Vitamin B-12 status and neurologic function in older people: a cross-sectional analysis of baseline trial data from the Older People and Enhanced Neurological Function (OPEN) study[1,2]

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ABSTRACT

Background: Aging is associated with a progressive decline in vitamin B-12 status. Overt vitamin B-12 deficiency causes neurologic disturbances in peripheral and central motor and sensory systems, but the public health impact for neurologic disease of moderately low vitamin B-12 status in older people is unclear. Evidence from observational studies is limited by heterogeneity in the definition of vitamin B-12 status and imprecise measures of nerve function.

Objective: We aimed to determine whether vitamin B-12 status is associated with electrophysiologic indexes of peripheral or central neurologic function in asymptomatic older people with moderately low vitamin B-12 status.

Design: We used a cross-sectional analysis of baseline data from the Older People and Enhanced Neurological Function study conducted in Southeast England. This trial investigated the effectiveness of vitamin B-12 supplementation on electrophysiologic indexes of neurologic function in asymptomatic older people (mean age: 80 y) with moderately low vitamin B-12 status (serum vitamin B-12 concentrations $\geq$107 and $<$210 pmol/L without anemia, n = 201). Vitamin B-12 status was assessed with the use of total vitamin B-12, holotranscobalamin, and a composite indicator of vitamin B-12 status (cB-12). Electrophysiologic measures of sensory and motor components of peripheral and central nerve function were assessed in all participants by a single observer.

Results: In multivariate models, there was no evidence of an association of vitamin B-12, holotranscobalamin, or cB-12 with any nerve conduction outcome. There was also no evidence of an association of vitamin B-12 status with clinical markers of neurologic function.

Conclusion: This secondary analysis of high-quality trial data did not show any association of any measure of vitamin B-12 status with either peripheral or central neurologic function or any clinical markers of neurologic function in older people with moderately low vitamin B-12 status. The results of this study are unlikely to be generalizable to a less healthy older population with more severe vitamin B-12 deficiency. This trial was registered at www.controlled-trials.com as ISRCTN54195799. *Am J Clin Nutr* 2016;104:790–6.

Keywords: neurologic, older people, vitamin B-12, peripheral conduction, central nerve conduction

INTRODUCTION

Aging is associated with a decline in vitamin B-12 status, and vitamin B-12 deficiency is relatively common in older people (1–3). In the United Kingdom, 5% of adults aged 65–74 y and 10% of adults aged $\geq$75 y have low vitamin B-12 concentrations (defined as vitamin B-12 $<$150 pmol/L) or metabolically significant vitamin B-12 deficiency [defined as vitamin B-12 $<$200 pmol/L and a homocysteine (Hcy) concentration $>$20 mmol/L] (1). Because intakes of vitamin B-12 are mostly adequate (4), poor status in older people is largely attributable to age-related malabsorption of vitamin B-12 (5).

Vitamin B-12 is required for initiating and maintaining myelination of the nervous system (6). The classic manifestation of overt vitamin B-12 deficiency, subacute combined degeneration of the spinal cord, involves demyelination of the posterior and lateral tracts of the spinal cord (6, 7). Neurologic disturbances associated with B-12 deficiency can affect peripheral motor and sensory systems and include ataxia, gait disturbance, symmetric paresthesia, numbness, impaired vibration or position sensation, abnormal balance, reflexes, and weakness (3, 6, 7).

Although impaired neurologic function is a characteristic feature of overt vitamin B-12 deficiency, the neurologic and public health impact of moderately low vitamin B-12 status in older people is currently unclear. Neurologic signs and symptoms associated with moderately low vitamin B-12 status can be nonspecific and are often undetected because they are attributed to "old age," yet they can have an important impact on physical function. A recent systematic review (8) evaluated the association of vitamin B-12 status with neurologic function and clinically

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[2] Supplemental Figure 1 and Supplemental Tables 1–5 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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relevant neurologic outcomes in older people. Evidence from observational studies was limited, and the heterogeneity and quality of the available studies precluded definitive conclusions. Some studies used electrophysiologic measures of nerve conduction, which are the most sensitive and objective measure of neurologic function relevant to vitamin B-12 status. Many studies were constrained by bias, and a few reported composite measures of vitamin B-12 status that included both a biomarker of circulating vitamin B-12 and a functional biomarker [methyl malonic acid (MMA) or Hcy], as now recommended (9).

