Further Evidence on the Effects of Vitamin B_{12} and Folate Levels on Episodic Memory Functioning: A Population-Based Study of Healthy Very Old Adults

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Background: *The relationship between vitamin status and cognitive functioning has been addressed in several recent studies with inconclusive results. The purpose of this study was to examine separate and combined effects of serum vitamin B_{12} and folic acid on episodic memory functioning in very old age.*

Methods: *Four study groups were selected from a population-based sample of healthy very old adults (90–101 years of age): normal B_{12}/normal folic acid, low B_{12}/normal folic acid, normal B_{12}/low folic acid, and low B_{12}/low folic acid. Cutoff levels were set at 180 pmol/L for vitamin B_{12} and at 13 nmol/L for folic acid. Subjects completed two episodic recall tasks (objects and words) and two episodic recognition tasks (faces and words).*

Results: *Neither vitamin affected recognition or primary memory. Most interesting, although B_{12} was unrelated to recall performance, subjects with low folic acid levels showed impairment in both word recall and object recall.*

Conclusions: *These results replicate and extend previous findings that folic acid may be more critical than B_{12} to memory functioning in late life. The selective effects of folic acid on episodic recall were discussed in terms of encoding and retrieval mechanisms, as well as in relation to brain protein synthesis.* Biol Psychiatry 1999;45: 1472–1480 © *1999 Society of Biological Psychiatry*

Key Words: Vitamin status, B_{12}, folic acid, episodic memory, population-based, very old

Introduction

Episodic memory deals with conscious retrieval of information that is encoded in a particular place at a particular time (Tulving 1983). This form of memory is extremely sensitive to a variety of conditions (e.g., amnesia, dementia), which may leave other forms of memory (e.g., semantic, primary, implicit) relatively unaffected (Bäckman et al in press; Nyberg and Tulving 1996; Squire 1987; Tulving and Schacter 1990). Age-related deficits in episodic memory functioning are well documented (see Kausler 1994; Salthouse 1991, for reviews). A variety of factors are potentially capable of influencing the cognitive aging process, or of adding substantially to the variation in cognitive functioning among elderly persons. One class of variables that is very relevant in this context may be subsumed under the general heading of physical health (for a review, see Elias et al 1990).

A health-related topic that has received renewed interest in recent years concerns vitamin status. Normal aging is associated with decline in both episodic memory functioning (Kausler 1994; Light 1991) and vitamin status (Crystal et al 1994; van Goor et al 1995; Pennypacker et al 1992). In particular, two vitamins are known to be related to brain functioning, namely vitamin B_{12} and folic acid (Abou-Saleh and Coppen 1986; Martin 1988).

Vitamin B_{12} and folic acid are water-soluble vitamins. Vitamin B_{12} is found almost exclusively in foods of animal origin (Stabler 1995), whereas occurrence of folic acid is rich in fresh leafy vegetables, fruits, yeast, and liver (Babior 1990). The link between food intake and cellular utilization of these vitamins is, however, under multifactorial influence. For example, old age is often associated with atrophic gastritis, which is the most common cause of hypo- or achlorhydria in elderly people, causing malabsorption of vitamins. Prevalence rates for atrophic gastritis among persons aged 70 years or more have been estimated as high as 50% (Krasinski et al 1986). Other causes of vitamin B_{12} and/or folic acid deficiency include inadequate ingestion, increased excretion, increased requirements, as in hemolytic anemias, and destruction of red blood cells (Herbert 1987).

The relationship between vitamin status and cognitive functioning has been addressed in several recent studies. In perhaps the most cited study on healthy individuals, Goodwin et al (1983) found that persons with low levels of vitamin B_{12} or folate showed cognitive deficits as com-

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pared with the remaining sample; however, in subsequent research, results have been somewhat equivocal. For example, several studies have reported no effects of low serum B_{12} levels in various memory tasks and global cognitive status exams (Crystal et al 1994; La Rue et al 1997; Metz et al 1996; Riggs et al 1996; Wahlin et al 1996), although negative effects of low serum B_{12} levels were found in a task requiring spatial copying (Riggs et al 1996), and in the Stroop Color-Word Test (Bohnen and Degenaar 1992). The results are equally mixed concerning the effects of low folic acid on cognitive performance in old age. Thus, negative effects of low folic acid level have been reported with regard to episodic memory (Wahlin et al 1996) and various other cognitive tasks (La Rue et al 1997; Riggs et al 1996), although La Rue et al and Riggs et al found no folate-related effects on memory, and Tucker et al (1990) failed to find any effects of folic acid in a variety of cognitive tasks.

When studying vitamin B_{12} and folic acid it is important to consider that these vitamins are interrelated (Beck 1983; Herbert and Zalusky 1962). Despite this fact, very few studies have examined concomitant effects of deficiency of these vitamins on cognitive functioning. In a recent study, Wahlin et al (1996) examined such concomitant effects. Wahlin et al found that low levels of folic acid were related to poor episodic memory performance, and that this deficit was most pronounced in persons with low levels of both vitamin B_{12} and folic acid. By contrast, low levels of B_{12} alone had no impact on memory performance. These results suggest that folic acid may be more sensitive as a marker of cognitive deficits in old age than vitamin B_{12}; however, this finding should be viewed cautiously, given the difficulties associated with selecting appropriate cutoffs. Laboratory cutoffs adjusted for old age are still lacking, and current definitions of a cutoff for at-risk elderly subgroups appear to be arbitrary (Bell et al 1990). In research practice, elderly persons classified as being deficient in vitamin B_{12} typically have serum levels of less than 250 pmol/L (e.g., Lindenbaum et al 1994; Pennypacker et al 1992). Concerning folic acid, the threshold of deficiency in old age is less well specified, although a cutoff around 14 nmol/L is typically employed (e.g., Ortega et al 1996).

