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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Abstract

Apolipoprotein (APOE), vitamin B_{12}, and folate were examined in relation to free recall among 167 community-based older adults (M=82.81 years). Cognitive support at encoding and retrieval was also taken into account. Participants were classified as APOE ϵ4 or non-ϵ4 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 ϵ4 genotype in respect to free recall, but only in circumstances of low cognitive support. This result was retained having removed incident dementia cases up to six years following 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.

Introduction

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 pre-existing 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 non-genetic 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 non-demented 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 as earlier work (Bunce, 2001a, b) suggests the tasks most sensitive to underlying physiological mechanisms in older adults, are those placing the greatest demands on cognitive processes (i.e., low cognitive support). Here, we ask if 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, ϵ2, ϵ3, and ϵ4. There is clear evidence that possession of the ϵ4 allele is a risk factor for dementia (for a review see Farrer, Cupples, Haines et al., 1997). However, work investigating whether the presence of the ϵ4 allele confers a greater vulnerability to cognitive impairment among non-demented older adults, is more equivocal. For example, individuals possessing the ϵ4 allele exhibited more precipitous decline in face and word recognition (Small, Basun, & Bäckman, 1998), delayed word recall (Hyman, Gomez-Isla, Briggs et al., 1996), factor scores for episodic memory and processing speed (Hofer, Christensen, MacKinnon 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, Whiteman, Pattie et al., 2002). By contrast, there is work suggesting no

association between the ϵ4 allele and decline in fluid intelligence (Pendleton, Payton, van den Boogard et al., 2002), composite visuospatial and language factors (Mayeux et al., 2001), and proxy measures of IQ (Deary, Whalley, St. Clair 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, Wilson, Schneider et al., 2003). Regarding measures of global cognitive performance, some studies suggest decline to be greater in ϵ4 carriers (e.g., Bretsky, Guralnik, Launer, Albert, & Seeman, 2003; Fillenbaum, Landerman, Blazer et al, 2001; Jonker et al., 1998), whereas others show no such differentials (Winnock, Letenneur, Jacqmin-Gadda et al., 2002). Given those equivocal 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, Bowen, Rajaram 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, Romero, Koehler 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 et al., 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 developing Alzheimer's disease (e.g., Wang, Wahlin, Basun et al., 2001). More broadly, there is evidence suggesting 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 due to 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 aged 75 years and over. 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, aged 75 years or 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 a total of 528 individuals. Of this number, 130 were diagnosed according to criteria in the Diagnostic and Statistical Manual of Mental Disorders III-R (American Psychiatric Association, 1987) as suffering dementia, and a further 33 depression. As inclusion of those individuals would affect interpretation of our results, they were removed from the statistical analyses. A further 37 were removed due to incomplete vitamin B_{12} or folate data, and also 16 participants who were taking vitamin B_{12} or folate supplements. Inspection of the remaining data revealed 32 individuals to have abnormally high folate levels. As 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 numbered 167, with a mean age of 82.81 years (SD = 5.68), was 80.24 percent female, and had a mean number of years education of 8.85 (SD = 2.98). A Table providing descriptive data for persons included in, and eliminated from, the sample can be viewed at [Hyperlink here]. For a minority of cases in the statistical analyses reported below, 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 subjects. The interim interval was 1 s. Participants were told to remember as many words as possible for a subsequent recall test. Immediately following presentation, two minutes 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 two minutes to remember as many of the words as possible. A cued recall condition followed where 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. As cardio-, and cerebrovascular factors may be independently associated with APOE, vitamin B_{12} 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 five-year period prior to cognitive testing. Admissions for any of the following complaints were recorded: diabetes, cerebrovascular diseases, stroke (hemorrhage, ischemic, or non-specific), transient ischemic attack, ischemic heart disease, heart failure, myocardial infarction, angina, arrhythmia, and arterial fibrillation.

Vitamin B_{12} and 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 blind to 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 of this procedure).

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 $B_{12} \leq 250$ pmol/liter; low folate ≤ 12 nmol/liter. Remaining participants, with values above 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 1. Before the statistical analyses of primary interest were undertaken, several preliminary procedures were carried out. First, between-group differences in the biographical variables listed in Table 1 were subjected to a series of 2 x 2 ANOVAs where 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, $F(1,163) = 7.74$, $\eta^2 = .045$, $p = .006$, and B_{12}, $F(1,163) = 19.22$, $\eta^2 = .105$, $p<.001$, but the APOE x B_{12} interaction was nonsignificant ($p > .46$). Members of the non-ϵ4 groups were older (83.61 versus 81.11 years), as were those in the low B_{12} groups (84.33 versus 80.39 years). Regarding years of education, the main effect for APOE, and the APOE x B_{12} interaction were statistically unreliable (ps>.71). However, persons with normal B_{12} levels had significantly more years of education (9.42 versus 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 below. Although a greater proportion of women made up the sample, gender was also entered as a covariate.