The OPEN (Older People and Enhanced Neurological Function) study (ISRCTN54195799) afforded an opportunity to test whether there was an association of vitamin B-12 status with neurologic function in a high-quality data set derived from asymptomatic older people with moderately low vitamin B-12 status. The aim of this study was to determine whether vitamin B-12 status is associated with electrophysiologic indexes of peripheral or central neurologic function or clinical markers of neurologic function in older people with moderate vitamin B-12 deficiency.

METHODS

Recruitment and procedures

This study was a secondary analysis of cross-sectional baseline data from the OPEN study, the protocol of which has been published (10). OPEN was a randomized, double-blind, placebo-controlled trial that reported no benefits of dietary supplementation with oral vitamin B-12 for 12 mo on electrophysiologic indexes of neurologic function in older people with moderate B-12 deficiency (11).

Participants aged ≥75 y were recruited from 7 general practices in Southeast England. Individuals with diabetes, dementia, epilepsy, alcohol addiction, pacemakers or other implanted metallic devices, residents of nursing homes, or a previous diagnosis of pernicious anemia were excluded. Those who reported current consumption of vitamin B-12 supplements or who had received a vitamin B-12 injection in the previous 6 mo were also excluded, as were potential participants with considerable cognitive impairment. Individuals with moderate vitamin B-12 deficiency who did not have anemia [serum vitamin B-12 concentrations ≥107 and <210 pmol/L (Beckman Coulter assay) and hemoglobin concentrations ≥110 g/L for women and ≥120 g/L for men] were eligible to join the OPEN study. Screening took place between November 2008 and February 2010.

Baseline data from 201 participants enrolled in OPEN were used in this secondary analysis (**Supplemental Figure 1**). The sample size for the trial was determined by a sample size calculation designed to achieve 90% power to detect a ≥28% difference in the primary nerve function outcome (with 5% significance) between arms of the original trial.

Participants attended King's College Hospital at study baseline and provided a blood sample and undertook a series of neurophysiologic function tests. At the baseline appointment, data were also collected on educational history, current prescribed medication (including statins and proton pump inhibitors), dietary habits, and frequency of alcohol consumption. BMI (in kg/m^2) was also calculated. Blood samples were analyzed for serum concentrations of vitamin B-12 (microbiologic assay; CV range: 5–7%); holotranscobalamin (HoloTC) (Axis-Shield radioimmunoassay; CV range: 5–8%), total Hcy (Abbott IMx analyzer; CV range: 2–3%), and folate (chloramphenicol-resistant microbiologic assay; CV range: 5–8%) in a single laboratory. The Beckman Coulter method (12) was used to assess vitamin B-12 status to screen participants for study eligibility. A microbiologic assay was used at study baseline to assess the vitamin B-12 status of study participants. A full blood count was analyzed for hematologic markers, including hemoglobin, hematocrit, and mean corpuscular volume.

A single expert physician conducted a battery of peripheral nerve conduction tests (including motor and sensory nerve conduction in the right median, ulnar, superficial peroneal, sural, common peroneal, and tibial nerves) and central motor conduction tests. These standard techniques used surface electrodes. Because nerve conduction in peripheral nerves is sensitive to the temperature of the limbs (13), skin temperature of the dorsum of the foot and hand were measured to allow for appropriate adjustments in the analyses.

The sensory action potential (SAP) amplitude (maximum deviation of the electrical response) and conduction velocity (distance divided by onset latency) were measured in the median, ulnar, superficial peroneal, and sural nerves. Common peroneal, tibial, median, and ulnar motor conduction were measured by recording from the extensor digitorum brevis, abductor hallucis (AH), abductor pollicis brevis, and abductor digiti minimi (ADM), respectively. Supramaximal stimuli were used at proximal and distal sites to ensure that all nerve fibers within the nerve were activated. Conduction velocity was calculated, and compound muscle action potential (CMAP) amplitude, distal motor latency, and F-wave latency (a measure of conduction time from the distal stimulation site to the spinal cord) were also measured.