In the studies reviewed above, many neuropsychological tests reflecting very different aspects of cognitive functioning have been used, and mixed results have often been obtained. In this study, the focus is on episodic memory, because this form of memory is particularly sensitive to a variety of conditions that affect normal brain functioning (e.g., LaRue et al 1995; Morris and Kopelman 1986; Nyberg and Tulving 1996). The main purpose was to obtain further evidence concerning the effects of low levels of serum vitamin B_{12} and folate status on episodic memory performance using a population-based sample of persons aged 90 years and above. As noted, low vitamin levels become more prevalent in very old age (Bell et al 1990; Crystal et al 1994; van Goor et al 1995; Pennypacker et al 1992). Thus, it becomes especially interesting to study the relationship between vitamin status and cognitive functioning in a sample of very old adults. Also, in very old age, memory functioning may be more vulnerable to vitamin deficiency as compared to what is true for younger elderly adults, given that the cognitive reserve capacity diminishes in old age (Bäckman 1991; Kliegl et al 1989).

Further, several diseases that are common in late adulthood may have negative repercussions to cognitive functioning. For example, epilepsy, diabetes, and thyroid dysfunctions have been found to have negative effects on cognitive performance (e.g., Coenen et al 1995; Mennemeier et al 1993; Tun et al 1990). A careful health screening is therefore imperative to be able to isolate potential vitamin-related effects on cognitive functioning from those resulting from other health conditions. In the present study, a rigorous health screening was undertaken to achieve this objective.

Methods and Materials

Participants

Participants were selected from a total population of very old people (aged 90 years and older) registered in the St. Göran parish in Stockholm, Sweden ($n = 379$). Fifty-six persons refused to participate in the study, and 1 individual was already involved in another study. The remaining sample of 322 persons underwent an extensive medical examination by physicians, answered a structured questionnaire by nurses, and completed the Mini-Mental State Examination (MMSE; Folstein et al 1975) as well as a battery of memory tests (for a detailed description of the study protocol, see Bäckman et al 1996).

A total of 155 persons received a dementia diagnosis according to DSM-III-R criteria (American Psychiatric Association 1987). These individuals were not included in this study. Further, those who had major depressive disorder according to the DSM-IV (American Psychiatric Association 1994) were excluded ($n = 15$).

Twenty persons were eliminated, because they did not participate in the memory assessment, or failed to complete this assessment. Eight of these 20 persons died shortly after entering into the study; 2 persons had poor Swedish language skills, and a native tongue other than Swedish (i.e., German); and 10 persons refused to participate.

Of the 132 remaining persons, 43 were excluded either because of insufficient sensory capacity that might interfere with the encoding of the stimulus materials, or because of various diseases known to affect cognitive functioning. Thus, those who had insufficient visual capacity, as determined by means of Snellen's Visual Acuity Test, or insufficient auditory capacity, as

Table 1. Subject Characteristics across Vitamin Groups

	Subgroup (B_{12}/folic acid)			
	B_{12}-N/FOL-N (n = 34)	B_{12}-L/FOL-N (n = 10)	B_{12}-N/FOL-L (n = 19)	B_{12}-L/FOL-L (n = 8)
Age (years)				
Mean	91.41	91.50	92.68	91.62
SD	2.00	1.43	2.93	2.07
Range	90–96	90–94	90–101	90–96
Gender (% female)	73	60	89	37
Years of education				
Mean	8.00	8.60	6.89	6.87
SD	2.65	3.41	2.05	1.55
Range	4–16	4–15	4–12	5–9
Vitamin B_{12}[a]				
Mean	324.65	152.20	335.63	117.37
SD	161.35	24.19	231.28	47.06
Range	180–856	109–179	180–948	58–177
Folic acid[b]				
Mean	26.68	27.40	10.47	9.50
SD	14.88	20.43	1.22	1.69
Range	13–54	13–76	8–12	7–12

B_{12}, vitamin B_{12}; FOL, folic acid; N, normal; L, low.
[a]pmol/L.
[b]nmol/L.

assessed by whispering a word at a distance of 5 m from the person's ear, were eliminated (n = 19). In addition, 1 person who had tactile problems, and persons suffering from epilepsy (n = 3), diabetes (n = 9), anemias other than vitamin-related (n = 2), and thyroid-related diseases (n = 21) were excluded. Subjects who were not assessed with regard to B_{12} and/or folic acid status in the medical examination were then eliminated (n = 4). Finally, subjects with vitamin B_{12} values above 1000 pmol/L were eliminated from further analyses (n = 2). These subjects were receiving vitamin substitution. The remaining sample consisted of 71 persons free of overt diseases.

