

**Impact of baseline vitamin B12 status on the effect of vitamin B12 supplementation on neurologic function in older people: secondary analysis of data from the OPEN randomised controlled trial.**

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**Trial registration** [www.isrctn.com](http://www.isrctn.com) ISRCTN54195799

## **Abstract**

### **Background**

The available evidence from randomised controlled trials suggests that vitamin B12 supplementation does not improve neurologic function in older people with marginal but not deficient Vitamin B12 status. This secondary analysis used data from the Older People and Enhanced Neurological function (OPEN) randomised controlled trial to assess whether baseline vitamin B12 status or change in vitamin B12 status over 12 months altered the effectiveness of dietary vitamin B12 supplementation on neurologic function in asymptomatic older people with depleted vitamin B12 status at study entry.

### **Methods**

Vitamin B12 status was measured as serum concentrations of vitamin B12, holotranscobalamin, homocysteine and via a composite indicator (cB12). Neurological function outcomes included eleven electrophysiological measures of sensory and motor components of peripheral and central nerve function. Linear regression analyses were restricted to participants randomised into the intervention arm of the OPEN trial (n=91).

### **Results**

Analyses revealed an inconsistent pattern of moderate associations between some measures of baseline vitamin B12 status and some neurological responses to supplementation. The directions of effect varied and heterogeneity in effect across outcomes could not be explained according to type of neurologic outcome. There was no evidence of differences in the neurological response to vitamin B12 supplementation according to change from baseline over 12 months in any indicator of B12 status.

### **Conclusions**

This secondary analysis of high quality data from the OPEN trial provides no evidence that baseline (or change from baseline) vitamin B12 status modifies the effect of vitamin B12 supplementation on peripheral or central nerve conduction among older people with marginal vitamin B12 status. There is currently insufficient evidence of efficacy for neurological function to support population-wide recommendations for vitamin B12 supplementation in healthy asymptomatic older people with marginal vitamin B12 status.

## Introduction

Ageing is associated with a decline in vitamin B12 status, and prevalence of vitamin B12 deficiency increases with age<sup>1</sup>. As dietary intakes are usually adequate in healthy populations<sup>2</sup>, the age-related decline in vitamin B12 status is usually attributed to atrophic gastritis which reduces absorption of vitamin B12<sup>3</sup>. Adequate vitamin B12 is necessary for optimal neurologic function. Symptoms of peripheral neuropathy associated with vitamin B12 deficiency commonly include symmetric paresthesias, numbness or gait problems, impaired position and cutaneous sensation, impaired vibration sense and weakness<sup>4,5</sup>.

In the light of the high prevalence of preclinical deficiency, routine vitamin B12 supplementation has been proposed in older people<sup>6,7</sup>. Indeed, US adults aged >50 years are advised to meet their recommended daily allowance of 2.4 µg vitamin B12 per day mainly by consuming either food fortified with vitamin B12 or a vitamin B12-containing supplement<sup>7</sup>. Yet surprisingly few studies<sup>8-12</sup> are available to help understand whether vitamin B12 supplementation improves neurological function in older people and overall, these studies (while heterogeneous in size, effect and quality) do not suggest that there is strong evidence that vitamin B12 supplementation improves neurologic function in older people in the absence of frank vitamin B12 deficiency.

It is however possible that improvement in neurologic function resulting from vitamin B12 supplementation may be apparent only in non-deficient people with the most marginal vitamin B12 status. For example, while primary analyses from the randomised controlled trial (RCT) by Hvas *et al.*<sup>9</sup> showed no difference in neurologic outcomes between intervention (1mg cyanocobalamin administered intramuscularly weekly for four weeks) and

control arms, there was evidence of effectiveness of treatment in individuals with the lowest vitamin B12 status at study entry. There is similar evidence to support greater benefits of vitamin B12 supplementation in people with the lowest vitamin B12 status from studies on cognitive outcomes. The VITACOG RCT reported that administering high doses of folic acid, vitamin B6 and vitamin B12 over two years on measures of brain atrophy and several other cognitive outcomes was more beneficial in individuals with the highest baseline plasma tHcy levels <sup>13, 14</sup>.

The Older People and Enhanced Neurological function (OPEN) RCT provides an opportunity to explore further, in a high quality dataset, whether improvement in neurologic function from vitamin B12 supplementation is limited to people with the lowest baseline vitamin B12 status. The OPEN study was a double-blind placebo-controlled RCT investigating the effects of 1mg vitamin B12 oral supplementation for 12 months on electrophysiological indices of neurologic function in older people aged 75+ years with marginal vitamin B12 status. The primary results from the trial demonstrated no effect of vitamin B12 supplementation on any measure of nerve function<sup>11</sup>. This secondary analysis explores whether differences in baseline vitamin B12 status or change in vitamin B12 status over 12 months of intervention alters the effectiveness of vitamin B12 supplementation on electrophysiological indices of neurologic function in asymptomatic older people with marginal vitamin B12 status at study entry.