Transcranial magnetic stimulation, which painlessly and noninvasively excites the motor cortex (14), was used to measure central motor conduction in the corticospinal tract. A 13-cm-diameter circular coil connected to a magnetic stimulator that provided a monophasic pulse was centered over the vertex to excite the hand area of the left motor cortex. A standard technique (15) determined the threshold for excitation. With the right ADM muscle partially activated voluntarily, 8 stimuli at 1.2 times the threshold were delivered to evoke motor-evoked potentials (MEPs), the mean amplitude and minimal latency of which were measured. Similarly, with the use of a double-cone coil, the leg area of the motor cortex was excited to measure MEPs evoked in AHs. Central motor conduction time was calculated by subtracting the time to response in a given muscle from an estimate of the peripheral nerve conduction time. A maximum of 70 brain stimuli were performed on any participant. Any participants shown to have substantial neurologic deficits were referred to their general practitioners.

Outcomes and exposures

In total, 19 nerve conduction outcomes were measured in the right side of the body. Peripheral nerve conduction outcomes were grouped in the analyses as follows: 4 SAP amplitudes in the sural, superficial peroneal, median, and ulnar nerves as an index of nerve fiber number; 4 sensory conduction velocities in the sural, superficial peroneal, median, and ulnar nerves to indicate the degree of myelination; 4 distal CMAP amplitudes in the tibial, common peroneal, median, and ulnar nerves that reflect the number of motor axons accessed by an electrical stimulus, which in turn reflects muscle strength (16, 17); and 4 motor conduction velocities in the tibial, common peroneal, median, and ulnar nerves to indicate the degree of myelination. Reduced sensory or motor

conduction velocity is a sign of demyelination (18). The remaining 3 outcomes assessed central nerve conduction: mean right ADM MEP amplitude and central motor conduction time to the right AH and ADM (the latter 2 were grouped in the analyses). We also assessed 4 clinical measures of neurologic function at baseline: the presence or absence of right knee and ankle jerks and of joint position sense and vibration sense in the right great toe.

Vitamin B-12 and HoloTC were used as measures of vitamin B-12 status. In view of the limited sensitivity and specificity of individual biomarkers of vitamin B-12, experts have advocated the combined use of ≥1 biomarker of circulating vitamin B-12 (serum vitamin B-12 or HoloTC) together with 1 functional biomarker [methyl malonic acid (MMA) or Hcy] (9) as a composite indicator of vitamin B-12 status (cB-12). The use of cB-12 is a novel approach that combines measures of vitamin B-12, HoloTC, Hcy, and MMA into 1 indicator (19). cB-12 can be derived with the use of equations that allow for an incomplete set of indicators, i.e., based on 2 or 3 of these markers (19). In this study, 3 markers (vitamin B-12, HoloTC, and Hcy) were used to derive cB-12 values.

Ethics

The OPEN study was reviewed and approved by the National Research Ethics Committee and the London School of Hygiene and Tropical Medicine Ethics Committee. The secondary analyses presented herein were approved by the London School of Hygiene and Tropical Medicine Ethics Committee.

Statistical analyses

All statistical analyses were conducted with the use of Stata version 14 (StataCorp). Descriptive statistics for all exposures, outcomes, and known or potential confounders were generated. Scatter plots were used to visually explore the nature of any potential associations present. The functional form of any potential relations was also explored by producing locally weighted scatterplot smoother curves (20). Three measures of vitamin B-12 status and 2 nerve conduction outcomes had >10% missing data, but a preliminary analysis suggested that there was no reason to assume that these were not missing at random, so all analyses covered all available cases.

Nerve conduction outcomes were grouped in multivariate regression models. Multivariate regression differs from multiple regression in that several dependent variables are jointly regressed on the same independent variables (21). Nerve conduction outcomes were grouped (as defined previously) according to the component of nerve function they reflect to minimize multiple testing of several outcomes. All multivariate models were bootstrapped to allow for nonnormal distributions, and results were presented as appropriate effect sizes with bias-corrected 95% CIs. Clinical marker outcomes were analyzed separately with the use of logistic regression; results were presented as ORs with 95% CIs. Because the analyses involved multiple comparisons, P values were interpreted with caution, with a stringent P value of <0.01 chosen to test for statistical significance.

