**Effects of vitamin B12 supplementation on neurological and cognitive function in older people: a randomized controlled trial**

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**Running head** Vitamin B12 for neurological and cognitive function

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**Abbreviations**

ADM: Abductor digiti minimi

AD: Abductor hallucis

CMAP: Compound muscle action potential

CMCT: Central motor conduction time

CVLT: California Verbal Learning Test

MEP: Motor evoked potentials

MMSE: Mini-Mental State Examination

SAP: Sensory action potentials

**Abstract**

**Background** Moderate vitamin B12 deficiency is relatively common in older people. There is currently little robust evidence on the effect of vitamin B12 supplementation on neurological and cognitive outcomes in later life.

**Objective** To investigate whether vitamin B12 supplementation benefits neurological and cognitive function in moderately vitamin B12 deficient older people.

**Design** We conducted a double-blind randomized placebo-controlled trial in 7 general practices in the South-East of England, UK. Study participants were aged 75+ years with moderate vitamin B12 deficiency (serum vitamin B12 levels: 107-210pmol/L) in the absence of anemia and received 1mg of crystalline vitamin B12 or matching placebo as a daily oral tablet for 12 months. Peripheral motor and sensory nerve conduction, central motor conduction, clinical neurological examination and cognitive function were assessed before and after treatment.

**Results** 201 participants were enrolled in the trial and 191 provided outcome data. Allocation to vitamin B12 was associated with a 177% increase in serum concentrations of vitamin B12 (641 vs 231pmol/L), a 331% increase in serum holotranscobalamin (240 vs 56pmol/L) and 17% lower serum homocysteine (14.2 vs 17.1µmol/L). In intention-to-treat analysis of covariance models, adjusting for baseline neurological function, there were no evidence of effect on the primary outcome of posterior tibial compound muscle action potential amplitude at 1 year (mean difference -0.2mV; 95% CI –0.8 to 0.3). There was also no evidence of effect on any secondary peripheral nerve or central motor function outcomes or on cognitive function or on clinical examination.

**Conclusions** The results of the trial do not support the hypothesis that correction of moderate vitamin B12 deficiency, in the absence of anemia and of neurological and cognitive signs or symptoms, has beneficial effects on neurological or cognitive function in later life.

**Introduction**

Vitamin B12 deficiency, frequently due to age-related gastric atrophy, is relatively common in later life and affects about one fifth of people aged over 75 years in the UK ([1](#_ENREF_1)). Vitamin B12 is required for the methylation of myelin, neurotransmitters and membrane phospholipids and is essential for the integrity of the central and peripheral nervous system ([2](#_ENREF_2), [3](#_ENREF_3)). Severe deficiency of vitamin B12 typically presents as sensory disturbances in the extremities (tingling and numbness) and loss of vibration and joint position sense, together with motor problems and abnormalities of gait, impaired cognition and depression ([2](#_ENREF_2), [3](#_ENREF_3)). The neurological and cognitive manifestations of severe vitamin B12 deficiency are largely responsive to treatment with vitamin B12 (repeat intramuscular injection), although improvement may take time ([4](#_ENREF_4)) and the severity and duration of neurologic abnormalities influence the degree of recovery ([2](#_ENREF_2), [3](#_ENREF_3)).

Neurological, cognitive and psychological abnormalities also occur in individuals with moderate vitamin B12 deficiency (serum vitamin B12 levels: 107-210pmol/L) ([5](#_ENREF_5)), although the evidence of a direct association between vitamin B12 status and neurological function ([6-9](#_ENREF_6)) or cognitive function ([10](#_ENREF_10), [11](#_ENREF_11)) is mixed. Oral supplementation with crystalline vitamin B12 is routinely used to correct hematological parameters of moderate deficiency ([2](#_ENREF_2), [3](#_ENREF_3), [12](#_ENREF_12)), but there is currently no evidence on the efficacy of treatment for neurological or cognitive function in older people with moderate vitamin B12 deficiency ([2](#_ENREF_2), [3](#_ENREF_3)).

The aim of the present trial was to determine whether daily supplementation for 12 months, with 1mg of vitamin B12 or placebo, of older people with moderate vitamin B12 deficiency in the absence of anemia, would have beneficial effects on peripheral and central neurological function, and on cognitive function.