## **Methods**

This study is a secondary analysis of data from the OPEN study, the protocol of which has been published<sup>15</sup> ([www.isrctn.com](http://www.isrctn.com); ISRCTN54195799). Screening for participation in the

OPEN study took place between November 2008 and February 2010. Participants were aged 75+ years and recruited from 7 general practices in South East England. Individuals with dementia, epilepsy, alcohol addiction, diabetes, pacemakers or other implanted metallic devices, residents of nursing homes, or a previous diagnosis of pernicious anaemia were excluded. Potential participants with significant cognitive impairment or who reported current consumption of vitamin B12 supplements or who had received a vitamin B12 injection in the previous 6 months were excluded. Individuals with moderate vitamin B12 deficiency [serum vitamin B12 concentrations  $\geq 107$  and  $< 210$  pmol/L (using a Beckman Coulter assay)] who did not have anaemia (haemoglobin concentrations  $\geq 110$  g/L for women and  $\geq 120$  g/L for men) were eligible to join the study.

Participants, who provided informed consent, were enrolled in the trial and randomised to treatment (n=99) or placebo arms (n=102). Allocation to treatment was balanced by age and sex, and all participants and investigators were blind to treatment allocation. Allocated treatment consisted of a single tablet containing 1mg vitamin B12 (cyanocobalamin) daily for 12 months. 91 participants in each arm of the trial provided follow-up data on the OPEN trial primary outcome: posterior tibial compound muscle action potential (CMAP) amplitude. The OPEN study was designed to achieve 90% power to detect a  $\geq 28\%$  difference in the primary outcome (with 5% significance) between arms of the trial.

At baseline and after 12 months follow-up, participants provided a blood sample and undertook a series of neurophysiological function tests. Blood samples were analysed for serum concentrations of vitamin B12 (using a microbiologic assay); holotranscobalamin (holoTC; Axis-Shield radioimmunoassay; Axis-Shield plc) and total homocysteine (tHcy;

Abbott IMx analyzer; Abbott Laboratories) in a single laboratory in Trinity College Dublin.

The microbiologic assay for vitamin B12 (used at study entry) typically provides estimates of serum vitamin B12 concentration that are ~25% higher than those produced by the Beckman Coulter method (used at initial screening). Median (and interquartile range) values for serum vitamin B12 (estimated using the microbiologic assay by the same laboratory in Trinity College Dublin) among older people in Ireland have recently been published as 277 (216-369) pmol/L<sup>16</sup>. 88% of OPEN study participants had vitamin B12 status below the median value for the microbiologic assay reference standard (derived from a random sample of 470 from nationally representative adults in the Irish National Adult Nutrition Survey) (personal communication Dr Anne Molloy, 2013), indicating that OPEN study participants had marginal vitamin B12 status at study entry.

A single expert neurophysiologist (KM) conducted a battery of peripheral nerve conduction tests and central motor conduction tests at baseline and follow-up. Standard techniques were used involving surface electrodes. Skin temperature of the dorsum of the foot and hand was measured to allow for appropriate adjustments in the analyses because nerve conduction in peripheral nerves is sensitive to temperature of the limbs<sup>17</sup>. Posterior tibial CMAP amplitude evoked by distal stimulation was the primary trial outcome. The seven secondary peripheral nerve outcomes were common peroneal CMAP amplitude (also evoked by distal stimulation); posterior tibial and common peroneal conduction velocities measured by recording from the adductor hallucis (AH) and extensor digitorum brevis muscles respectively; and sensory action potential (SAP) amplitude (maximum deviation of the electrical response) and conduction velocity measured in the sural and superficial peroneal nerves. Together these outcomes represent each component of peripheral nerve

function: posterior tibial and common peroneal CMAP reflects the number of motor axons that can be accessed by an electrical stimulus which in turn reflects muscle strength<sup>18, 19</sup>; sural and superficial peroneal SAP amplitudes are indices of nerve fibre number; and sensory (sural and superficial peroneal nerve) or motor (posterior tibial and common peroneal) conduction velocity is an indicator of myelination<sup>20</sup>. All nerve conduction outcomes were measured on the right side of the body.