Age and sex are known confounders of the relation between vitamin B-12 status and neurologic function and were thus adjusted for in our analyses. In addition, alcohol frequency, hemoglobin, hematocrit, mean corpuscular volume, and the use of statins or proton pump inhibitors were assessed as potential confounders. If a potential confounder was found to be associated with both an exposure and outcome and its inclusion altered the effect size by ≥10%, then it was included in the final model.

Skin temperature is a known confounder for nerve conduction outcomes, specifically hand skin temperature for nerve conduction parameters in nerves of the upper limbs (median and ulnar) and foot skin temperature for equivalent parameters in nerves of the lower limbs (tibial, common peroneal, sural, and superficial peroneal). The analyses presented herein combined outcomes in upper and lower limbs, so including both hand and foot skin temperature in the models was considered. However, hand and foot skin temperature were strongly positively correlated, so only foot skin temperature was included in the final models because of collinearity concerns.

Sensitivity and subgroup analyses were conducted to test the robustness of the findings. Sensitivity analyses were performed that excluded participants with clinical (previously decompressed nerves) or neurophysiologic evidence (a median nerve sensory conduction velocity <40 m/s and an ulnar sensory conduction velocity ≥10 m/s faster and/or a median distal motor latency of >4.5 m/s) of carpal tunnel syndrome because this syndrome is known to affect median sensory and motor nerve conduction parameters. Subgroup analyses were conducted to explore whether any association between vitamin B-12 and neurologic function differed by age or folate status by testing for interactions between age and vitamin B-12 status and folate and vitamin B-12 status on nerve conduction outcomes.

RESULTS

The mean age of the 201 study participants was 80 y, and men made up 47% of the population (**Table 1**). At study baseline, 88% of the recruited participants had a vitamin B-12 status below the median value of 301 pmol/L for the microbiologic assay reference standard (derived from a random sample of 470 nationally representative adults in the Irish National Adult Nutrition Survey) (A Molloy, Trinity College Dublin, personal communication, 2013), indicating that study participants had moderately low vitamin B-12 status. **Table 2** shows nerve conduction outcomes and clinical markers of the study participants. Participants displayed sural nerve SAP amplitudes in line with available reference ranges (3.1 ± 1.2 μV) for older people (22). Furthermore, the clinical markers of neurologic function show that it was suboptimal among participants. In particular, 66% of participants had absent right great toe vibration sense, and 28% had absent right ankle jerks.

In multivariate models, there was no evidence of an association of any measure of vitamin B-12, HoloTC, or cB-12 with any of the nerve conduction outcomes in either unadjusted (**Supplemental Table 1**) or adjusted analyses (**Table 3**). Results were consistent across all measures of peripheral and central nerve conduction and all measures of vitamin B-12 status. Coefficients were very close to zero, and the direction of effects was inconsistent within each group of nerve function outcomes. Likewise, there was no evidence of an association of any measure of vitamin B-12 status with any clinical markers of neurologic function (**Supplemental Table 2**). Overall, there was no evidence to support an association of any measure of vitamin B-12 status with any measure of central or peripheral sensory or motor nerve function.

A sensitivity analysis that excluded 31 participants with carpal tunnel syndrome did not alter these conclusions (**Supplemental**

TABLE 1
Demographic characteristics and blood biochemical measures of study participants[1]

	Values	Total participants, *n*
Demographic characteristics		
Age, y	80.0 ± 3.6[2]	201
Sex, *n* (%)		201
Men	94 (47)	
Women	107 (53)	
Age at leaving education, y	18.1 ± 6.0	201
Educational achievement, *n* (%)		198
None	54 (27)	
Basic or clerical	34 (17)	
Advanced or university	52 (26)	
Other	58 (29)	
BMI, kg/m^2	26.8 (24.0, 29.3)[3]	201
<18.5, *n* (%)	1 (0)	
18.5–24.9, *n* (%)	69 (34)	
25.0–29.9, *n* (%)	87 (43)	
≥30, *n* (%)	44 (22)	
Statin use, *n* (%)	67 (41)	162
Proton pump inhibitor use, *n* (%)	53 (33)	162
Frequency of alcohol consumption, *n* (%)		195
Daily	68 (35)	
>1 time/wk	63 (32)	
~1 time/fortnight	19 (10)	
Rarely or never	45 (23)	
Frequency of meat consumption, *n* (%)		191
>1 time/wk	139 (73)	
Blood biochemical measures		
Vitamin B-12,[4] pmol/L	225.5 (196.0, 269.6)	165
HoloTC, pmol/L	49.3 (38.8, 64.8)	159
Hcy, μmol/L	16.2 (13.8, 19.5)	162
Folate, nmol/L	17.6 (4.8, 25.4)	164
cB-12	−0.2 ± 0.4	159
Hematocrit, %	40.8 ± 3.1	177
Hemoglobin, g/L	139.3 ± 12.0	177
Mean corpuscular volume, fL	88.6 ± 4.3	177

