

Original Research

Serum Vitamin B₁₂, C and Folate Concentrations in the New Mexico Elder Health Survey: Correlations with Cognitive and Affective Functions

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Key words: vitamin B₁₂ (cobalamin), vitamin C (ascorbic acid), folic acid, Hispanic elderly, cognitive function, depression

Objectives: 1) To compare serum vitamin B₁₂, C and folate concentrations in a randomly selected sample of elderly (age 65 years or older) male and female Hispanics and nonHispanic whites (NHW) and 2) to examine associations between serum B₁₂, C and folate concentrations compared to measures of cognitive and affective (depression) functions.

Methods: Equal numbers of male and female Hispanics and NHW were randomly sampled from the Health Care Financing Administration (Medicare) registrant list for Bernalillo County, New Mexico, and asked to volunteer for a paid home interview followed by a paid comprehensive interview/examination covering health and health-related issues. In addition to serum determinations of B₁₂, C and folate, associations were examined between these vitamins and measures of cognitive and affective functions.

Results: Males and Hispanics had lower serum vitamin B₁₂, C and folate concentrations than females and NHW respectively. Participants taking a multivitamin supplement (MVI) had higher serum vitamin concentrations than those not taking MVI. There were significant associations between serum folate concentrations and measures of cognitive function, not seen with B₁₂ or C, nor between any of the vitamins and affective function.

Conclusions: Hispanics, even after adjustments for gender, age, vitamin supplementation, vitamin content of dietary foods, education and household income, had lower serum concentrations of B₁₂, C and folate than NHW. The most significant associations observed were those between serum folate and various measures of cognitive function, even after adjusting for presence of depression.

INTRODUCTION

In the past, recommended vitamin intakes have often been based on levels that were adequate to prevent clinical deficiencies from developing. Once these levels were reached, clinicians and nutrition scientists generally attributed little value to higher vitamin intakes from supplements or food sources.

More recently, evidence has continued to mount showing that the intakes and serum concentrations of certain vitamins,

e.g. vitamins B₁₂, folate and C, above those necessary to prevent clinical deficiencies, might importantly influence health status. Supplements of vitamin B₁₂ and folate lower serum homocysteine concentrations, and even minor elevations of serum homocysteine increase the risk of vascular disease [1–8]. Although the body of evidence demonstrating the vascular protective effects of higher B₁₂ and folate intake have been accumulating, this evidence was considered too preliminary for incorporation into the Dietary Reference Intakes for

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the B vitamins [9]. Similarly, an inverse association has been observed between vitamin C intake and/or serum concentrations and the presence of atherosclerotic vascular disease, notably stroke [10,11].

Poorer cognitive function has been reported in individuals with lower serum concentrations of B₁₂ and/or folate (and higher serum concentrations of homocysteine) [12–16], and vitamin C [17]. Depression has been reported to be the most common neuropsychiatric manifestation of folate deficiency [18–21] and needs to be considered when evaluating cognitive status, as it is well recognized that depression can mimic impaired cognitive function (pseudodementia). Little information is available comparing serum vitamin B₁₂, C and folate in elderly Hispanics and non-Hispanic whites. A recent publication [22] from the National Health and Nutrition Examination Survey (1988–91) reports that serum and red cell folates were significantly lower in younger Mexican Americans compared to non Hispanic whites.

The New Mexico Elder Health Survey (NMEHS) was a study of health and health-related issues in nearly equal numbers of elderly (65 years of age or older) Hispanic and non-Hispanic white (NHW) males and females randomly selected from the Health Care Financing Administration (Medicare) rolls of Bernalillo County (Albuquerque), New Mexico [23,24]. One of the objectives of the NMEHS was to compare nutritional status in these two ethnicities, both by obtaining detailed information on dietary intake and supplements and by quantifying serum concentrations of vitamin B₁₂, folate and C. A database also was available on cognitive and affective (depression) functions, which allowed us to make comparisons between vitamin nutritional status and these functions.