Vitamin Status

All blood analyses were performed by the same laboratory. For the analyses of vitamin B_{12} and folic acid, the radioimmunoassay method was used (see Chen et al 1982, for a description). Specific cutoff values on the two critical variables, vitamin B_{12} and folic acid, were employed. These cutoff scores were chosen after inspecting the distribution of the vitamin data, to ensure a sufficient number of subjects in each subgroup. For this reason, the cutoff for vitamin B_{12} was set at 180 pmol/L, and the cutoff for folic acid was set at 13 nmol/L. Following the analytic procedure of Wahlin et al (1996), the sample was further divided into four subsamples: normal B_{12}/normal folic acid (n = 34); low B_{12}/normal folic acid (n = 10); normal B_{12}/low folic acid (n = 19); and low B_{12}/low folic acid (n = 8). Altering the cutoffs for B_{12} and folic acid in either direction did not change the basic pattern of results to be reported in this article.

There were no group differences with regard to age or education ($ps > .05$). Table 1 shows demographic and vitamin characteristics across groups.

Analyses of the B_{12} data showed, as expected, significant group differences, $F(3,67) = 6.59$, $MS_E = 25354.47$, $p < .001$, $\omega^2 = .19$. This was because the two groups with normal B_{12} levels differed from the two groups with low B_{12} levels. Similar results were obtained in the corresponding analysis of the folic acid data, $F(3,67) = 9.35$, $MS_E = 165.89$, $p < .0001$, $\omega^2 = .26$, where the group differences resulted from the fact that the two groups with normal folic acid levels differed from the two groups with low folic acid levels.

Materials and Procedure

The participants were tested individually. The memory tasks were presented in the following order for all participants: face recognition, immediate word recall, object recall, delayed word recall, and word recognition. These tasks have been described in detail elsewhere (Bäckman et al 1996), and only a brief description will be provided here.

FACE RECOGNITION. The materials to be remembered in the face recognition task consisted of 20 black and white photographs of unfamiliar faces presented at a rate of 6 sec per face. In the self-paced immediate recognition test, the 20 target faces were presented along with 20 distractor faces in randomized order, and participants responded orally.

IMMEDIATE AND DELAYED FREE RECALL OF WORDS. A word list comprised of 12 concrete nouns from different taxonomic categories was prepared. At study, the words were presented in a booklet, one at a time, and read aloud simultaneously by the experimenter. Immediately following presentation, participants were given 3 min for an oral free recall test. A delayed free recall test was given after the object recall test (i.e., 20 min after initial word presentation).

Table 2. Recognition and Recall Data across Vitamin Groups

	Subgroup (B_{12}/folic acid)							
	B_{12}-N/FOL-N		B_{12}-L/FOL-N		B_{12}-N/FOL-L		B_{12}-L/FOL-L	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Object recall								
Total recall	25.26	7.82	23.60	5.62	20.89	6.31	19.50	7.45
L-T retrieval	19.56	9.93	17.40	7.32	13.05	8.16	12.00	7.75
List learning	15.70	9.97	13.20	9.07	9.95	7.55	9.00	7.33
S-T retrieval	5.67	3.42	6.20	2.57	7.84	3.44	7.38	2.50
Word recall								
Immediate	5.26	1.81	5.70	0.82	3.79	1.40	4.13	1.55
Delayed	2.00	2.06	2.30	1.16	1.32	1.86	0.13	0.35
Face recognition								
Hits	14.06	4.37	12.70	4.08	14.00	4.12	15.13	2.95
False alarms	4.21	3.87	3.70	3.06	4.21	3.49	5.63	4.10
d'	1.65	0.85	1.45	0.85	1.66	0.78	1.51	0.58
C	0.15	0.55	0.28	0.42	0.16	0.57	−0.02	0.54
Word recognition								
Hits	9.64	1.97	8.80	2.10	7.84	2.34	8.75	2.55
False alarms	2.73	3.02	1.90	2.96	1.89	2.42	2.75	2.82
d'	2.07	0.99	2.04	1.16	1.91	1.09	1.86	0.79
C	−0.05	0.61	0.18	0.48	0.34	0.51	0.09	0.67

B_{12}, vitamin B_{12}; FOL, folic acid; N, normal; L, low.

OBJECT RECALL. The object recall task was administered in accordance with the Fuld Object-Memory Evaluation procedure (Fuld 1981). The materials involved 10 common objects (e.g., spoon, comb, zipper, glasses), presented consecutively from a bag for tactile naming. A series of four recall trials were then administrated. Selective reminding was provided at the end of each recall trial, such that the experimenter repeated the names of the objects participants failed to recall. Participants were given 2 min for each recall trial.

Four measures were derived from this test: a) total recall, which refers to all objects correctly recalled across the four trials; b) long-term retrieval, denoting the total of objects correctly recalled on at least two consecutive trials without reminding; c) list learning, indicating the total number of objects consistently recalled without further reminding; and d) short-term retrieval, denoting all objects recalled with reminding from the previous trial.

WORD RECOGNITION. After the delayed free recall task, a self-paced yes–no recognition test was administered, in which the 12 target words were presented along with 12 distractors. The recognition test took about 3 min to complete. No participant reported requiring additional time in any memory test.

Results

Recall

OBJECT RECALL. The results for the four measures derived from the Fuld Object-Memory Evaluation are presented in Table 2. A 2 (B_{12} level: normal, low) × 2 (folic acid level: normal, low) analysis of variance (ANOVA) was conducted on each of the dependent measures from the object recall task (total recall, long-term retrieval, list learning, and short-term retrieval). The analyses showed no main effects of B_{12} level (Fs < 1), and no interaction effects between B_{12} level and folic acid level (Fs < 1). On the other hand, there were significant effects of folic acid in total recall $F(1,67) = 4.60$, $MS_E = 50.82$, $p < .05$, $\omega^2 = .05$, long-term retrieval $F(1,67) = 5.79$, $MS_E = 79.96$, $p < .05$, $\omega^2 = .06$, and list learning $F(1,67) = 3.98$, $MS_E = 80.95$, $p = .05$, $\omega^2 = .04$. These effects were all due to the fact that the groups with normal levels of folic acid performed better than the groups with low levels of folic acid.