**Methods**

*Participants*

Details of the trial protocol have been published ([13](#_ENREF_13)). Participants, aged 75 years or older, were enrolled at 7 general practices in South-East England that were members of the Medical Research Council General Practice Research Framework or the National Institute of Health Research Primary Care Research Network. Potentially eligible participants were identified by a computer search after excluding those with diabetes, dementia or epilepsy. An additional manual check of health records was carried out by trained nurses to exclude individuals with alcohol addiction, pacemakers or other implanted metallic devices (for whom central neurophysiological testing is contraindicated), residents of nursing homes and anyone with a prior diagnosis of pernicious anemia. After confirmation by their general practitioner, eligible individuals were invited by mail to participate in the trial. Those reporting current consumption of vitamin B12 supplements or who had received a vitamin B12 injection in the previous 6 months were excluded. Interested eligible participants were invited to attend their general practice for a screening appointment where research nurses clarified any queries and administered the MMSE ([14](#_ENREF_14)) to exclude significant cognitive impairment. Participants with an MMSE score of 24 or greater (maximum score 30) were asked to provide a blood sample to assess serum vitamin B12 and hemoglobin concentrations. Individuals with very low vitamin B12 levels (<107pmol/l – Beckman Coulter assay – a cut-off typically used for deficiency) or who were found to have anemia (hemoglobin concentration <110g/L for women and <120g/L for men) were excluded and referred to their general practitioner for further assessment. Individuals with moderate vitamin B12 deficiency who did not have anemia (serum vitamin B12 levels >107 and <210pmol/l – Beckman Coulter assay ([1](#_ENREF_1)); and hemoglobin levels >110g/L for women, >120g/L for men) were eligible to join the trial and were invited to attend a baseline appointment at King’s College Hospital, London.

*Procedures*

At the baseline appointment, the study manager discussed the trial with potential participants and obtained written informed consent before random treatment allocation. Allocation codes were obtained from a central computerized randomization service. Allocation to treatment was balanced by age (75-79; >80 years) and sex. Allocated treatment consisted of a single tablet administered daily that was identical in size, shape, color, smell and taste for both the intervention and placebo, and packaged into identical pots each containing 70 tablets. The intervention tablets each contained 1mg vitamin B12 (cyanocobalamin). The dose was selected to be greater than the minimum recommended daily intake (2.5µg) required to correct vitamin B12 deficiency in older people ([15](#_ENREF_15)) and is safe ([16](#_ENREF_16)) (there is no defined dietary intake upper limit for vitamin B12). As approximately 1-2% of an oral dose of vitamin B12 is absorbed (by passive diffusion), this dose would be expected to provide 10-20µg/day in the absence of intrinsic factor required for active absorption ([12](#_ENREF_12)). All study personnel were blinded to treatment allocation.

Before the baseline appointment, participants were invited to complete a postal questionnaire collecting information on diet and alcohol consumption. Psychological health was also assessed by postal questionnaire at baseline and at 12 months using the 30-item General Health Questionnaire ([17](#_ENREF_17)). At the baseline appointment, data were collected on educational history and history of prior stroke or myocardial infarction. Data were also collected on current prescribed medication. At baseline and 12 months after randomization, height (to the nearest 0.1cm) and weight (to the nearest 0.1kg) were measured and the timed up-and-go test ([18](#_ENREF_18)) was administered to assess mobility.

*Assessment of neurological function*

At baseline and after 12 months, a single physician (KM) assessed clinical measures of neurological function (presence or absence of knee and ankle jerks, and of joint position sense and vibration sense in the great toe) and conducted a standard battery of peripheral nerve conduction tests (including motor and sensory nerve conduction in the right superficial peroneal, sural, common peroneal, and tibial nerves), and central motor conduction tests. Skin temperature of the dorsum of the foot was measured, allowing correction for temperature differences between the first and second visits. SAP amplitude (maximum deviation of the electrical response) and conduction velocity (distance divided by onset latency) were measured. Common peroneal, tibial, and ulnar motor conduction were measured by recording from extensor digitorum brevis, AH and ADM muscles, respectively. Nerves were stimulated supramaximally at proximal and distal sites and conduction velocity calculated. 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.