METHODS

Study Design/Subjects

Twenty-two hundred prospective participants (equal numbers of Hispanic and non Hispanic white males and females) were randomly selected from 50,700 HCFA registrants (Medicare recipients), age 65 years or older, residing in Bernalillo County (Albuquerque), New Mexico. After eliminating those who had died and moved from the county, those who could not be located either because only a post office box address was available or they did not respond to notes left at their homes, and those who were ineligible because they clearly did not meet criteria to qualify as Hispanic or NHW, 1,666 eligible participants were contacted. Eleven hundred and thirty (67.8%) participated in home interviews. An additional 29 interviewees were thereafter found not qualified for the survey because they either did not meet ethnicity standards (self identification and three of four grandparents Hispanic or NHW respectively) or had died or moved after the home interview and before an examination could be completed. Of the 1101 individuals interviewed at home and found eligible, 883 (80.2%) participated

in a four-hour interview/examination by a nurse practitioner, nurse and nutritionist. There were no other exclusionary criteria as long as informed consent could be obtained from the participant or legal guardian. All participants gave a written informed consent and the research was approved by the Human Research Review Committee of the University of New Mexico Health Sciences Center. Further details on the design and survey instruments used in this study and demographic characteristics of the recruited participants are published elsewhere [24].

Serum Vitamin B₁₂ and Folate

Between May 1993 and March 1994, serum vitamin B₁₂ and folate concentrations were determined on 318 samples using the SimulTRAC Radioassay (RA) kit from Becton-Dickinson (Orangeburg, NY), Cat #262226. Samples collected after March 1994 (n=515) were assayed by the Quantaphase II RA kit from Bio-Rad (Hercules, CA), Cat #191-1041. The introduction of the Quantaphase II RA in 1994 was due to issues associated with the Life Sciences Research Office Report [25], on the assessment of radioassays used to measure serum folate, citing consistently a 30% elevation in folate levels in current radioassays. Because of this factor, a new, but properly standardized procedure, the Quantaphase II RA, was implemented in our laboratory. In comparing folate levels measured by the SimulTRAC RA with the Quantaphase II RA, it was determined that a correction factor (of 0.735) needed to be employed to adjust SimulTRAC RA values to those results obtained using the Quantaphase II RA. In comparing the adjusted results of 80 samples from the SimulTRAC RA (adjusted by a factor of 0.735) with the new Quantaphase II RA, a linear correlation of 0.99 was achieved. In the process of establishing the new B₁₂/folate methodology, it was also noted that a correction factor had to be established for the vitamin B₁₂ levels using the Quantaphase II RA. In comparing 80 samples by both methodologies, the SimulTRAC RA showed B₁₂ levels consistently higher, by 6.4%, when compared to the new Quantaphase II B₁₂ RA. As a result of this comparison, the 318 samples assayed for B₁₂ levels by the SimulTRAC RA were corrected for by a 6.4% factor.

Employing the standardized methodology, B₁₂ and folate concentrations were determined on the remaining 515 serum samples by simultaneously using the ¹²⁵I/⁵⁷Co Quantaphase II Radioassay from Bio-Rad Corp (Hercules, CA), Cat #191-1041. The assay's minimum detectable concentration of B₁₂ is 15 pmol/L (20 pg/mL) and for folate is 0.2 nmol/L (0.1 ng/mL). The normal range, as established by the manufacturer for vitamin B₁₂ is 96 to 567 pmol/L (130–770 pg/mL) and for folate is 0.7–9.5 nmol/L (1.5–20.6 ng/mL) with the coefficient of variation of the inter-assay precision being 4.0% to 5.9% and 3.8% to 5.2%, respectively. The rationales for selection of "cutpoints" of 221 pmol/L (300 pg/mL) to separate participants

with normal vs. low and low normal serum vitamin B₁₂ concentrations, and 11 nmol/L (5 ng/mL) for serum folate concentrations is outlined in the discussion.

Vitamin C (ascorbic acid) analysis was performed on serum samples utilizing the procedure described by Garry *et al.* [26]. The normal range established by this procedure is 28 to 85 $\mu\text{mol/L}$ (0.5 to 1.5 mg/dL) with an assay sensitivity of 14 $\mu\text{mol/L}$ (0.25 mg/dL). The coefficient of variation of the inter-assay precision is 1.5% to 9.6% (n=36 assays).