Central motor conduction tests were measured using transcranial magnetic stimulation, which painlessly and noninvasively excites the motor cortex<sup>21</sup>. Further secondary outcomes were mean abductor digiti motor (ADM) motor evoked potential (MEP) amplitude, and ADM and AH central motor conduction time (CMCT). With the right ADM muscle partially activated voluntarily, stimuli were delivered to evoke MEPs, and mean amplitudes were measured. Similarly, the leg area of motor cortex was excited to measure MEPs evoked in the AH muscle. ADM and AH CMCT were calculated by subtracting the time to response in each muscle from an estimate of the peripheral nerve conduction time.

Vitamin B12 and holoTC were used as measures of vitamin B12 status. In addition, cB12 was used as a composite indicator of vitamin B12 status combining vitamin B12, holoTC and tHcy<sup>22</sup>. Although tHcy alone does not have good specificity as an indicator of vitamin B12 status, it is also included as an indicator of vitamin B12 in this study for exploratory purposes.



### *Statistical analysis*

Secondary analyses were performed to explore whether baseline or change in vitamin B12 status altered the impact of dietary vitamin B12 supplementation on neurologic function. Analyses were restricted to the intervention arm (n=91), because the placebo arm did not receive any vitamin B12 supplementation and accordingly, change in vitamin B12 status was negligible<sup>11</sup>, and minor changes in nerve conduction were assumed to be due to variability in repeated measures. Analyses were exploratory in nature and aimed to identify consistent patterns in findings rather than applying stringent p-values to test for statistical significance.

The effects of supplementation on vitamin B12 status according to baseline status were explored by baseline quartiles of vitamin B12, holoTC, tHcy and cB12 as indicators of vitamin B12 status. Linear regression models were used to test for associations between baseline and change in vitamin B12 status (measured by vitamin B12, holoTC, tHcy and cB12) and neurologic response to vitamin B12 supplementation. Eleven nerve conduction outcomes were used, consistent with the outcomes used in the primary analyses from the OPEN study<sup>11</sup>. For each nerve conduction outcome, linear regression models tested for associations between baseline vitamin B12 status and change in the outcome (in response to supplementation), adjusted for the corresponding baseline measure of nerve conduction, age, sex and change in skin temperature. Similarly, linear regression models tested for associations between change in vitamin B12 status and change in the outcome (in response to supplementation) for the same nerve conduction outcomes; adjustments included baseline measures of vitamin B12 in addition to those listed above. All linear regression models were boot-strapped to allow for non-normal distributions of exposures and outcomes. Results are presented as mean change in outcome with bias-corrected 95%

confidence intervals. Because the analyses involved multiple comparisons, p-values have been interpreted with caution. Statistical analyses were conducted using STATA (version 14 StataCorp, Texas USA).

### *Ethics*

The OPEN study was reviewed and approved by the National Research Ethics Committee (08/H0305/18) and the London School of Hygiene & Tropical Medicine Ethics Committee (LSHTM) (no. 5298). The secondary analyses presented here were approved by the LSHTM Ethics Committee (no. 7176).

### **Results**

Participants included in the current analysis had a mean age of 79.9 years and 46.5% were male. Comparing baseline with 12 months, oral supplementation was effective in increasing vitamin B12 status: vitamin B12, holoTC and cB12 increased (mean change 409.6 pmol/L, 184.3 pmol/L and 1.5 respectively) and tHcy (mean change -2.8  $\mu$ mol/L) decreased at 12 month follow-up (**Table 1**). Levels of vitamin B12 and cB12 at follow-up were constant across quartiles of baseline status (vitamin B12: F test  $p=0.44$ ; cB12: F test  $p=0.21$ ), suggesting a plateau effect (**Figure 1**). In contrast, the effect of vitamin B12 supplementation on tHcy and holoTC differed across baseline quartiles (tHcy: test for trend  $p<0.001$ ; holoTC: test for trend  $p=0.01$ ).

Linear regression models found no evidence of a difference in impact of vitamin B12 supplementation on the primary trial outcome (posterior tibial CMAP amplitude) (**Table 2**). For the other nerve function outcomes, effect sizes were generally small and patterns of

effect inconsistent. There was evidence of an association of baseline vitamin B12 status with common peroneal CMAP amplitude, with higher baseline vitamin B12 being associated with a smaller change in common peroneal CMAP amplitude in response to supplementation [ $\beta=-0.01$  (-0.01 - -0.00),  $p=0.02$ ]; ( $p>0.05$  for all other measures of vitamin B12 status).

There was evidence of inverse associations of baseline vitamin B12 and cB12 with change in tibial motor conduction velocity; participants with the lowest baseline status tended to have a greater change in motor conduction velocity in response to supplementation over 12 months. In contrast, weak associations were detected between baseline holoTC and cB12 and change in common peroneal motor conduction velocity in response to supplementation that suggest a greater change in motor conduction velocity with a higher baseline status.