WORD RECALL. The word recall data were first analyzed with a 2 (B_{12} level: normal, low) × 2 (folic acid: normal, low) × 2 (time of testing: immediate, delayed) mixed ANOVA, with repeated measures on the last factor. This ANOVA showed that B_{12} level had no effect on word recall ($F < 1$), and that none of the interaction effects were significant (ps $> .05$); however, the effect of folic acid level was again significant, $F(1,67) = 12.74$, $MS_E = 4.46$, $p < .01$, $\omega^2 = .14$. This effect reflected that the groups with low folic acid levels performed reliably worse than the groups with normal folic acid levels at both immediate recall ($p < .01$) and delayed recall ($p < .05$). Further, time of testing had a significant effect on recall, $F(1,67) = 227.24$, $MS_E = 1.24$, $p < .001$, $\omega^2 = .76$, such that more words were recalled at immediate testing (mean = 4.80) than at delayed testing (mean = 1.65). These data are shown in Table 2.

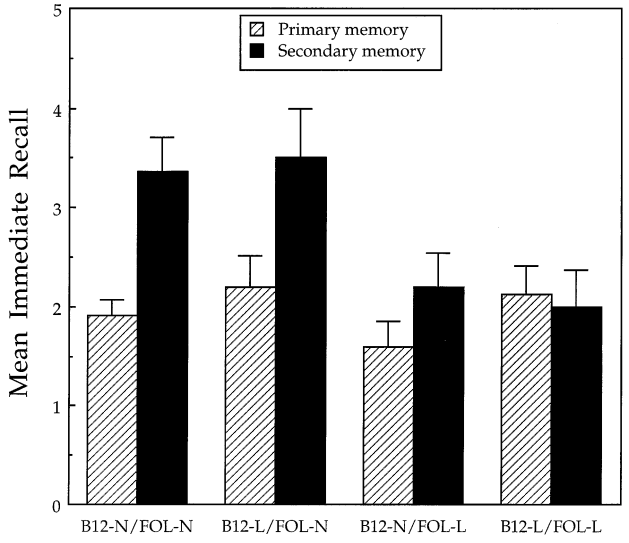

Figure 1. Relative contribution of primary and secondary memory to immediate word recall across vitamin groups. B_{12}, vitamin B_{12}; FOL, folic acid; N, normal; L, low. (Error bars represent standard errors around the means.)

The immediate recall data were then partitioned into the relative contribution of primary memory (PM) and secondary memory (SM), using the lag method devised by Tulving and Colotla (1970). This classification of recall is derived from the order in which items are presented and recalled. An item is assumed to be retrieved from PM if not more than seven items intervene between its presentation and recall. Other items are classified as part of SM.

A 2 (B_{12} level: normal, low) × 2 (folic acid: normal, low) ANOVA showed no effects of vitamin levels for the PM data ($ps > .05$). For SM, the ANOVA revealed no effect of B_{12} and no interaction effect ($ps > .05$); however, a reliable effect of folic acid level on SM was obtained, $F(1,67) = 7.21$, $MS_E = 3.16$, $p < .01$, $\omega^2 = .10$, reflecting a negative effect of low level of folic acid on SM. PM and SM scores across vitamin group are portrayed in Figure 1.

Although there were no significant group differences in education, inspection of Table 1 reveals that the two groups with low levels of folic acid had received less education than the remaining two groups. To control for the possibility that the effects of folic acid observed in word recall and object recall were due to differences in education, analyses of covariance (ANCOVAs) were conducted, with years of education as a covariate. These ANCOVAs yielded identical patterns of effects as the ANOVAs previously reported. Thus, it was concluded that the observed effects of folic acid on recall performance did not result from group differences in education.

Recognition

Word and face recognition data are presented in Table 2. Correct recognitions (hits) and false alarms were transformed into d' scores (Hochhaus 1972). In addition, to determine whether the four vitamin groups differed in response bias, we used the C measure devised by Snodgrass and Corwin (1988). An important advantage of C compared to related measures of response bias is that it is unrelated to recognition accuracy. C is computed according to the following formula:

$$C = Z_{FA} - d'/2 = 0.5\,(Z_F A + Z_H)$$

where a C value of 0 indicates completely neutral bias, a positive value indicates conservative bias, and a negative value indicates liberal bias.

FACE RECOGNITION. A 2 (B_{12} level: normal, low) × 2 (folic acid: normal, low) ANOVA was conducted on each of the dependent variables (hits, false alarms, d', and C). The analysis showed no main effects or interaction effects ($ps > .10$).

WORD RECOGNITION. A 2 (B_{12} level: normal, low) × 2 (folic acid: normal, low) ANOVA on the word recognition variables also revealed a lack of reliable effects ($ps > .10$).

