ANCOVA, the amount of variance explained by the *APOE* × Folate cross-product interaction term approached conventional levels of statistical significance ($p = .08$). Finally, the small ε4–low-folate group meant that we could not lower the threshold further to less than 11 nmol/L as some researchers have done. Therefore, in the ANCOVAs it is possible that our low-folate groups were not defined by sufficiently low values. Together, these considerations suggest that the differing findings for vitamin B_{12} and folate were related to the small number of participants recording low folate values rather than a dissociation in biochemical processes.

Given the foregoing, the findings build on earlier work demonstrating the association between B vitamins and cognitive performance in old age (e.g., Hassing et al., 1999; Riggs et al., 1996; Wahlin et al., 1996). In the current study, the significant three-way

interaction suggested that the recall performance of persons with low-B_{12}–ϵ4 benefited more from increased cognitive support (2- to 5-s encoding time). Because recall conditions were identical (2 min) after both 2- and 5-s encoding, it appears that the more limited study time deleteriously affected the encoding processes of ϵ4 carriers with low B_{12} vitamin levels. Bäckman (e.g., 1995) argues that the benefits of cognitive support to older adults depend on experimental factors (e.g., type of support provided and cognitive ease with which it can be used) and individual differences (e.g., verbal ability). Also, Bunce (2003) indicates that cognitive support aids episodic memory performance in older adults of lower, relative to higher, neuropsychological function. The current findings add to this work in two ways. First, in nondemented adults, it appears that cognitive support also moderates biologically based individual differences in respect to episodic memory. Second, when the statistical analyses were rerun only on persons who became demented up to 6 years after testing, the *APOE* $\times$ B_{12} $\times$ Cognitive Support interaction became nonsignificant. Although on a smaller sample, this reanalysis suggests that the pathological progression of neurodegenerative diseases may reach a point at which cognitive support is no longer of benefit. Further work is required to explore such limitations in more detail.

Our findings have some important theoretical implications. Structural neuroimaging studies suggest that nondemented *APOE* ϵ4 carriers have smaller hippocampi (Plassman et al., 1997) and suffer greater hippocampal atrophy (Cohen, Small, Lalonde, Friz, & Sunderland, 2001; Moffat, Szekely, Zonderman, Kabani, & Resnick, 2000). Moreover, studies show a greater magnitude and extent of brain activation among *APOE* ϵ4 carriers in the prefrontal cortex, hippocampus, and parietal cortex during a challenging memory task (Bookheimer et al., 2000). It is suggested that such differences may represent compensatory recruitment of additional brain regions by *APOE* ϵ4 carriers while encoding into episodic memory in demanding conditions (Burggren, Small, Sabb, & Bookheimer, 2002). In addition, although the biological mechanisms by which B vitamins affect cognitive function are uncertain, two hypotheses have emerged (Calvaresi & Bryan, 2001). The *hypomethylation* hypothesis suggests that low levels of B_{12} and folate interact to inhibit methylation throughout the central nervous system. Among other effects, this inhibits the metabolism of the neurotransmitters dopamine, norepinephrine, and serotonin to the detriment of cognitive function. Alternatively, the *homocysteine* hypothesis proposes impaired neurocognitive function because of elevated levels of homocysteine arising from low vitamin B levels and related cerebrovascular changes. From the current perspective, the notable feature is that both hypotheses suggest physiological mechanisms by which neurological processes either are impaired or damaged. Together, the deleterious influence of low B vitamin levels on neurological processes and structures, in combination with the compromised neuroanatomical structures reported in ϵ4 carriers, may explain the free-recall deficits we identified in ϵ4 carriers with low vitamin B_{12} levels.

Such an explanation is consistent with the vulnerability hypothesis, and it is also worth noting the link with the concept of brain reserve (e.g., Cummings, Vinters, Cole, & Khachaturian, 1998; Katzman, 1993; Mortimer, 1988; Satz, 1993; Skoog, 2000; Stern, 2002) commonly used to explain the later onset of dementia among persons of higher education. Brain reserve is determined by the integrity of neuroanatomical structures and neural processes and provides protection against the pathological progression of neurodegenerative disease in old age. In the current context, the neuroimaging work described earlier suggests that *APOE* ϵ4 carriers may have compromised or more vulnerable neuroanatomical reserves relative to non-ϵ4 carriers. Therefore, if an additional factor such as low B vitamin levels further depletes those reserves, the threshold at which cognitive deficits occur is more likely to be reached in ϵ4 than non-ϵ4 carriers. Our data support this possibility and highlight the value of future research investigating associations between *APOE* and cognitive performance in older adults while taking into account additional physiological factors that may influence that relationship.

Several investigators have argued that associations between the *APOE* ϵ4 allele and cognitive performance reflect the preclinical phase of dementia (e.g., Bondi, Salmon, Galasko, Thomas, & Thal, 1999; Small et al., 2000). Our findings, however, suggest the picture may be more complex. Here, after removing incident dementia cases up to 6 years after testing, the findings remained statistically significant. It is possible, therefore, that in certain complex circumstances *APOE* exerts an influence on cognitive performance independent of future dementia. The evidence here indicates that the deleterious influence of an additional physiological factor, in combination with high cognitive demands, is one such circumstance. However, there is also empirical research showing that *APOE*-related cognitive deficits disappear when future dementia is taken into account (Bondi et al., 1999). Given the lengthy preclinical phase of the disease, we cannot dismiss the possibility that the 6-year period we took into account was insufficiently long to identify all eventual dementia cases. Until further research is produced demonstrating that *APOE*-related cognitive deficits exist having controlled for future dementia, our conclusions should be treated with caution.

The current study is not without its limitations. First, data relating to homocysteine levels were not available. Inclusion of such information would have helped demonstrate the extent to which low vitamin values were indicative of true deficiencies. Second, the advantage of a population-based study such as this is that participant selection bias is limited. The downside, however, is that analyses are restricted to the data available in that population. Here, the effects were twofold. After stratification by *APOE* and vitamin level, the group sizes were restricted, particularly in the case of folate. This not only limited statistical power but also meant that we were unable to examine the ϵ4 dose effect.

Practically, the research suggests that a subsample of the nondemented elderly population (i.e., *APOE* ϵ4 carriers) may derive relatively greater benefits to cognitive performance from B_{12} and folate supplements, particularly when task demands are high. Research using transgenic mice (Kruman et al., 2002) has demonstrated depleted folate levels to be associated with the formation of the amyloid plaques found in Alzheimer's disease, and work in humans also suggests a link between vitamin B_{12} and folate and Alzheimer's disease (Wang et al., 2001). Such findings, together with those of the current study, confirm that there is good reason to consider inclusion of vitamin B_{12} and folate supplements as part of preventive health regimens for older persons.

The main conclusion of this study is that brain reserve may vary as a function of *APOE* genotype and that ϵ4 carriers may be particularly vulnerable to cognitive impairment in the presence of an additional factor that deleteriously influences neuroanatomical

structures and processes. The current study suggests vitamin B deficiencies to be one such factor. The findings appear unrelated to impending dementia up to 6 years after testing. It is clearly important that population- and laboratory-based research further explores associations between *APOE* and cognitive performance in nondemented older adults while taking into account additional factors that may influence the vulnerability of neuroanatomical reserves.

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