Neuropsychological Assessment

Cognitive test measures and the functions they were intended to estimate were 1) the Mini-Mental State Exam (MMSE) (orientation, recall, attention, language, and visual graphic ability) [27], 2) WAIS-R Digits Forward (attention and immediate memory) [28], 3) Fuld Object Memory Evaluation (learning and secondary memory) [29], 4) clock drawing (visuoconstruction) [30] and 5) two Color Trail Making Tests (psychomotor speed and cognitive flexibility) [31]. How these measures were used in this population are described in more detail elsewhere [32].

Three indicators of depression were obtained. First, a self report of a past history of depression was available from the interview. Second, a list of all current medications was obtained and coded by a pharmacist so that antidepressants could be identified by a single code number. Third, the 15-question short version of the Yesavage Geriatric Depression Scale (GDS) was administered in both Spanish and English versions [33]. Both the total number of questions answered to indicate depression and percent of participants with greater than six answers indicating depression are reported.

Additional Information

Dietary intakes for the three vitamins were obtained from the Health Habits and History Questionnaire, a food frequency questionnaire, using methods previously described [34]. Information on vitamin supplementation also was obtained. Participants were asked if they had taken any multivitamins (MVI) in the last 12 months (those answering affirmatively were the participants included in the MVI category), how many of the last 12 months had they taken MVI and how many days per week they took them. Of the individuals taking MVI, 85.1% took them daily and 91.5% took them at least every other day; 81.6% had taken them every month for the 12 months prior to the interview. For vitamin C, participants were included in the vitamin supplement category if they took either MVI or vitamin C supplements. Education was quantified both in years of education and whether or not participants had a high school education. Household income was separated into incomes less than \$15,000 per year (poverty level) and those \$15,000 per year or more.

Statistical Methods

Participants with any component of the necessary database missing were excluded from that analysis. This meant from 751 to 816 participants were included in the four tables. Continuous variables were tested for normality in distribution. A logarithmic transformation was applied to skewed variables, e.g. vitamin intake from food, to normalize distributions before statistical analysis. Group comparisons were conducted using general linear models for continuous variables adjusting for the effects of gender, ethnicity, vitamin supplement usage, age (five-year intervals) education (high school graduate vs. non graduate) and household income (less than \$15,000 vs. equal to or greater than \$15,000) (Table 2). Also examined was the effect in these models of adjusting for vitamin intake from food. For categorical variables, e.g. serum vitamin concentrations above and below certain cutoff levels (Table 3), and use/nonuse of multivitamin supplements (Table 4), multivariate logistic regression models were fitted for each of the primary outcomes (cognitive and affective tests) adjusting for gender, ethnicity, age (years), education (years), and household income (<\$15,000 vs. \leq \$15,000). Also examined were the effects on each of the cognitive tests of adjusting for each of the three measures of depression. All analyses were done using SAS software [35].

RESULTS

Table 1 shows the number of participants and mean serum concentrations of vitamin B₁₂ (cobalamin), folic acid and vitamin C (ascorbic acid) for the four ethnic/gender groups studied. The mean age of the participants studied was 74.1 years. Participants were divided into those taking a vitamin supplement (MVI) and those who did not (no MVI). Whereas 48% of the nonHispanic white population took a MVI, only 32% of the Hispanic population did. Table 2 shows multivariate linear models predicting the serum vitamin concentrations using age (five-year intervals), ethnicity, gender, multivitamin usage, education and income. The models show that, when adjusted for each of the other variables, men had lower serum concentrations of folate and vitamin C, but not vitamin B₁₂ compared to women, and Hispanics had lower serum vitamin concentrations for all three vitamins than nonHispanic whites. Individuals taking a vitamin supplement had higher serum concentrations of all three vitamins compared to those who did not. No significant associations were found, when adjusted, between the three vitamin concentrations and level of education. A significant association was found between serum folate and vitamin C, and annual household income with participants having an income above poverty levels (greater than \$15,000) having higher vitamin concentrations.