[1]cB-12, composite indicator of vitamin B-12 status; Hcy, homocysteine; HoloTC, holotranscobalamin.
[2]Mean ± SD (all such values).
[3]Median; IQR in parentheses (all such values).
[4]Vitamin B-12 status was assessed with the use of a microbiologic assay.

Table 3). There was also no evidence of an interaction between age and vitamin B-12 status or between folate and vitamin B-12 status for any electrophysiologic measure of nerve function (**Supplemental Tables 4** and **5**).

DISCUSSION

Key findings

This study identified no evidence of an association of vitamin B-12 status with a suite of measures of peripheral or central neurologic function or any measures of clinical markers of neurologic function in older people with moderately low vitamin B-12 status. The null results were consistent in all categories of vitamin B-12 status and across all neurologic outcomes. There was also no evidence of an interaction between folate and vitamin B-12 status for any electrophysiologic measure of nerve function.

Comparison with other studies

Few other studies to our knowledge have assessed neurologic function with the use of nerve conduction tests. In a longitudinal study (23), no association was reported between vitamin B-12 status and CMAP and nerve conduction velocity measured between the fibular head and ankle. Results from 2 cross-sectional studies were mixed. Vitamin B-12-deficient individuals in one study had a lower CMAP and nerve conduction velocity (measured between popliteal fossa and ankle) (24), but it is notable that vitamin B-12 deficiency was defined as vitamin B-12 <260 pmol/L and elevated MMA, the latter of which might be important. A second cross-sectional study measured sensory and motor nerve conduction velocity in the median nerve and reported no association with vitamin B-12 status (depletion defined as serum B-12 <148 pmol/L) (25). Nevertheless, a recent pre- and posttreatment study in asymptomatic older adults with serum vitamin B-12 <120 pmol/L (26) reported an improvement in sensory latency (peripheral nerve conductivity) for left and right sural nerves and of the right median nerve, but not SAP amplitudes for these nerves, 4 mo after a single dose of an intramuscular treatment of 10 mg vitamin B-12, 100 mg pyridoxine, and 100 mg thiamine. Heterogeneity in assays and cutoffs used to assess vitamin B-12 status (27) constrains a fair comparison

TABLE 2
Neurologic function of study participants[1]

Neurologic function	Values	Total participants, *n*
SAP amplitudes,[2] μV		
Median	8.2 (5.6, 11.9)[3]	200
Ulnar	6.6 (4.1, 9.1)	200
Sural	3.8 (1.6, 6.3)	199
Superficial peroneal	2.9 (1.1, 5.4)	199
Sensory nerve conduction velocities, m/s		
Median	45.1 ± 5.5[4]	194
Ulnar	44.7 ± 4.6	192
Sural	40.4 ± 5.3	172
Superficial peroneal	41.1 ± 5.6	159
CMAP amplitudes, mV		
Median	3.8 (2.7, 5.0)	200
Ulnar	9.7 (8.5, 11.2)	200
Tibial	4.6 (2.1, 7.3)	199
Common peroneal	2.4 (1.1, 3.6)	199
Motor nerve conduction velocities, m/s		
Median	51.3 ± 5.2	200
Ulnar	54.5 ± 5.2	200
Tibial	40.0 ± 5.1	193
Common peroneal	42.8 ± 4.3	189
Central motor conduction		
Left hemisphere ADM CMCT, ms	5.5 ± 1.3	198
Left hemisphere AH CMCT, ms	13.6 ± 3.4	182
Left hemisphere mean ADM MEP amplitude, mV	3.4 (2.1, 4.4)	200
Clinical markers, *n* (%)		201
Absent right knee jerk	21 (10)	
Absent right ankle jerk	57 (28)	
Absent right great toe position sense	14 (7)	
Absent right great toe vibration sense	132 (66)	

[1]ADM, abductor digiti minimi; AH, abductor hallucis; CMAP, compound muscle action potential; CMCT, central motor conduction time; MEP, motor-evoked potential; SAP, sensory action potential.