Table 1 about here

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 the following 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 for 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 78.09 percent of the explained variance. The diagnoses groupings related to stroke (hemorrhage, ischemic, and non-specific), 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 the factor scores arising from each of those four factors were correlated significantly with any of the cognitive variables. Neither did univariate analysis of variance (APOE and either 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 1 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 x 2 x 2 Analyses of Covariance (ANCOVA), where B_{12} level (low/normal) and APOE genotype (ϵ4/non-ϵ4) formed the between-subject factors, and cognitive support the within-subjects factor (see below 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 1.

Free recall of semantically unrelated words following 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 below 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, while the main effect for APOE was not statistically reliable ($p>.87$), that for B_{12} was significant, $F[1,160] = 16.86$, $\eta^2 = .095$, $p = .001$; Table 1 suggests persons with normal B_{12} levels recalled a greater number of words. The main effect for time support was also statistically reliable, $F(1,163) = 4.17$, $\eta^2 = .025$, $p = .043$; greater time at encoding

was associated with superior recall. With the exception of that involving APOE and B_{12}, ($F[1,160] = 8.70$, $\eta^2 = .052$, $p = .004$), all two-way interactions were nonsignificant (ps>.27). However, that significant two-way interaction was modified by a statistically reliable APOE x B_{12} x Time support interaction, $F(1,163) = 5.72$, η^2=.034, $p = .018$. Consideration of Table 1, 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, $F(1,164) = 4.81$, η^2=.028, p =.030. 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, $F(1,165) = 8.30$, η^2= .048, p =.005; the recall performance of ϵ4 carriers with low B_{12} levels significantly benefited when encoding time was increased from 2s 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 either were semantically unrelated (low support), or organizable into four taxonomic categories (higher support). Main effects were significant for vitamin group, $F(1,160) = 6.66$, η^2= .040, $p = .011$, and the level of support available, $F(1,163) = 38.06$, η^2= .189, p =.000; 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, $F(1,163) = 261.33$, η^2= .616, $p = .000$; cued recall produced higher scores than free recall. However, all other statistics were nonsignificant (ps>.12).

In sum, the above analyses suggest the detrimental effect of ϵ4 in combination with low B_{12} levels was manifest in the most demanding condition of the present experiment (i.e.,

free recall following 2 s encoding time for semantically unrelated words). This can be seen in Table 1 by examining the third data column; 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 the foregoing, 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 three and six years following cognitive testing. In order to eliminate the possibility that our findings reflected the preclinical phase of the disease, all ANCOVAs were repeated, with incident dementia cases removed from the analyses. Regarding the second factor, investigators have used various cut-offs to define low vitamin B_{12} levels. To confirm our results were not an artifact of the cut-off we adopted, statistical analyses were also re-run but with low B_{12} status defined as $\leq$200 pmol/liter. Analyses were repeated having removed incident dementia cases three and six years following testing.

Table 2 about here

The results of those additional analyses are summarized in Table 2. Significance levels for cut-offs $\leq$250 and $\leq$200 pmol/liter are presented to the left and right of the Table, respectively. Overall, the pattern of results from the re-analyses were remarkably consistent with those from the original, regardless of cut-off used to define low B_{12} status, or whether incident dementia cases were excluded from the analyses. Specifically, for analyses where low B_{12} was defined as $\leq$250 pmol/liter, when data three years following testing were taken into account, 20 demented, and 20 deceased participants were removed from the sample. An additional seven individuals declined participation at follow-up. The resulting sample numbered 120, were 79.17 percent female, had a mean age of 82.28 (SD = 5.08) years, and an average of 9.22 (SD = 3.29) years education. As age varied significantly according to B_{12} group ($p < .001$), and years of education was marginal to conventional levels of statistical significance ($p = .051$), both variables, together with gender, were again entered as covariates into the analyses.

It can been seen in the third column of Table 2 that 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 ($p = .024$), while 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 x B_{12} interaction was statistically reliable ($p = .032$), and the other two-way interactions were nonsignificant (p>.08). However, the APOE x B_{12} x 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 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 either in the preclinical phase of dementia, deceased, or who refused participation three years following testing, did not affect our original results.