Also examined using these models was the effect of vitamin intake from food (Food Frequency Questionnaire) (log transformation of a continuous variable), but this was not included

Table 1. Number of participants (n) and mean±SD serum vitamin B₁₂, folate and vitamin C concentrations in 795 participants with and without a multivitamin supplement (MVI) for the 4 ethnic/gender groups in the New Mexico Elder Health Survey

	Hispanic Males		NHW Males		Hispanic Females		NHW Females	
	n	Mean±SD	n	Mean±SD	n	Mean±SD	n	Mean±SD
Vitamin B ₁₂ (pmol/L)								
No MVI	148	289 ± 136	125	319 ± 139	103	310 ± 141	96	339 ± 169
MVI	51	389 ± 158	101	418 ± 214	67	396 ± 192	104	466 ± 232
Folate (nmol/L)								
No MVI	148	17 ± 9	125	21 ± 13	103	18 ± 15	96	22 ± 16
MVI	51	36 ± 24	100	43 ± 28	67	36 ± 22	103	56 ± 40
Vitamin C (μmol/L)								
No MVI or Vit C	121	49 ± 19	93	57 ± 23	89	60 ± 24	72	70 ± 24
MVI and/or Vit C	74	70 ± 25	133	80 ± 28	81	80 ± 31	130	89 ± 26

Table 2. Estimates of regression coefficients (betas), standard errors of the estimate (S.E.E.) and *p* values for the effects of gender, ethnicity, vitamin supplement usage, age (5 year intervals), education (high school graduate vs. non graduate) and annual household income (>\$15,000 vs. ≤\$15,000) on serum vitamin B₁₂, folate and C concentrations

	Vitamin B ₁₂			Folate			Vitamin C		
	Beta	S.E.E.	<i>p</i>	Beta	S.E.E.	<i>p</i>	Beta	S.E.E.	<i>p</i>
Intercept	6.20	0.65	<.001	2.71	.085	<.001	.033	.052	.533
Gender (F vs M)	.057	.035	.109	.103	.046	.025	.195	.029	<.001
Ethnicity (H vs NHW)	-.092	.041	.025	-.227	.053	<.001	.065	.033	.051
Vitamin supplement (+ vs -)	.291	.035	<.001	.755	.046	<.001	.313	.028	<.001
Age group (65-69 vs. 80+)	.025	.054	.336	-.006	.070	.192	.018	.044	.434
(70-74 vs. 80+)	.076	.052	Group	.058	.068	Group	.051	.043	Group
(75-79 vs. 80+)	.079	0.57	Effect	.123	.074	Effect	.063	.047	Effect
Education (HS graduate vs not)	.055	.047	.239	-.097	.061	.112	.072	.038	.060
Earnings (>\$15,000 vs. ≤\$15,000)	-.053	.043	.217	.191	.056	<.001	-.143	.035	<.001
R-square, adjusted	.120			.339			.272		

The effects of each variable on serum vitamin B₁₂, folate and C concentrations is adjusted for each of the other variables in 751 participants in the New Mexico Elder Health Survey with complete data sets.

in the models in Table 2 because vitamin intake from food was not available for all participants. In models adjusted for all of the variables in Table 2, and including both those taking and not taking vitamin supplements (n=657), there was a positive association between vitamin intake from food and serum levels of folate (*p*<0.001), and vitamins B₁₂ and C (*p*<0.05). In the models including vitamin intake from food, there was still a significant association of ethnicity with serum vitamin levels (*p*<0.05). In a similar model including only those not taking vitamin supplements (n=385), the significance levels for the association of vitamin intake from food with serum levels were *p*<.001 for vitamin B₁₂ and folate and *p*<.05 for vitamin C.

Table 3 compares the results of cognitive and affective (depressive) function tests in participants with low and low normal serum concentrations of the three vitamins compared to those with values more clearly within the normal range. Using multivariate logistic regression models to adjust for differences in age, ethnicity, gender and education (years), none of these differences reached levels of statistical significance for B₁₂ or C. For folate, however, participants with low (and low normal) serum concentrations were associated with lower cognitive performance scores when compared to participants with normal

folate levels. Lower scores were associated with low serum folates in the Mini Mental Status Exam, the Digits Forward, the Fuld Object Memory test, (number retrieved, number of names, number recalled) and one of the two Color Trails tests, but not in the Clock Face test. Using lower cutoff points to define a vitamin deficiency state, i.e. 200 pg/mL for B₁₂ and 3 ng/mL for folate, did not change the findings. Those individuals with low normal serum B₁₂ concentrations did not have different mean corpuscular volumes compared to those with normal B₁₂ concentrations (mean±SD 90.6±5.5 vs. 90.5±5.2).