[2]Percentage of absent (SAP amplitude = 0) responses = 3 for median, 4 for ulnar, 14 for sural, and 20 for superficial peroneal nerves.

[3]Median; IQR in parentheses (all such values).

[4]Mean ± SD (all such values).

between similar studies. This study is the first to our knowledge to assess vitamin B-12 status as measured by cB-12 and its relation with neurologic function and offers a high-quality data set with which to investigate the relation between vitamin B-12 status and neurologic function. The results presented herein are consistent with a conclusion of no association of moderately low vitamin B-12 status with nerve function.

Strengths and weaknesses

A strength of this study is the use of several measures of vitamin B-12 status. Of particular note, HoloTC (which measures the active fraction of vitamin B-12) has been proposed as appropriate for use in subclinical situations (28, 29). Furthermore, the use of cB-12 has tested a novel approach to assess vitamin B-12 status. This approach has an advantage over single biomarker tests because it also includes a functional biomarker of vitamin B-12 status (Hcy in this case). However, cB-12 is more reliable when based on 4 markers, which was not possible in this study because MMA was not measured. When using 3 markers, having MMA missing is less reliable than having any of the other 3 markers missing (19). Furthermore, renal function, which is known to affect Hcy (30), was not measured in the OPEN study. In fact, cB-12 has recently been reported to be independently associated with renal function (31). There is uncertainty about the most appropriate measures or cutoffs for assessing vitamin B-12 status. It has been suggested that age- and sex-specific reference cutoffs may be needed (27).

The OPEN study exclusion criteria resulted in study participants with moderately low vitamin B-12 status at study entry. The exclusion criteria reflect the intention of the OPEN study to be relevant to population health in older people. However, it is possible that the participants, although moderately deficient, were too replete in vitamin B-12 to be able to detect any associations between vitamin B-12 status and neurologic function. Furthermore, the sample of older people recruited for the study was not selected at random and may be in better health than a representative sample of older people in the United Kingdom. Participants also had relatively high levels of educational achievement, suggesting that the sample was not fully representative of older

TABLE 3
Association between vitamin B-12 status and nerve conduction outcomes[1]