We then repeated those analyses having removed individuals diagnosed as demented six years following testing (see Table 2, Column 4). The consequent sample was 86. With this reduced sample (both low vitamin APOE groups numbered 12) the pattern of results was virtually the same, and the APOE x B_{12} x Time Support interaction attained significance, $F(1,82) = 3.96$, $\eta^2 = .046$, $p = .050$. Although of reduced effect size, this again suggests our findings were uninfluenced by individuals in the preclinical phase of dementia during the six years following testing. We repeated this analysis on persons who were removed from the sample due to incident dementia in the six years following testing. T-tests revealed this group were significantly older (83.81 versus 81.91 years, p<.05), and had significantly fewer years' education (8.10 versus 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 though, the APOE x B_{12} x Time Support interaction became nonsignificant (p>.065) 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 to 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 all other main effects and interactions were nonsignificant. This was the case when incident dementia cases three years, and also six years, following testing were removed from the sample.

Turning to statistical analyses where the B_{12} cut-off was lowered to ≤200 pmol/liter (as in the earlier analyses the normal group was defined as B_{12} >250 pmol/liter), the sample was reduced to 136 individuals. As statistically significant between-group differences existed in age, and years of education, those variables were again entered, with gender, as covariates into analyses. Table 2, Column 5 shows that the analyses, with one exception, replicated the

results obtained using the higher cut-off. The single statistic that differed was in the analysis of free recall following 2 s or 5 s encoding; the main effect for cognitive support that was significant in the original analysis, became nonsignificant. Removing participants either demented (n=11), deceased (n=16), or who declined further involvement in the study (n=7), three years later (consequent N = 102), rendered the main effects for B_{12} and time support nonsignificant for free recall following 2 s or 5 s encoding. All other statistics were as before. Although removal of individuals demented six years following 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 cut-off 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 ≤12 nmol/liter were designated as the low folate group, and those with values >12 as normal. Age, years of education, and gender were entered as covariates into the analyses.

For the ANCOVA comparing 2 s and 5 s encoding time, with the exception of a statistically significant main effect for cognitive support ($F[1,163] = 6.79$, $\eta^2 = .040$, $p = .010$), none of the other main effects or interactions were statistically reliable (ps>.12). However, there was a nonsignificant trend in the data suggesting low folate-ϵ4 carriers to benefit from time support (2 s to 5 s) at encoding to a greater extent than other groups (see Table 3). We elected to explore this trend further through hierarchical multiple regression where the APOE x Folate cross-product interaction term was entered into the regression at the third step following 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 percent increment to the variance explained ($p = .083$), whereas for all other conditions that interaction was nonsignificant (ps>.21). Therefore, although unreliable at conventional levels of statistical 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 ($p<.001$). All other main effects and interactions were nonsignificant. As in the earlier analyses, providing cognitive support improved free recall performance. As the ϵ4-low folate group numbered only 15 persons, removing those who were demented, deceased, or who refused participation three years or more following testing, rendered that cell too small for meaningful analyses. A similar problem was encountered when, following investigators elsewhere, a cut-off of ≤10 nmol/liter 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 present sample.

Table 3 about here

Discussion

In this study, we have produced evidence that low B vitamin 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, where 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 non-genetic 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 six years following 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 cut-offs used to define low B_{12} levels, as they remained having lowered that cut-off. Those factors can therefore, be eliminated as potential confounds to our findings.

As 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 if any associations with APOE are mediated by the same, or differing, biochemical mechanisms. Our data suggested a dissociation, as 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 though, consideration should be given to the following. First, in the statistical analyses involving ANCOVA, although the APOE x Folate x 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 where 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 employed instead of ANCOVA, the amount of variance explained by the APOE x Folate cross-product interaction term approached conventional levels of statistical significance ($p = .083$). Finally, the small ϵ4-low folate group meant that we could not lower the threshold further to $\leq$10 nmol/liter 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 upon earlier work demonstrating the association between B vitamins, and cognitive performance in old age (e.g., Hassing et al., 1996; Riggs et al., 1996; Wahlin et al., 1996). In the present study, the significant three-way interaction suggested the recall performance of low B_{12} ϵ4-carrying persons benefited more from increased cognitive support (2 s to 5 s encoding time). As recall conditions were identical (2 minutes) following both 2 s 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 the benefits of cognitive support to older adults depend upon experimental factors (e.g., the type of support provided, and the cognitive ease with which it can be utilized), and individual differences (e.g., verbal ability). Also, recent work (Bunce, in press) indicates that cognitive support aids episodic memory performance in older