Central motor conduction in the corticospinal tract was measured by transcranial magnetic stimulation which painlessly and non-invasively excites motor cortex ([19](#_ENREF_19)). A 13 cm diameter circular coil connected to a magnetic stimulator providing a monophasic pulse was centered over the vertex to excite the hand area of left motor cortex. The threshold for excitation was determined using a standard technique ([20](#_ENREF_20)). With the right ADM muscle partially activated voluntarily, eight stimuli at 1.2 times threshold were delivered to evoke MEPs, the mean amplitude and minimal latency of which were measured. The time to response in a given muscle was subtracted from an estimate of the peripheral nerve conduction time to calculate the CMCT. Similarly, using a double cone coil, the leg area of motor cortex was excited to measure MEPs evoked in AH. Each participant received a maximum of 70 brain stimuli. Any individuals found to have significant neurological deficit were referred to their general practitioner for further assessment.

The primary outcome of the trial was the posterior tibial CMAP amplitude evoked by distal stimulation. The negative peak amplitude of the peripherally evoked CMAP reflects the number of motor axons that can be accessed by an electrical stimulus which in turn reflects muscle strength ([21](#_ENREF_21), [22](#_ENREF_22)). Among the ten secondary neurological outcomes, three assessed motor nerve conduction (posterior tibial conduction velocity, common peroneal CMAP amplitude, common peroneal conduction velocity); four assessed sensory nerve conduction (sural SAP amplitude, sural conduction velocity, superficial peroneal SAP amplitude, superficial peroneal conduction velocity); and three assessed central nerve conduction (mean right ADM MEP amplitude, central motor conduction time to the right ADM, central motor conduction time to the right AH).

*Assessment of cognitive function*

At baseline and after 12 months the study manager administered a range of cognitive function tests. In accordance with international guidance ([23](#_ENREF_23)), the main cognitive outcome was a test of memory, a 16-item word list from the CVLT ([24](#_ENREF_24)). The sum of words recalled after each of three repeats and the number of words recalled after a 20 minute delay formed the main cognitive outcome. The identical version of the CVLT was used at baseline and after 12 months. Other cognitive outcomes were: processing speed assessed using the oral version of the symbol letter modality test ([25](#_ENREF_25)) for which the outcome was number correct in 90 seconds; simple and choice reaction time ([26](#_ENREF_26)) assessed using an electronic reaction timer providing 20 single (simple reaction time) or 40 multiple (choice reaction time) challenges; and executive-function assessed using a verbal fluency test (animal naming) over 60 seconds ([27](#_ENREF_27)).

*Adherence to randomized intervention*

Adherence to allocated treatment was measured by counting the number of tablets returned at the end of the study. At baseline and 12 months after randomization, blood samples were collected to measure serum concentration of vitamin B12 (microbiological assay; CV range 5-7%), holotranscobalamin (holoTC: AXIS-Shield radioimmunoassay; CV range 5-8%), total homocysteine (tHcy: Abbott IMx analyzer; CV range 2-3%) and folate (chloramphenicol-resistant microbiological assay; CV range 5-8%). A full blood count was analyzed for hemoglobin. Baseline appointments were held on average 63 [38, 119] (median [IQR]) days after the screening appointment. At baseline, vitamin B12 was assessed using a microbiological assay (that estimates serum concentrations about 25% higher than the Beckman Coulter method used at the screening appointment), and confirmed that those randomized had low vitamin B12 status at study entry (88% fell below the median value for the microbiological assay) and did not have anemia. There were no preset criteria for participant withdrawal during the trial. Any participants who stopped taking study medication were invited to attend their scheduled follow-up assessment at 12 months.

*Statistical analysis*

A sample size of 100 individuals for each allocated treatment group was selected which, with an assumed 30% drop-out over 12 months, would give 90% power to detect at least a 28% change in the primary outcome of posterior tibial CMAP amplitude with 5% significance. Posterior tibial CMAP amplitude is a marker of foot muscle strength and a 28% increase is likely to be associated with clinically relevant improvements in physical coordination and balance in older people ([28](#_ENREF_28), [29](#_ENREF_29)). The primary analysis was carried out on an intention to treat basis. Analysis of covariance models adjusted for baseline measures. Adjusted models further allowed for age and sex and in the case of the primary outcome we also adjusted for skin temperature. All models with continuous outcomes were boot-strapped to allow for non-normal distributions. Odds ratios for binary variables were calculated using logistic regression. Variables that were not normally distributed are presented as medians with inter-quartile ranges. No sub-group analysis was pre-specified. An independent Data Monitoring and Safety Committee assessed safety data. The results are presented as appropriate effect sizes with 95% confidence intervals.

This study was reviewed and approved by the National Research Ethics Committee (08/H0305/18) and the London School of Hygiene & Tropical Medicine ethics committee (no. 5298).