Because others [18–21] have found an association between serum folate concentrations and evidence of depression and because depression can lower cognitive scores (pseudodementia), depression was entered into additional multivariate logistic regression models as an additional variable examining cognitive outcomes. Several methods were available to determine the presence or absence of depression. The 15 question Yesavage Geriatric Depression Scale (GDS) with a score >6 consistent with depression was used as the primary method, but information also was utilized on self report of a past medical history of depression and on current use of prescription antidepressants. Of 861 participants who provided this information, 96 (11.1%)

Table 3. Effect of low and low normal serum vitamin B12, folic acid and vitamin C on various cognitive and affective testing in 783 participants in the New Mexico Elder Health Survey using multivariate logistic regression models

	Serum vitamin B ₁₂			Serum Folate			Serum vitamin C		
	<221 pmol/L Mean±SD	≥221 pmol/L Mean±SD	<i>p</i> value	<11.1 nmol/ L Mean±SD	≥11.1 nmol/ L Mean±SD	<i>p</i> value	<57 μmol/L Mean±SD	≥57 μmol/L Mean±SD	<i>p</i> value
Number of participants (%)	166 (21.1)	619 (78.9)		142 (18.1)	641 (81.9)		257 (32.8)	526 (67.2)	
Age, years	73.6 ± 6.3	73.4 ± 5.8	0.30	73.4 ± 6.0	73.6 ± 5.9	0.64	73.9 ± 6.2	73.2 ± 5.7	0.20
Education, years	11.5 ± 4.5	12.2 ± 4.1	0.63	10.6 ± 4.2	12.4 ± 4.1	0.007	10.7 ± 4.1	12.8 ± 4.0	<.001
Cognitive testing									
Mini Mental State exam (30)	26.7 ± 2.4	27.0 ± 2.7	0.37	25.9 ± 2.8	27.1 ± 2.5	0.002	26.4 ± 2.9	27.2 ± 2.4	0.90
Fuld object-memory									
Number retrieved (10)	6.73 ± 1.5	6.83 ± 1.5	0.77	6.55 ± 1.69	6.86 ± 1.50	0.05	6.60 ± 1.58	6.92 ± 1.51	0.21
Total recalled (10)	7.46 ± 1.5	7.50 ± 1.7	0.34	7.16 ± 1.82	7.56 ± 1.58	0.02	7.19 ± 1.66	7.66 ± 1.59	0.06
Number of names	15.7 ± 4.9	16.2 ± 5.4	0.60	14.4 ± 4.8	16.5 ± 5.3	0.005	14.6 ± 4.6	16.8 ± 5.4	0.17
Digits forward (10)	5.92 ± 2.17	6.37 ± 2.33	0.92	5.50 ± 2.31	6.44 ± 2.27	0.07	5.62 ± 2.17	6.60 ± 2.28	0.09
Clock score (8)	6.22 ± 1.6	6.21 ± 1.6	0.31	5.98 ± 1.73	6.26 ± 1.55	0.94	6.12 ± 1.67	6.28 ± 1.52	0.48
Color trails 1 (25)	20.9 ± 5.0	21.5 ± 4.8	0.84	20.2 ± 5.3	21.6 ± 4.7	0.14	20.6 ± 5.2	21.8 ± 4.6	0.91
Color trails 2 (25)	12.9 ± 4.6	13.3 ± 4.8	0.60	11.8 ± 5.1	13.5 ± 4.6	0.04	12.1 ± 4.6	13.8 ± 4.8	0.18
Affective testing									
Geriatric Depression Scale (15)	2.07 ± 2.46	2.03 ± 2.51	0.27	2.42 ± 2.69	1.96 ± 2.44	0.55	2.34 ± 2.51	1.88 ± 2.46	0.28
GDS (% >6)	10.4	9.1	0.93	12.7	8.7	0.45	9.8	9.0	0.59
History of depression (% positive)	19.9	24.1	0.08	31.0	21.6	0.10	28.8	20.2	0.04
Currently on antidepressants (% positive)	5.4	4.9	0.48	5.0	5.0	0.61	4.7	5.1	0.59