	Vitamin B-12, pmol/L	HoloTC, pmol/L	cB-12
Sensory SAP amplitudes,[2] μV			
n	164	158	158
Median	−0.01 (−0.02, 0.00)	−0.02 (−0.05, 0.02)[3]	−1.05 (−3.19, 1.06)[3]
Ulnar	−0.01 (−0.02, −0.00)	−0.01 (−0.04, 0.02)[3]	−0.72 (−2.10, 0.52)[3]
Sural	−0.00 (−0.01, 0.01)	−0.01 (−0.04, 0.02)[3]	−0.17 (−1.72, 1.42)[3]
Superficial peroneal	0.00 (−0.01, 0.01)	−0.01 (−0.03, 0.02)[3]	0.26 (−1.11, 1.45)[3]
P value	0.12	0.87	0.60[3]
Sensory nerve conduction velocities, m/s			
n	115	110	110
Median	0.01 (−0.01, 0.02)	0.03 (−0.00, 0.08)	2.80 (0.37, 5.59)
Ulnar	−0.01 (−0.02, 0.01)	−0.02 (−0.06, 0.02)	−0.77 (−2.60, 1.04)
Sural	−0.01 (−0.03, 0.01)	0.01 (−0.03, 0.05)	−0.56 (−2.83, 1.47)
Superficial peroneal	−0.01 (−0.02, 0.01)	0.01 (−0.03, 0.05)	0.20 (−2.54, 2.72)
P value	0.28	0.12	0.05
Motor CMAP amplitudes, mV			
n	164	158	158
Median	−0.01 (−0.01, −0.00)	−0.00 (−0.02, 0.01)	−0.17 (−0.72, 0.48)
Ulnar	0.01 (−0.00, 0.01)	0.01 (−0.01, 0.03)	0.73 (−0.19, 1.66)
Tibial	−0.01 (−0.02, 0.01)	−0.00 (−0.03, 0.02)	−0.14 (−1.79, 1.24)
Common peroneal	0.00 (−0.00, 0.01)	0.01 (−0.01, 0.02)	0.54 (−0.23, 1.22)
P value	0.02	0.49	0.11
Motor nerve conduction velocities, m/s			
n	153	148	148
Median	−0.00 (−0.02, 0.01)	0.00 (−0.05, 0.04)	−0.14 (−2.22, 2.40)
Ulnar	0.00 (−0.02, 0.02)	0.01 (−0.03, 0.06)	0.84 (−1.82, 3.01)
Tibial	0.00 (−0.01, 0.02)	0.00 (−0.04, 0.05)	0.54 (−1.53, 2.84)
Common peroneal	−0.01 (−0.02, 0.01)	−0.02 (−0.05, 0.01)	−0.33 (−2.03, 1.65)
P value	0.80	0.66	0.86
Central motor conduction			
n	147	142	142
ADM CMCT, ms	0.00 (−0.00, 0.01)	−0.00 (−0.01, 0.01)	0.20 (−0.29, 0.67)
AH CMCT, ms	0.00 (−0.01, 0.01)	−0.00 (−0.03, 0.03)	−0.09 (−1.90, 1.80)
P value	0.41	0.72	0.66
Mean ADM MEP amplitude, mV	−0.00 (−0.00, 0.00)[4]	−0.00 (−0.01, 0.01)[4]	0.05 (−0.53, 0.59)[4]
n	164	158	158
P value	0.99[4]	0.92[4]	0.86[4]

[1]Values are adjusted coefficients (95% CIs) unless otherwise indicated. Multivariate regression analyses were adjusted for age, sex, and skin temperature (foot) unless otherwise stated. ADM, abductor digiti minimi; AH, abductor hallucis; CMAP, compound muscle action potential; CMCT, central motor conduction time; HoloTC, holotranscobalamin; MEP, motor-evoked potential; SAP, sensory action potential.

[2]Percentage of absent (SAP amplitude = 0) responses = 3 for median, 4 for ulnar, 14 for sural, and 20 for superficial peroneal nerves.

[3]Mean corpuscular volume confounded the relation between HoloTC and cB-12 with SAP amplitudes; these models have therefore been adjusted for age, sex, skin temperature (foot), and mean corpuscular volume.

[4]Adjusted for age, sex, and skin temperature (hand).

people in the United Kingdom. The results of this study are unlikely to be generalizable to a less healthy older population with more severe vitamin B-12 deficiency.

An important strength of this study was the use of nerve conduction tests to measure neurologic function. Nerve conduction tests provided objective measures of neurologic function with the use of state-of-the-art methods, and all testing was conducted by a single neurophysiologist, which eliminated interobserver variability. A wide range of neurologic outcomes was used to allow both sensory and motor components of nerve function in upper and lower limbs to be assessed. Age-related changes to nerve conduction outcomes are mostly restricted to sensory SAP amplitudes (32), and the available age-specific reference ranges suggest OPEN study participants had little or only mild neurologic impairment.

The risk of bias from confounding was minimized by conducting an extensive exercise to identify potential confounders. Sensitivity and subgroup analyses were also conducted to test the reliability of study findings.

Policy relevance and research needs

In conclusion, this study did not identify an association between vitamin B-12 status and peripheral or central neurologic function or clinical markers of neurologic function in moderately vitamin B-12-deficient older people. The robustness of this finding is supported by the use of a composite measure of vitamin B-12 status and a wide range of nerve conduction tests to measure neurologic function. On a population level, these findings cast

doubt over concerns about moderately low vitamin B-12 status in older people in relation to neurologic function.

Nevertheless, vitamin B-12-dependent impairment of neurologic function in less healthy and more vitamin B-12-deplete populations cannot be excluded. Impaired nerve function as a result of lower vitamin B-12 status could remain undetected at the population level and therefore may still have implications for public health.

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