Correlational Analyses

After having established that folic acid, but not B_{12}, was related to performance in specific episodic memory tasks using group comparisons, it was judged important to assess these relationships using vitamin status as a continuous variable. These correlational analyses revealed only one significant relationship, namely that folic acid was reliably related to immediate word recall ($r = .24$; $p < .05$). Thus, for most of the variables that were affected by folic acid in the group analyses (i.e., total recall, list learning, long-term retrieval, immediate and delayed word recall), there was no reliable linear relationship.

Discussion

The purpose of this study was to assess potential effects of low levels of vitamin B_{12} and folic acid, separately and combined, on episodic memory functioning in very old age. To achieve this objective, a population-based sample of very old people, rigorously screened for various diseases, completed a memory battery comprising recognition of faces and words, immediate and delayed recall of words, and The Fuld Object Memory Evaluation. To examine the separate and combined influence of B_{12} and folic acid on memory performance, four groups were

constructed based on combinations of normal and low levels of these vitamins, respectively.

In general, the results showed no effects of vitamin B_{12} level in any of the memory tasks nor any interaction effects between vitamin B_{12} and folic acid. The lack of interaction effects between the two vitamins indicates that there were no joint influences of B_{12} and folic acid in the present study sample. Level of folic acid, on the contrary, had clear effects in some of the memory tasks. Specifically, negative effects of low levels of folic acid were found in immediate and delayed word recall as well as in object recall. These effects were due to folate-related deficits in secondary memory functioning. No effects of folic acid were seen in primary memory or in any of the recognition tasks.

The fact that there were no effects of serum vitamin B_{12} level on episodic memory in this sample of healthy very old adults is consistent with several previous studies using healthy older individuals (Crystal et al 1994; La Rue et al 1997; Riggs et al 1996; Wahlin et al 1996), although contrary results were reported in an early study by Goodwin et al (1983). The reasons for the mixed findings are unclear but may reflect differences across studies with regard to cutoff levels as well as in the exclusion criteria employed (e.g., in terms of concomitant diseases).

In addition, as noted by some authors, measuring serum B_{12} levels may not be the most sensitive method to diagnose B_{12} deficiency (see Stabler 1995; van Goor et al 1995, for a discussion), because it is possible to have serum B_{12} within normal limits, although being metabolically deficient in B_{12}. Therefore, it has been suggested that methylmalonic acid and homocysteine in serum may be more reliable indicators of B_{12} deficiency (e.g., Allen et al 1990). Unfortunately, such analyses were not possible to conduct in this population-based sample. Concerning diagnosis of folic acid deficiency, serum levels indicate levels during the past few days, whereas erythrocyte folate levels indicate the balance during the past few months and may be a better indicator of total folate storage in the body (e.g., Babior 1990); however, the strong relationship between folic acid level and some of the episodic memory tasks found in the present study indicates that folic acid level in serum may be an important factor for episodic memory functioning in very old age.

The present results are in agreement with those recently reported by Wahlin et al (1996). On the other hand, some studies have reported no folate-related effects on memory performance (Goodwin et al 1983; La Rue et al 1997; Riggs et al 1996; Tucker et al 1990), although negative effects of low levels have been found in tests assessing other cognitive functions (Goodwin et al 1983; La Rue et al 1997; Riggs et al 1996). Interestingly, most of the studies that report no effects of folic acid level on memory functioning (La Rue et al 1997; Riggs et al 1996; Tucker et al 1990) have employed correlational analyses. This may not be a sensitive way to detect an association, if the relationship is nonlinear, but rather is restricted to those at the lower end of the distribution (Goodwin et al 1983). This line of reasoning received support in the Wahlin et al (1996) study, where an association was found between folic acid status and memory functioning only when data were analyzed using group comparisons, but not when linear regression analyses were employed. Likewise, in the present study, only one of the memory measures proved to be significantly associated with folic acid level using correlational analyses, whereas effects were seen for six variables using group comparisons. The important conclusion to be drawn from these comparisons is that the choice of analytical method may partly determine whether or not folate-related effects on memory will be observed. In particular, the difference in outcome between the two statistical procedures suggest that there may be a critical level above which the relationship in question disappears.

Regarding the nature of the memory deficits associated with low folic acid levels in the current study, a consistent pattern was found in that there were no group differences in the measures that reflect primary memory, whereas low levels of folic acid were associated with deficits in all measures of secondary memory. This pattern was seen not only in object recall, where low levels of folic acid had a clear effect on long-term retrieval and list learning and no effect on short-term retrieval, but also in the word recall task, where low levels of folic acid had a negative influence on SM but not on PM. Thus, the current data suggest that low levels of folic acid may result in problems in transferring information to some form of permanent representation, but not with regard to the temporary holding of information in consciousness.

An interesting finding in this study was that the effect of folic acid found in the recall tasks disappeared in the recognition tasks. The crucial difference between free recall and recognition is the amount of retrieval support provided. In a recognition task, a substantial amount of retrieval support is provided compared with free recall. Thus, the finding that folate-related effects on memory were eliminated in recognition suggests that retrieval problems may have contributed to the impairment observed (Craik and McDowd 1987; Schonfield and Robertson 1966). Thus, bringing together all the different findings concerning the effects (or lack thereof) of folic acid demonstrated in this study, the interesting conclusion is that low levels of this vitamin in very old age may influence encoding and retrieval processes, while leaving primary memory unaffected.