Not included in the analysis were 34 participants with MMSE scores <18. *p* values are adjusted for ethnicity, gender, age (years), and years of education by adjusting each variable for all of the other variables.

had a GDS consistent with depression (>6); only 16 (16.7%) were currently on antidepressants. Another 28 of 765 participants (3.7%), not clinically depressed by the GDS, also currently were on antidepressants. When one examined the 194 participants with a past history of depression, only 12.9% were currently on antidepressants. There was a highly significant correlation between the Mini Mental Status Exam and each of these three measures of depression (*p*<.001).

Adding each of the three measures of depression as a variable into the multivariate logistic regression models, serum folate concentrations continued to show significant associations with measures of cognitive function. Using the dichotomized level of GDS >6 vs. ≤6 in the model, significant associations still were found between serum folate concentrations and the MMSE and number of names (*p*<.01), and Fuld (total recalled) and color trails #2 tests (*p*≤.05). Using a history of depression as the variable instead of the GDS gave very similar results, except the last two tests of cognitive function were now only marginally significant (*p*=.06).

Table 4 compares those participants on MVI with those not on MVI. No significant association of cognitive or affective function with MVI use was observed after adjusting for differences in age, ethnicity, gender and years of education. The participants taking MVI had more education than those not taking MVI, even after adjusting for ethnic differences where more NHW took MVI than Hispanics.

Table 4. Associations between taking or not taking a daily multivitamin supplement (MVI) and mean±SD scores for various components of the cognitive and affective testing in 816 participants in the New Mexico Elder Health Survey using a multivariate logistic regression model

	No MVI Mean±SD	MVI Mean±SD	<i>p</i> value
Number of participants (%)	483 (59.2)	333 (40.8)	
Age, years	73.6 ± 6.0	73.6 ± 5.8	0.82
Education, years	11.5 ± 4.2	12.8 ± 4.1	0.003
Cognitive testing			
Mini Mental State exam (30)	26.7 ± 2.6	27.3 ± 2.4	0.47
Fuld object-memory			
Number retrieved (10)	6.80 ± 1.49	6.92 ± 1.44	0.54
Total recalled (10)	7.40 ± 1.65	7.66 ± 1.54	0.22
Number of names	15.7 ± 5.1	16.8 ± 5.4	0.65
Digits forward (10)	6.08 ± 2.23	6.58 ± 2.36	0.81
Clock score (8)	6.24 ± 1.53	6.24 ± 1.54	0.10
Color trails 1 (25)	21.1 ± 4.9	21.8 ± 4.6	0.91
Color trails 2 (25)	12.9 ± 4.7	13.6 ± 4.8	0.88
Affective testing			
Geriatric Depression Scale (15)	2.03 ± 2.44	1.92 ± 2.41	0.81
GDS (% >6)	8.8	8.8	0.58

Not included in this analysis were 34 participants with MMSE scores <18. *p* values are adjusted for ethnicity, gender, age (years), and years of education by adjusting each variable for all other variables.

DISCUSSION

We are unable to find previous reports on randomly sampled studies of serum vitamin B₁₂, folate and vitamin C concentrations in elderly Hispanic populations. We have previously reported on food sources of folate for the elderly [36] and have noted qualitative differences in food sources of folate and vitamin C for elderly Hispanic and NHW participants in the NMEHS [34]. The present study goes beyond observations of food sources to document differences in blood levels of nutrients. Significantly lower serum concentrations of all three vitamins were found in Hispanics, even after adjusting for differences in age, gender, food vitamin intake, vitamin supplement use, education and household income. This suggests this large minority population may be more susceptible to subtle or marginal deficiencies than Anglo populations more traditionally studied. Furthermore, this could have implications on various measures of health status, such as cognitive status and mood disorders in this minority population.