**Results**

*Study participants*

Participants were screened between November 2008 and February 2010. Invitation letters were sent to 3,071 potential participants and 487 (16%) agreed to attend a screening appointment (**Figure 1**). After screening, 262 potential participants were found to be ineligible, largely because their serum vitamin B12 levels were out of range. Among the 209 participants who were randomly allocated to the study between January 2009 and May 2010, eight were randomized in error (as a result of protocol deviations) and provided no further data. Valid data at baseline were available on 201 participants. Six participants withdrew from the study (2 from vitamin B12 arm, 4 from placebo arm) and one participant died. There were no other reported serious adverse events. Three individuals who continued study medication (all allocated to vitamin B12) did not provide any data at 12 months. Outcome data on 191 participants (95% of randomized) were available after 12 months of intervention. At baseline, the socio-demographic variables, medical history, use of prescribed medication, diet and serum B-vitamin status were similar between the allocated groups (**Table 1**). The study arms were well matched at baseline for neurological (**Table 2**), cognitive and psychological (**Table 3**) function outcomes.

*Intervention participation*

Participants were provided with 420 tablets over the course of the study and remained on randomized treatment for an average 389 days. There was no difference between trial arms in the number of capsules returned at the end of the study (mean of 37 in vitamin B12 arm, 39 in placebo arm). The number of capsules apparently consumed closely matched the number of days on the study confirming a very high level of adherence with allocated treatment (>97%). Blood samples were available from 151 participants at both baseline and 12 months (**Table 4**). Allocation to vitamin B12 was associated with a 177% increase in serum vitamin B12 (641 vs 231pmol/L), a 331% increase in serum holotranscobalamin (240 vs 56pmol/L) and 17% lower serum homocysteine (14.2 vs 17.1µmol/L). In comparison, allocation to placebo was associated with small changes (0-5%) in biochemical parameters (Table 4).

*Effects on neurological function*

Among participants allocated to placebo, the nerve conduction test-retest correlation for the primary outcome of posterior tibial compound muscle action potential (CMAP) amplitude was 0.82, demonstrating a high level of reliability of nerve conduction measurements. Change in the primary outcome over the course of the study was small in both the vitamin B12 and placebo arms. There was no evidence of effect on the primary outcome by allocated treatment at 12 months (mean difference -0.2mV; 95% CI –0.8 to 0.3) or on any secondary peripheral nerve or central motor function outcomes or on clinical examination (**Table 5**). Further adjustment for age and sex did not alter these findings (Table 5).

*Effects on cognitive function and other secondary outcomes*

Change in main cognitive function outcome of the California Verbal Learning Test (CVLT) over the course of the study was small in both the vitamin B12 and placebo arms. There was no evidence of effect by allocated treatment on CVLT at 12 months (mean difference -1.4 words; 95% CI -2.9 to 0.1) or on any other cognitive function outcome or on psychological health (**Table 6**).

**Discussion**

We randomized 201 non-anemic adults aged 75 years and over with moderate vitamin B12 deficiency to receive 1mg vitamin B12 or placebo daily for 12 months. At baseline, participant characteristics, the primary outcome, and secondary neurological and cognitive outcomes were well matched by allocated treatment groups, and loss to follow-up over 12 months was less than 5%. The substantial changes in blood levels of vitamin B12, holotranscobalamin and homocysteine in response to the allocated treatments demonstrated a high level of adherence to the allocated study treatment over 12 months. However, the results of this trial found no evidence of effect on any measure of peripheral or central nerve conduction, or of cognitive function, by allocated treatment and do not provide any evidence that correction of moderate vitamin B12 deficiency in the absence of anemia has beneficial effects on neurological or cognitive function.

We identified trial participants with moderate vitamin B12 status using standard clinical testing procedures (serum vitamin B12 concentration) ([3](#_ENREF_3)). This was done in order to enhance relevance of our trial for population health in older people although the appropriateness of various hematological tests to assess vitamin B12 status is currently under review ([30](#_ENREF_30)). We selected neurological and cognitive assessments for our trial that relate to strength, coordination, mobility, memory, processing speed and executive function and are highly relevant to population health and quality of life in older people. The nerve conduction tests in particular provided objective measures of neurological function using state-of-the-art methods and all testing was conducted by a single expert clinician both at baseline and study end-point thereby eliminating inter-observer variability. We collected clinical measures of neurological function (e.g. knee and ankle jerks and others) to support the relevance of the study. The study protocol was designed to minimize inconvenience and disturbance for participants and we had high participant retention.