The biochemical basis for the neurological changes that occur in vitamin B deficiency remains unclear (see Green

and Kinsella 1995). Likewise, additional studies are needed to delineate the mechanisms that underlie the association between vitamin status and cognitive performance that was documented in the present study; however, it is well known that both vitamin B_{12} and folic acid are necessary for the metabolism of nucleid acid that controls the rate at which protein synthesis takes place (e.g., Robinson 1966; Venkataraman et al 1967). Given that protein synthesis in the brain is an essential process for the encoding of information in episodic memory (Davis and Squire 1984), it is tempting to speculate that the vitamin-related memory deficits observed were due to alterations in brain protein metabolism. Moreover, the present finding that folic acid was related to memory performance, whereas vitamin B_{12} was not, may reflect that the permeability of the blood–brain barrier changes in persons with low levels of folic acid (Botez 1989). As a result, folic acid levels in the blood may reflect more accurately the availability of this vitamin in the brain than what is true for vitamin B_{12}.

Another possibility that cannot be ruled out is that the folate-related effects on memory observed were due to an overrepresentation of persons in a preclinical phase of dementia or depression in the groupings with low folic levels. It is known that both Alzheimer's disease (Linn et al 1995; Small et al 1997; Tierney et al 1996) and depression (Berger et al 1998; Henderson and Jorm 1997; Kivelä et al 1996) may have relatively long preclinical periods in late life during which cognitive deficits are detectable. It is also known that folic acid plays a role in regulating the level of homocysteine (Chu and Hall 1988; Brattström et al 1988; La Rue et al 1997); high homocysteine is a risk factor for vascular disease (e.g., vascular dementia), and concomitant vascular disease is often seen in very old persons with Alzheimer's disease (Snowdon et al 1997). In addition, low folic acid levels have been linked to depression (Botez et al 1984; Schlegel and Nieber 1989). Although the present sample was carefully screened for dementia and depression, the low folic acid groups may involve more persons with subclinical dementia and depression than the remaining groups. A follow-up of this sample is currently undertaken. Information from this follow-up will reveal whether incidence rates for dementia and depression are higher in the groups with low folic acid than in the groups with normal levels.

Given the present relationship between folic acid and episodic memory, an interesting question concerns whether replacement therapy would result in improved memory performance among persons with low folate levels. Research addressing this issue has yielded mixed results, with some studies reporting improved cognitive performance following supplementation (Botez et al 1984; Rapin et al 1988) and others not (Joyal et al 1993; Kral et al 1970). There is some evidence that both the duration of cognitive symptoms and the length of the treatment period influence the probability of obtaining positive effects of replacement therapy in persons with vitamin B deficiency (Martin et al 1992).

A potential limitation of the present study is that the sample sizes were relatively small. Small sample sizes influence the power of a study to detect a potential relationship between variables. The reasons for the small sample sizes were a) the very old age of the participants, and b) the strict health screening employed to rule out possible effects of other conditions (e.g., depression, dementia, diabetes, thyroid disease) on performance. Larger sample sizes could have been obtained in this study by applying less strict inclusion criteria; however, by relaxing the inclusion criteria other problems would have emerged, associated with separating the effects of vitamin status from those resulting from other health-related conditions.

To summarize, in this study of healthy very old adults, there were no effects of serum vitamin B_{12} level on episodic memory functioning. Neither were there any interactive effects of low levels of B_{12} and folic acid. On the other hand, low levels of folic acid were associated with poorer performance in object recall and word recall. The effects of low folic acid levels were seen in measures reflecting secondary memory, whereas no effects were found in measures reflecting primary memory. Contrary to the findings in the episodic recall tasks, there were no vitamin-related effects in any of the recognition tasks. Most prior research concerning the relationship between vitamin status and cognitive functioning has involved younger cohorts (Crystal et al 1994; La Rue et al 1997; Riggs et al 1996). The present results replicate previous findings that folic acid may be more critical than vitamin B_{12} to memory functioning in late life (Wahlin et al 1996), and extend those findings into very old age.

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References

Abou-Saleh MT, Coppen A (1988): The biology of folate in depression: Implications for nutritional hypotheses of the psychoses. *J Psychiatr Res* 20:91–101.

Allen RH, Stabler SP, Savage DG, Lindenbaum J (1990): Diagnosis of cobalamin deficiency: Usefulness of serum methylmalonic acid and total homocysteine concentrations. *Am J Hematol* 34:90–98.

American Psychiatric Association (1987): *Diagnostic and Statistical Manual of Mental Disorders*, 3rd ed rev. Washington, DC: American Psychiatric Press.

American Psychiatric Association (1994): *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed. Washington, DC: American Psychiatric Press.

Babior BM (1990): Metabolic aspects of folic acid and cobalamin. In: Williams WJ, Beutler E, Erslev AJ, Lichtman MA, editors. *Hematology*. New York: McGraw Hill, pp 339–354.

Bäckman L (1991): Recognition memory across the adult life span: The role of prior knowledge. *Mem Cogn* 19:63–71.

Bäckman L, Hassing L, Forsell Y, Viitanen M (1996): Episodic remembering in a population-based sample of nonagenarians: Does major depression exacerbate the memory deficits seen in Alzheimer's disease? *Psychol Aging* 11:649–657.

Bäckman L, Small BJ, Larsson M (in press): Memory. In: Evans JG, Williams TF, Beattie BL, Michel J-P, Wilcock GK, editors. *Oxford Textbook of Geriatric Medicine*, 2nd ed. Oxford: Oxford University Press.