Other observations, e.g., that males had significantly lower serum concentrations compared to females, that daily vitamin supplements increased serum vitamin concentrations, that a direct association existed between food vitamin intake and serum vitamin concentrations (exclusive of vitamin supplements) and that participants with less education and incomes below the poverty level had lower serum vitamin concentrations were more predictable based on previous publications [37–41]. While not all participants in the multivitamin supplement category took a multivitamin daily for all 12 months prior to the testing done, such a high percentage did that this should not affect the ability to find significant differences between the groups receiving multivitamin supplements and those not receiving them, if the effects on cognition and mood really existed.

Although ranges of “normal” values are generally established in clinical laboratories, the rationales for these levels are not always clear. With no clear, independent, objective markers for clinical B₁₂, C and folate deficiencies, the distinctions between true early biochemical deficiencies of these vitamins and the lower limits of normal become somewhat arbitrary. Florid presentations of vitamin deficiencies, e.g., the megaloblastic anemia and neurologic dysfunction seen with pernicious anemia (B₁₂ deficiency), are becoming rare. More often marginal or mild deficiencies are detected by screening serum or red cell concentrations of the vitamins or by finding evidence of accumulation of a metabolite; for example, an increase in serum methylmalonic acid (MMA) can be indicative of a B₁₂ deficiency, or an increase in serum homocysteine can be related to a deficiency of either folate or B₁₂. Some of these metabolites may be responsible for subtle pathophysiologic changes, such as the excessive cardiovascular mortality now well documented to result from an increase in serum homocysteine

concentrations [1–8]. It therefore becomes important to identify these early or marginal deficiencies and to identify populations that may be at increased risk.

An extensive literature exists on the neuropsychiatric manifestations of cobalamin (B₁₂) deficiency that often are present without the macrocytic anemia seen with classic pernicious anemia due to a deficiency of intrinsic factor. This has been comprehensively reviewed recently by Van Goor, *et al.* [18]. Malabsorption of cobalamin due to intrinsic factor deficiency can be measured by the Schilling test. In elderly individuals, low serum cobalamins often are found with normal Schilling tests and appear to be the result of protein-bound cobalamin malabsorption. Here the release of cobalamin from its dietary protein-bound state is impaired, often but not necessarily due to achlorhydria [42]. In recognition of the fact that 20 to 30 percent of elderly persons may malabsorb food-bound B₁₂, it has been recommended that those older than 50 years meet the Recommended Dietary Allowance (RDA) for B₁₂ (2.4 micrograms/day) from supplements and/or from foods fortified with B₁₂ (such as breakfast cereals) [9].

Memory deficits and slowing of mental processes are the most commonly reported cognitive disturbances in cobalamin deficiency, but organic mental changes resulting in paranoia, hallucinations and delirium also have been described [18]. Such patients with an organic psychosis and cognitive dysfunction have been reported to show a complete recovery with cobalamin therapy [43–45].

Despite the widespread use of the serum B₁₂ concentrations in epidemiologic studies, and as a screening procedure, it has been recognized that there are formidable problems of sensitivity and specificity with this test [46,47]. A low serum B₁₂ does not necessarily indicate a deficiency state exists, and a “normal” B₁₂ does not rule out a deficiency. Metz, *et al.* [48] showed that 90% of older patients with serum B₁₂ <150 pmol/L showed evidence of tissue B₁₂ deficiency. They felt the deficiency became manifest at relatively higher levels of serum B₁₂ in older patients, possibly because of lower levels of holotranscobalamin II in older patients. Most cobalamin is bound to transcobalamin I, which has little functional significance; the 10% to 20% that is bound to transcobalamin II is the functionally active component, and this tends to be low in the elderly. Since there are no good commercial techniques to quantify transcobalamin II available, the best alternative to identify B₁₂-deficient individuals appears to be to measure the serum concentrations of MMA and homocysteine and the response to therapy with B₁₂. Stabler [47], in reviewing a study on a cohort of geriatric outpatients [49], felt the conventional practice of setting the lower limits of normal for B₁₂ at 200 pg/mL (147 pmol/L) missed approximately 50% of B₁₂-deficient individuals. Many subjects with “low normal” levels (between 200 and 300 pg/mL) had metabolites (MMA and/or homocysteine) elevated more than two standard deviations above the mean which subsequently responded to treatment with B₁₂. Bernard *et al.* [19], on the other hand, reported that