adults of lower, relative to higher, neuropsychological function. The present findings add to this work in two ways. First, in non-demented 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 six years following testing, the APOE x B_{12} x Cognitive support interaction became nonsignificant. Although on a smaller sample, this re-analysis 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 ϵ4carriers have smaller hippocampi (Plassman, Welsh-Bohmer, Bigler et al., 1997), and suffer greater hippocampal atrophy (Cohen, Small, Lalonde, Friz, & Sunderland, 2001; Moffat, Szekely, Zonderman, Kabini, & Resnick, 2000). Moreover, there is work showing 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, Strojwas, Cohen 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 (Burggen, 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 low levels of B_{12} and folate interact to inhibit methylation throughout the central nervous system. Amongst other effects, this inhibits the metabolism of the neurotransmitters dopamine, norepinephrine, and serotonine, to the detriment of cognitive function. Alternatively, the homocysteine hypothesis proposes impaired neurocognitive function due to elevated levels of homocysteine arising from low vitamin B levels, and related cerebrovascular changes. From the present 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, 2002; 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 present 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 carriers 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, Graves, McEvoy et al., 2000). Our findings however, suggest the picture may be more complex. Here, having removed incident dementia cases up to six years following testing, the findings remained statistically significant. It is possible therefore, that in certain complex circumstances, APOE exerts an influence on cognitive performance, independently of future dementia. The evidence here indicates 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 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 six-year period we took into account, was insufficiently long to identify all eventual dementia cases. Until further research is produced demonstrating APOE-related cognitive deficits having controlled for future dementia, our conclusions should be treated with caution.

The present 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 advantages of a population-based study such as this, is that participant selection bias is limited. The downside though, 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 non-demented 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. Recent research using transgenic mice (Kruman, Kumaravel, Lohani 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 present study, confirm there is good reason to consider inclusion of vitamin B_{12} and folate supplements as part of preventive health regimes 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 present study suggests vitamin B deficiencies to be one such factor. The findings appear unrelated to impending dementia up to six years following 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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Table 1. Biographical and memory variables as a function of apoE and vitamin B_{12}

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

Table 2. Significance levels for statistical analyses with B_{12} cut-offs at <250 or <200 pmol/liter

	Cut-off ≤250			Cut-off ≤200		
2s Vs 5s	Original	3yr rem	6yr rem	Original	3yr rem	6yr rem
	p=	p=	p=	p=	p=	p=
ApoE						
B_{12}	.001			.004		
ApoE x B_{12}	.004	.032	.004	.007	.025	.003
CS	.043	.024	.018			.044
ApoE x CS						.042
B_{12} x CS						
ApoE x B_{12} x CS	.018	.010	.050	.045	.026	.042
5s Vs Org						
ApoE						
B_{12}	.011					
ApoE x B_{12}						
CS	.000	.000	.000	.000	.000	.000
ApoE x CS						
B_{12} x CS						
ApoE x B_{12} x CS						
Org Vs Cued						
ApoE						
B_{12}						
ApoE x B_{12}						
CS	.000	.000	.000	.000	.000	.000
ApoE x CS						
B_{12} x CS						
ApoE x B_{12} x CS						
N_{1-6}	167	120	86	136	102	74

Notes. Empty cell denotes statistic was nonsignificant
3 yr rem = Demented up to 3 years post-test removed
6 yr rem = Demented up to 6 years post-test 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

Table 3. Biographical and memory variables as a function of apoE and folate

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

Hyperlink Table.

Means and standard deviations for persons included in, and excluded from, the sample (Bunce, Kivipelto, & Wahlin)

Variable	Included	Excluded	Significance level
Age	82.81 (5.68)	85.05 (5.05)	<.01
% women	80.24	80.90	ns
Years education	8.85 (2.98)	8.44 (2.28)	ns
No. diseases	26 (15.57%)	98 (27.15%)	<.05
B_{12} (pmol/l)	279.77 (139.35)	370 (342.38)	<.01
Folate (nmol/l)	18.19 (8.71)	23.17 (16.10)	<.01
N	167	361	

Notes. ns = nonsignificant
The fourth column indicates if significant differences exist. Chi^2 tests were used for women and diseases, and T-tests otherwise.