Beck WS (1983): Metabolic aspects of vitamin B_{12} and folic acid. In: Williams WJ, Beutler E, Erslev AJ, Lichtman MA, editors. *Hematology*. New York: McGraw Hill, pp 311–331.

Bell IR, Edman JS, Marby DW, Satlin A, Dreier T, Liptzin B, et al (1990): Vitamin B_{12} and folate status in acute geropsychiatric inpatients: Affective and cognitive characteristics of a vitamin nondeficient population. *Biol Psychiatry* 27:125–137.

Berger A-K, Small BJ, Forsell Y, Winblad B, Bäckman L (1998): Preclinical symptoms of major depression in very old age: A prospective longitudinal study. *Am J Psychiatry* 155:1039–1043.

Bohnen N, Degenaar CP (1992): Lower blood levels of vitamin B_{12} are related to decreased performance of healthy subjects in The Stroop Color-Word Test. *Neurosci Res Com* 11:53–56.

Botez MI (1989): Neuropsychiatric illness and deficiency of vitamin B_{12} and folate. In: Zittoun JA, Cooper BA, editors. *Folate and Cobalamins*. Berlin: Springer-Verlag, pp 145–159.

Botez MI, Botez T, Maag U (1984): The Wechsler subtest in mild organic brain damage associated with folate deficiency. *Psychol Med* 14:431–437.

Brattström L, Israelsson B, Jeppsson JO, Hultberg B (1988): Folic acid — An innocuous means to reduce plasma homocysteine. *Scand J Clin Lab Invest* 48:215–221.

Chen IW, Silberstein EB, Maxon HR, Volle CP, Sohnlein BH (1982): Semiautomated system for simultaneous assays of serum vitamin B_{12} and folic acid in serum evaluated. *Clin Chem* 28:2161–2165.

Chu RC, Hall CA (1988): The total serum homocysteine as an indicator of vitamin B_{12} and folate status. *Am J Clin Pathol* 90:446–449.

Coenen AML, Konings GMLG, Aldenkamp AP, Reiner WO (1995): Effects of chronic use of carbamazepine and valproate on cognitive processes. *J Epilepsy* 8:250–254.

Craik FIM, McDowd JM (1987): Age differences in recall and recognition. *J Exp Psychol Learn Mem Cogn* 13:474–479.

Crystal HA, Ortof E, Frishman WH, Gruber A, Hershman D, Aronson M (1994): Serum vitamin B_{12} levels and incidence of dementia in a healthy elderly population: A report from the Bronx longitudinal aging study. *J Am Geriatr Soc* 42:933–936.

Davis HP, Squire LR (1984): Protein synthesis and memory: A review. *Psychol Bull* 96:518–559.

Elias MF, Elias JW, Elias PK (1990): Biological and health influences on behavior. In: Birren JE, Schaie KW, editors. *Handbook of the Psychology of Aging*, 3rd ed. San Diego, CA: Academic Press, pp 79–102.

Folstein MF, Folstein SE, McHugh PR (1975): "Mini-Mental State": A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 12:189–198.

Fuld PA (1981): *The Fuld Object Memory Test.* Chicago: Stoelting Instrument Company.

Goodwin JS, Goodwin JM, Garry PJ (1983): Association between nutritional status and cognitive functioning in a healthy elderly population. *JAMA* 249:2917–2921.

Green R, Kinsella LJ (1995): Current concepts in the diagnosis of cobalamin deficiency. *Neurology* 45:1435–1440.

Henderson AS, Jorm AF (1997): Some contributions to the epidemiology of dementia and depression. *Int J Geriatr Psychiatry* 12:145–154.

Herbert V (1987): Nutrition science as a continually unfolding story: The folate and vitamin B_{12} paradigm. *Am J Clin Nutr* 46:387–402.

Herbert V, Zalusky R (1962): Interrelation of vitamin B_{12} and folic acid metabolism: Folic acid clearance studies. *J Clin Invest* 41:1263–1276.

Hochhaus L (1972): A table for the calculation of d′ and β. *Psychol Bull* 77:375–376.

Joyal CC, Lalond R, Vikis-Freibergs V, Botez MI (1993): Are age-related behavioral disorders improved by folate administration? *Exp Aging Res* 19:367–376.

Kausler DH (1994): *Learning and Memory in Normal Aging.* San Diego, CA: Academic Press.

Kivelä SL, Kongäs-Saviaro P, Pahkala K, Kesti E, Laipalla P (1996): Health, health behavior, and functional ability predicting depression in old age: A longitudinal study. *Int J Geriatr Psychiatry* 11:871–877.

Kliegl R, Smith J, Baltes PB (1989): Testing-the-limits and the study of adult age differences in cognitive plasticity of a mnemonic skill. *Dev Psychol* 25:247–256.

Kral VA, Solyom L, Enesco H, Ledwidge B (1970): Relationship of vitamin B_{12} and folic acid to memory function. *Biol Psychiatry* 2:19–26.

Krasinski SD, Russel RM, Samloff IM, Jacob RA, Dallal GE, McGandy RB, et al (1986): Fundic atrophic gastritis in an elderly population: Effect on hemoglobin and several serum nutritional indicators. *J Am Geriatr Soc* 34:800–806.

La Rue A, Swan GE, Carmelli D (1995): Cognition and depression in a cohort of aging men. *Psychol Aging* 10:30–33.

La Rue A, Koehler KM, Wayne SJ, Chiulli SJ, Haaland KY, Garry PH (1997): Nutritional status and cognitive functioning in a normally aging sample: A 6-year assessment. *Am J Clin Nutr* 65:20–29.