The study exclusion criteria resulted in selection of relatively healthy and highly functioning participants who may have been less likely to benefit from vitamin B12 supplementation. There are no age-specific reference data for neurological function in older people ([31](#_ENREF_31)) and norms for cognitive function ([32](#_ENREF_32), [33](#_ENREF_33)) are rarely population specific making interpretation problematic. It may be that neurological and cognitive function was not impaired in study participants at baseline. However, this trial was designed to identify neurological and cognitive benefits from vitamin B12 supplementation among older people with moderate vitamin B12 deficiency irrespective of baseline function. Given the healthy nature of the trial participants, the results may not be fully generalizable to all older people in the population. It is also possible that the duration of treatment may have been too short, and that any effects of vitamin B12 supplementation may only become evident after several years of supplementation or follow-up ([34](#_ENREF_34)). It is noteworthy that no relevant trial with vitamin B12 supplementation of longer than two years has yet been conducted ([34](#_ENREF_34)). The dose of vitamin B12 used in the study may have been insufficient. We selected a safe dose that was within current guidelines and the intervention profoundly improved vitamin B12 and holotranscobalamin status. Finally, the sample might have been too small adequately to detect a small change. The study had adequate power to detect a 28% change in the primary neurological outcome assuming a test-retest correlation of 0.6. In fact, our test-retest correlation was 0.8, suggesting that we had power to detect a smaller change than originally planned.

Observational studies have reported inverse associations of vitamin B12 status with nerve conduction ([6](#_ENREF_6)) although this is not a consistent finding ([7](#_ENREF_7), [9](#_ENREF_9), [35](#_ENREF_35)). There has been no previous randomized controlled trial to assess the impact of vitamin B12 supplementation on neurological function in older people. Previous trials of the effect of vitamin B12 supplementation on cognitive function have largely found no benefits of supplementation but have been of variable quality, small size and short duration ([10](#_ENREF_10), [34](#_ENREF_34)). Some evidence of a benefit from multiple B vitamin supplementation has recently been reported especially among sub-groups of individuals with worse biochemical status when randomized at baseline ([36](#_ENREF_36), [37](#_ENREF_37)). Our trial contributes robust evidence on the effect of vitamin B12 on cognitive function in later life and our findings are consistent with a recent meta-analysis that found no effect of supplementation with vitamin B12 on cognitive aging ([38](#_ENREF_38)).

The present study did not detect any benefits of daily vitamin B12 supplementation over one year on neurological or cognitive function among asymptomatic, non-anemic older people with moderate vitamin B12 deficiency. These results are directly relevant to current clinical practice which identifies low vitamin B12 status especially in older people as being a risk factor for neurological and cognitive impairment. Moreover, the results of the present study cast doubt on the relevance of screening for moderate vitamin B12 deficiency in the absence of anemia and symptoms of neurological or cognitive impairment suggesting the need for more stringent definitions of vitamin B12 deficiency ([30](#_ENREF_30)). Our findings suggest that, with regard to peripheral motor and sensory nerve conduction, central motor conduction and cognitive function, concerns over moderate vitamin B12 deficiency in the absence of anemia in asymptomatic, older adults may not be justified.

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**Table 1: Baseline characteristics of OPEN study participants by allocated treatment**