elderly veterans with low B₁₂ levels, as defined by a serum B₁₂ level <200 pg/mL, had evidence of cognitive impairment compared to those above this level. When a broader definition of B₁₂ deficiency was used, i.e. <300 pg/mL and elevation of MMA and/or homocysteine, no significant differences were observed. We therefore used both the 200 and 300 pg/mL cutpoints to dichotomize our findings for analysis, but have reported only the latter. Using the 300 pg/mL cutpoint, 21% of our participants had low serum B₁₂ concentrations. Neither of these cutpoints showed any significant association between low B₁₂ concentrations and cognitive or affective (mood) function. Some of this failure may be related to the inability of the serum B₁₂ concentration to adequately identify B₁₂ deficient participants.

Low serum folate concentrations also have been associated with poorer function on neuropsychological assessment [16,17,20,50]. Goodwin *et al.* [50] reported lower test scores on a non-verbal test of abstract thinking ability and on a memory test for healthy elderly with low folate levels compared to those with normal levels. In a previous report on 137 elderly (age 66 to 90 years), well educated, well nourished community residents in the New Mexico Aging Process Study free of cognitive impairment, we reported no differences in tests of memory and visual perception between those individuals taking vitamin supplements and those not taking them [20]. However, on more complex measures of performance involving visuospatial skills, abstraction and non-verbal memory, those individuals taking supplements, including B₁₂, C and folate, generally scored significantly higher than those not taking supplements. Similarly, Riggs *et al.* [16] reported poorer spatial copying skills in those with low folates. The Framingham study [51], in their analysis of "low and low normal" serum folate concentrations, used a level less than 5 ng/mL (11.1 nmol/L), in contrast to the more traditional level distinguishing a deficiency as that below 3 ng/mL. Using the former cutpoint, 18% of our participants had low serum folate concentrations. Here we saw lower scores on a number of cognitive tests involving learning, memory and psychomotor speed in participants with low or low normal serum folate concentrations compared to those with normal serum concentrations.

Although we were unable to show any association between serum folate levels and the presence or absence of depression, an important consideration is to diagnose depression accurately, as it is well recognized that impaired cognitive status can be a result of depression (pseudodementia). In 1962, Herbert [18] reported an association between folate deficiency and depressive symptoms, experiencing himself insomnia, irritability, fatigue and forgetfulness after four months on a folate-deficient diet. Albert and Fava [19] review the literature showing the relationship between folate and neuropsychiatric disorders inferred from clinical observation and the current understanding of the role of folate in critical brain metabolic pathways. Depressive symptoms have been reported to be the

most common neuropsychiatric manifestations of folate deficiency. Conversely, low serum and/or red cell folate levels have been detected in 15% to 38% of adults diagnosed with depressive disorders [19–21] and low folate levels have been linked to poorer antidepressant response to selective serotonin reuptake inhibitors [19].

Perrig *et al.* [21] recently reviewed the effects of the antioxidant vitamins, including vitamin C, on cognitive function. Although there had been previous correlational studies in healthy older people suggesting that low serum vitamin C might be associated with poorer mental function [50], there was no clear effect of the antioxidant vitamins on cognitive performance, especially memory, in these previous studies. Perrig *et al.* [21], however, did find a positive correlation between plasma ascorbic acid concentrations and memory performance in people aged 65 and older. Although none of the cognitive tests in our study showed such a positive correlation, two tests of memory came close ($p < 0.1$).

Within the next few years, ongoing and planned randomized trials should help to resolve many of the uncertainties described above. At present, in recognition of absorption problems, the elderly are recommended to obtain the RDA for vitamin B₁₂ from supplements or fortified foods. However, the combined evidence does not support the routine use of higher supplement doses of vitamin B₁₂, or of folate or vitamin C supplements to protect against cognitive loss or mood disorders (depression). Individual choices and public policy decisions should await the results of large trials, which will provide more information on the efficacy and safety of these vitamins.

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