Light LL (1991): Memory and aging: Four hypotheses in search of data. *Annu Rev Psychol* 42:333–376.

Lindenbaum J, Rosenberg I, Wilson P, Stabler SP, Allen RH (1994): Prevalence of cobalamin deficiency in the Framingham elderly population. *Am J Clin Nutr* 60:2–11.

Linn RT, Wolf PA, Bachman DL, Knoefel JE, Cobb JL, Belanger AJ, et al (1995): The "preclinical phase" of probable Alzheimer's disease. *Arch Neurol* 52:485–490.

Martin DC (1988): B-12 and folate deficiency dementia. *Clin Geriatr Med* 4:841–852.

Martin DC, Francis J, Protetch J, Huff FJ (1992): Time dependency of cognitive recovery with cobalamin replacement: Report of a pilot study. *J Am Geriatr Soc* 40:168–172.

Mennemeier M, Garner RD, Heilman KM (1993): Memory, mood and measurement in hypothyroidism. *J Clin Exp Neuropsychol* 15:822–831.

Metz J, Bell AH, Flicker L, Bottiglieri T, Ibrahim J, Seal E, et al (1996): The significance of subnormal serum vitamin B_{12} concentration in older people: A case control study. *J Am Geriatr Soc* 44:1355–1361.

Morris RG, Kopelman MD (1986): The memory deficits in Alzheimer-type dementia: A review. *Q J Exp Psychol* 38A: 575–602.

Nyberg L, Tulving E (1996): Classifying human long-term memory: Evidence from converging dissociations. *Eur J Cogn Psychol* 8:163–183.

Ortega RM, Manas LR, Andrés P, Gaspar MJ, Agudo FR, Jiménez A, et al (1996): Functional and psychic deterioration in elderly people may be aggravated by folate deficiency. *J Nutr* 126:1992–1999.

Pennypacker LC, Allen RH, Kelly JP, Matthews LM, Grigsby J, Kaye K, et al (1992): High prevalence of cobalamin deficiency in elderly outpatients. *J Am Geriatr Soc* 40:1197–1204.

Rapin JR, Le Poncin M, Grebyl J (1989): Blood folates deficiencies and cognitive functions in aging. *Trends Biomed Gerontol* 1:221–223.

Riggs KM, Spiro A III, Tucker K, Ruh D (1996): Relations of vitamin B-12, vitamin B-6, folate, and homocysteine to cognitive performance in the Normative Aging Study. *Am J Clin Nutr* 63:306–314.

Robinson FA (1996): *The Vitamin Co-factors of Enzyme Systems.* New York: Pergamon Press.

Salthouse TA (1991): *Theoretical Perspectives on Cognitive Aging.* Hillsdale, NJ: Erlbaum.

Schlegel S, Nieber D (1989): Folic acid and cognition in the elderly depressed. *Biol Psychiatry* 25:976–977.

Schonfield D, Robertson EA (1966): Memory storage and aging. *Can J Psychol* 20:228–236.

Small BJ, Herlitz A, Fratiglioni L, Almkvist O, Bäckman L (1997): Cognitive predictors of incident Alzheimer's disease: A prospective longitudinal study. *Neuropsychologia* 11:413–420.

Snodgrass JG, Corwin J (1988): Pragmatics of measuring recognition memory: Applications to dementia and amnesia. *J Exp Psychol Gen* 117:34–50.

Snowdon DA, Greiner LH, Mortimer JA, Riley KP, Markesbery WR (1997): Brain infarction and the clinical expression of Alzheimer's disease. *JAMA* 277:813–817.

Squire LR (1987). *Memory and Brain.* New York: Oxford University Press.

Stabler SP (1995): Screening the older population for cobalamin (vitamin B_{12}) deficiency. *J Am Geriatr Soc* 43:1290–1297.

Tierney MC, Szalai JP, Snow WG, Fischer RH, Nores A, Nadon G, et al (1996): Prediction of probable Alzheimer's disease in memory-impaired patients: A prospective longitudinal study. *Neurology* 46:661–665.

Tucker DM, Penland JG, Sandstead HH, Milne DB, Heck DG, Klevay LM (1990): Nutrition status and brain function in aging. *Am J Clin Nutr* 52:93–102.

Tulving E (1983): *Elements of Episodic Memory.* Oxford: Oxford University Press.

Tulving E, Colotla V (1970): Free recall of trilingual lists. *Cogn Psychol* 1:86–98.

Tulving E, Schacter DL (1990): Priming and human memory systems. *Science* 247:301–306.

Tun PA, Nathan DM, Perlmuter LC (1990): Cognitive and affective disorders in elderly diabetics. *Clin Geriatr Med* 6:731–746.

van Goor LP, Woiski MD, Lagaay AM, Meinders AE, Tak PP (1995): Review: Cobalamin deficiency and mental impairment in elderly people. *Age Ageing* 24:536–542.

Venkataraman S, Walerych W, Johnson BC (1967): Methylation of t-RNA: Effect of vitamin B_{12} deficiency in rats. *Proc Soc Exp Biol Med* 124:204–207.

Wahlin Å, Hill RD, Winblad B, Bäckman L (1996): Effects of serum vitamin B_{12} and folate status on episodic memory performance in very old age: A population-based study. *Psychol Aging* 11:487–496.