|  |  |  |
| --- | --- | --- |
|  | Vitamin B12 | Placebo |
| Number of participants | 99 | 102 |
| Sex |  |  |
| Male n (%) | 46 (46.5) | 48 (47.1) |
| Age |  |  |
| Years1 | 79.9 (3.5) | 80.1 (3.7) |
| 75-79 years, n (%) | 56 (56.6) | 57 (55.9) |
| 80+ years, n (%) | 43 (43.4) | 45 (44.1) |
| Education |  |  |
| Age at leaving (yrs)1 | 18.3 (5.3) | 17.8 (6.6) |
| No qualifications, n (%) | 21 (21.4) | 33 (33.0) |
| Basic or clerical, n (%) | 16 (16.3) | 18 (18.0) |
| Advanced or university, n (%) | 33 (33.7) | 19 (19.0) |
| Other n (%) | 28 (28.6) | 30 (30.0) |
| Vascular health |  |  |
| Myocardial infarction in 5 yrs, n(%) | 1 (1.0) | 4 (3.9) |
| Stroke in 5 yrs, n (%) | 1 (1.0) | 0 |
| Body Mass Index (kg/m2) |  |  |
| Mean1 | 27.0 (5.6) | 27.5 (5.3) |
| <18.5, n (%) | 1 (1.0) | 0 |
| >30, n (%) | 18 (18.2) | 25 (24.8) |
| Mini-Mental State Examination score2 | 29 (28, 29) | 29 (28, 29) |
| Current prescription drugs3 |  |  |
| Statins (%) | 32 (38.1) | 35 (44.9) |
| Proton pump inhibitors (%) | 26 (31.0) | 27 (34.6) |
| Other relevant (%) | 1 (1.2) | 2 (2.6) |
| Dietary pattern |  |  |
| Meat >1 portion/wk, n (%) | 69 (73.4) | 70 (72.2) |
| Oily fish >1 portion/wk, n (%) | 16 (18.0) | 22 (23.4) |
| White fish >1 portion/wk, n(%) | 21 (21.9) | 18 (18.8) |
| Eggs >1 portion/wk, n (%) | 47 (48.5) | 38 (39.2) |
| Alcohol daily, n (%) | 33 (34.4) | 35 (35.4) |
| Blood biochemical measures |  |  |
| Number of participants | 86 | 84 |
| Vitamin B12 (pmol/L)2 | 222.9 [197.4, 268.9] | 228.0 [194.7, 271.0] |
| Holotranscobalamin (pmol/L)2 | 50.4 [38.2, 68.3] | 48.8 [39.8, 62.9] |
| Homocysteine (µmol/L)2 | 15.9 [14.0, 18.9] | 16.3 [13.3, 19.9] |
| Folate (nmol/L)2 | 17.7 [10.8, 25.4] | 17.5 [11.8, 25.4] |
| Hemoglobin (g/L)1 | 139.8 (11.1) | 138.9 (12.9) |

1Mean (SD)

2Median (inter-quartile range)

3Drug categories: statins (Simvastatin, Atorvastatin, Pravastatin, Rosuvastatin); proton pump inhibitors (Omeprazole, Lansoprazole, Esomeprazole, Rabeprazole, Pantoprazole); other relevant (Amiodarone, Metronidazole)

**Table 2: Neurological function at baseline, by allocated treatment**

|  |  |  |
| --- | --- | --- |
|  | Vitamin B12  n=99 | Placebo  n=1001 |
| **Motor nerve conduction** |  |  |
| Posterior tibial compound muscle action potential (CMAP) amplitude (mV)2 (**Primary outcome**) | 4.6 [0, 18.0] | 4.9 [0, 13.6] |
| Posterior tibial conduction velocity (m/s)3, 4 | 39.9 (5.0) | 40.1 (5.2) |
| Common peroneal CMAP amplitude (mV)2 | 2.2 [0, 8.8] | 2.5 [0, 8.2] |
| Common peroneal conduction velocity (m/s)3, 4 | 42.5 (4.6) | 43.0 (4.1) |
| **Sensory nerve conduction**  Sural sensory action potential (SAP) amplitude (µV)2 | 3.8 [0, 17.5] | 3.8 [0, 14.2] |
| Sural conduction velocity (m/s)3, 5 | 40.6 (5.2) | 40.2 (5.3) |
| Superficial peroneal SAP amplitude (µV)2 | 2.4 [0, 13.2] | 3.4 [0, 16.7] |
| Superficial peroneal conduction velocity (m/s)3, 6 | 41.2 (6.0) | 41.0 (5.2) |
| **Central motor conduction**  Mean right abductor digiti minimi (ADM) motor evoked potential amplitude (mV)3 | 3.3 (1.4) | 3.4 (1.5) |
| Central Motor Conduction Time (right ADM) (ms)3 | 5.5 (1.2) | 5.5 (1.4) |
| Central Motor Conduction Time (right abductor hallucis) (ms)3, 4 | 13.6 (3.3) | 13.6 (3.5) |
| **Clinical nerve outcomes**  Absent right leg knee jerk, n (%) | 11 (11.1) | 8 (8.0) |
| Absent right leg ankle jerk, n (%) | 33 (33.3) | 22 (22.0) |
| Absent right great toe position sense, n (%) | 4 (4.0) | 8 (8.0) |
| Absent right great toe vibration sense, n (%) | 66 (66.7) | 66 (66.0) |
| Timed up-and-go (seconds)3 | 10.4 (3.0) | 10.7 (3.5) |

1Two participants randomized to placebo provided no baseline nerve function data

2Median (range)

3Mean (SD)

4Small amounts of missing data (n<10 in each arm)

5Missing data (n=11 in vitamin B12 and n=16 in placebo)

5Missing data (n=23 in vitamin B12 and n=17 in placebo)

**Table 3: Cognitive and psychological function at baseline, by allocated treatment**1

|  |  |  |
| --- | --- | --- |
|  | Vitamin B12  n=99 | Placebo  n=102 |
| California Verbal Learning Test |  |  |
| Total words correct in first 3 trials | 22.8 (6.0) | 22.0 (6.5) |
| Words recalled at delayed recall | 7.3 (2.6) | 7.0 (3.1) |
| Symbol letter modality (number correct)2 | 41.1 (9.5) | 39.6 (12.5) |
| Reaction time (s) |  |  |
| Simple | 0.3 (0.1) | 0.3 (0.1) |
| Choice | 0.7 (0.1) | 0.7 (0.2) |
| Verbal fluency (number of animals named) | 21.4 (5.4) | 21.3 (6.0) |
| 30-item General Health Questionnaire score3 | 2.5 (4.7) | 2.9 (4.7) |

1Mean (SD)

2Missing data (n=1 in vitamin B12 and n=1 in placebo)

3Missing data (n=8 in vitamin B12 and n=9 in placebo)

**Table 4: Effects of vitamin B12 on serum concentrations of vitamin B12, holotranscobalamin, homocysteine, folate and haemoglobin1, 2**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Vitamin B12 arm | | |  | Placebo arm | | |  |
| Serum measures | n | Baseline | 12 months | % change from baseline | n | Baseline | 12 months | % change from baseline |
| Vitamin B12 (pmol/L) | 74 | 231.3 (52.0) | 640.9 (199.3) | 177 | 70 | 235.4 (60.7) | 235.7 (77.5) | 0 |
| Holotranscobalamin (pmol/L) | 71 | 55.7 (21.5) | 240.0 (162.9) | 331 | 70 | 51.8 (17.5) | 54.2 (29.5) | 5 |
| Homocysteine (µmol/L) | 73 | 17.1 (4.6) | 14.2 (4.2) | -17 | 70 | 17.2 (5.6) | 17.4 (6.0) | 1 |
| Folate (nmol/L) | 72 | 20.7 (12.3) | 20.2 (11.6) | -2 | 71 | 21.0 (13.8) | 20.4 (14.0) | -3 |
| Hemoglobin (g/L) | 78 | 140.5 (11.0) | 140.0 (10.7) | 0 | 71 | 137.9 (12.8) | 137.2 (12.6) | 0 |

1Values are mean (SD)

2Small amounts of missing data (n<10 per analyte)

**Table 5: Effects of vitamin B12 on the primary and secondary neurological function outcomes at 12 months**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Vitamin B12  n=91 | Placebo  n=91 | Unadjustedeffect size (95% CI)1, 2 | Adjustedeffect size (95% CI)2, 3 |
| **Motor nerve conduction**  Posterior tibial compound muscle action potential (CMAP) amplitude (mV)4 **Primary outcome** | 4.7 [0, 15.3] | 5.3 [0, 17.1] | -0.2 (-0.8, 0.3) | -0.2 (-0.9, 0.3) |
| Posterior tibial conduction velocity (m/s)5 | 39.1 (0.5) | 40.2 (0.5) | -0.7 (-2.0, 0.5) | -0.9 (-2.1, 0.6) |
| Common peroneal CMAP amplitude (mV)4 | 2.3 [0, 8.0] | 2.3 [0, 6.6] | 0.0 (-0.3, 0.3) | -0.0 (-0.3, 0.3) |
| Common peroneal conduction velocity (m/s)5 | 42.3 (0.5) | 43.1 (0.5) | -0.4 (-1.3, 0.7) | -0.4 (-1.6, 0.6) |
|  |  |  |  |  |
| **Sensory nerve conduction**  Sural sensory action potential (SAP) amplitude (µV)4 | 3.2 [0, 18.7] | 3.1 [0, 18.5] | -0.6 (-1.5, 0.2) | -0.5 (-1.4, 0.3) |
| Sural conduction velocity (m/s)5, 7 | 40.3 (0.5) | 40.9 (0.5) | -1.0 (-2.2, 0.3) | -1.1 (-2.5, 0.1) |
| Superficial peroneal SAP amplitude (µV)4 | 3.1 [0, 19.5] | 3.2 [0, 14.5] | 0.1 (-0.7, 1.0) | 0.1 (-0.7, 1.1) |
| Superficial peroneal conduction velocity (m/s)5, 8 | 40.8 (0.6) | 40.8 (0.5) | -0.6 (-2.3, 1.4) | -0.4 (-2.1, 1.2) |
| **Central motor conduction**  Mean right abductor digiti minimi (ADM) motor evoked potential amplitude (mV)5 | 3.5 (0.1) | 3.6 (0.1) | 0.0 (-0.3, 0.4) | 0.0 (-0.3, 0.4) |
| Central Motor Conduction Time (right ADM) (ms)5 | 6.2 (0.1) | 6.2 (0.1) | -0.0 (-0.4, 0.4) | -0.0 (-0.4, 0.4) |
| Central Motor Conduction Time (right abductor hallucis) (ms)5, 9 | 14.0 (0.3) | 14.0 (0.3) | 0.1 (-0.8, 1.1) | 0.1 (-0.8, 1.1) |
| **Clinical nerve outcomes**  Absent right leg knee jerk n (%) | 14 (15.4) | 9 (9.9) | 1.2 (0.4, 3.7)6 | 1.1 (0.3, 3.6)6 |
| Absent right leg ankle jerk n (%) | 33 (36.3) | 22 (24.2) | 0.8 (0.3, 2.0)6 | 0.8 (0.3, 2.0)6 |
| Absent right great toe position sense n (%) | 4 (4.4) | 4 (4.4) | 1.4 (0.4, 5.1)6 | 1.4 (0.4, 5.1)6 |
| Absent right great toe vibration sense n (%) | 57 (62.6) | 52 (62.6) | 0.8 (0.4, 1.4)6 | 0.8 (0.4, 1.4)6 |
| Timed up-and-go (seconds)5 | 10.4 (2.6) | 10.7 (3.2) | -0.12 (-0.6, 0.4) | -0.13 (-0.7, 0.4) |

1Analysis of covariance models adjusted for baseline neurological function

2Mean difference (95% Confidence Interval) unless otherwise stated

3Analysis of covariance models djusted for baseline neurological function, age and sex

4Median (range)

5Mean (SE)

6Odds ratio (95% Confidence Interval)

7Missing data (n=19 in vitamin B12 and n=15 in placebo)

8Missing data (n=22 in vitamin B12 and n=22 in placebo)

9Small amounts of missing data (n<10 in each arm)

**Table 6: Effects of vitamin B12 on cognitive and psychological function outcomes at 12 months**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Vitamin B121  n=91 | Placebo1  n=93 | Unadjustedeffect size (95% CI)2, 3 | Adjustedeffect size (95% CI)3,4 |
| California Verbal Learning Test |  |  |  |  |
| Total words correct in first 3 trials | 23.9 (0.7) | 24.6 (0.7) | -1.4 (-2.9, 0.1) | -1.4 (-2.9, 0.1) |
| Words recalled at delayed recall | 7.5 (0.3) | 7.7 (0.4) | -0.4 (-1.0, 0.2) | -0.4 (-1.0, 0.2) |
| Symbol letter modality (number correct) | 39.6 (1.1) | 40.1 (1.2) | -1.3 (-3.2, 0.6) | -1.3 (-3.2, 0.6) |
| Reaction time (s) |  |  |  |  |
| Simple | 0.3 (0.01) | 0.3 (0.01) | 0.01 (-0.02, 0.04) | 0.01 (-0.02, 0.04) |
| Choice | 0.7 (0.01) | 0.7 (0.02) | -0.003 (-0.03, 0.02) | -0.003 (-0.03, 0.02) |
| Verbal fluency (number of animals named) | 20.8 (0.5) | 19.9 (0.6) | 1.1 (-0.1, 2.2) | 1.1 (-0.1, 2.2) |
| 30-item General Health Questionnaire score5 | 2.4 (0.5) | 2.7 (0.5) | -0.1 (-1.2, 1.0) | -0.1 (-1.3, 1.1) |

1Mean (SE)

2Analysis of variance models adjusted for baseline cognitive function

3Mean difference (95% Confidence Interval) unless otherwise stated

4Analysis of variance models adjusted for baseline cognitive function, age and sex

5Missing data (n=5 in vitamin B12 and n=11 in placebo)

**Figure 1**: **CONSORT flow chart for OPEN study**