There is also evidence of a positive association of baseline vitamin B12 status with and a greater change in sensory sural conduction velocity in response to supplementation [ $\beta=0.02$  (0.00 – 0.03),  $p=0.05$ ]; ( $p>0.05$  for all other measures of vitamin B12 status).

In measure of central nerve conduction, vitamin B12 supplementation improved (i.e. decreases) AH CMCT in participants with lower baseline cB12 status [ $\beta=2.19$  (0.10 – 4.04),  $p=0.03$ ]. Results for change in ADM CMCT were consistent in direction of effect but smaller in magnitude and not statistically significant [ $\beta=0.53$  (-0.75 – 1.43),  $p=0.31$ ]. In contrast, results for mean ADM MEP amplitude suggest that a greater response was observed in participants with higher baseline vitamin B12 status (as measured by tHcy and cB12).

Linear regression analyses show that neurologic response to vitamin B12 supplementation did not differ by change in vitamin B12 status (**Table 3**). Null results are consistent across each measure of vitamin B12 status and all nerve conduction outcomes.

## **Discussion**

### *Key findings*

The secondary analyses presented here used high quality data from the OPEN RCT to explore the relevance of baseline (or change from baseline) vitamin B12 status on the effectiveness of vitamin B12 supplementation for neurological function in older people with marginal vitamin B12 status. The analysis did not identify an impact of baseline (or change from baseline) vitamin B12 status on the effect of vitamin B12 supplementation on the OPEN trial primary outcome (tibial compound muscle action potential amplitude). When considering secondary neurological outcomes, analyses using some indicators of vitamin B12 status (but not others) revealed suggestive evidence of associations between baseline vitamin B12 status and some peripheral and central neurologic responses to supplementation. However, directions of effect were inconsistent: an equal number of analyses showed suggestive evidence of greater neurologic improvement in response to supplementation in participants with *higher* baseline vitamin B12 status as there were analyses showing suggestive evidence of greater neurologic improvement in response to supplementation in participants with *lower* baseline vitamin B12 status. Heterogeneity in findings across neurologic outcomes could not be explained by the aspect of neurologic function each outcomes measures: there were no differences in findings in peripheral versus central nerve conduction; motor versus sensory peripheral nerve conduction; or conduction velocities versus CMAP or SAP amplitudes. Taken together, this secondary

analysis of OPEN trial data suggests that there is no evidence of differences in the effect of vitamin B12 supplementation on measures of nerve conduction based on baseline vitamin B12 status (vitamin B12, holoTC, tHcy or cB12). This analysis also finds no evidence of difference in neurologic response to vitamin B12 supplementation according to any indicator of *change* in vitamin B12 status.

### *Comparison with other studies*

Whilst previous reports<sup>9</sup> suggested that individuals with the lowest vitamin B12 status might benefit most from an intramuscular vitamin B12 intervention in terms of neurological symptoms, results from this study indicate that this does not extend to benefits of an oral vitamin B12 intervention or improvements in nerve conduction. This study also extends the primary findings of the OPEN RCT<sup>11</sup> by exploring whether the null findings of the intervention might be attributed to the relatively replete vitamin B12 status of participants. As the secondary analyses found no consistent evidence of a greater benefit of vitamin B12 supplementation in terms of neurologic function in those with the lowest vitamin B12 status, it remains unlikely that the OPEN trial intervention would have been effective if participants had poorer vitamin B12 status at baseline alongside absence of neurologic or haematological symptoms of deficiency.

### *Strengths and weaknesses*

The use of several measures of vitamin B12 status and measurement of neurologic function by nerve conduction are strengths of the study. In particular, holoTC measures the active fraction of vitamin B12 and has been proposed as appropriate to use in the subclinical situation<sup>23, 24</sup>. The use of cB12 has the advantage of combining biomarkers of circulating

vitamin B12 and a functional biomarker of vitamin B12 status<sup>22</sup>. Poor renal function can be a cause of elevated tHcy<sup>6</sup> and is also reported to be associated with cB12<sup>25</sup> but was not measured in the OPEN study. Nerve conduction tests use state-of-the-art methods and are objective measures. All baseline and follow-up testing was conducted by a single neurophysiologist thereby eliminating inter-observer variability.

It is recognised that this secondary analysis is limited in statistical power. It is possible that trends would have been more easily detected in a larger study. It is also possible that the statistically significant associations detected in this study were identified by chance as a result of multiple comparisons across several outcomes. Furthermore, it remains possible that the duration of the vitamin B12 supplementation was too short and that benefits of supplementation only become evident after several years of treatment.

#### *Policy relevance and research needs*

This secondary analysis of the OPEN trial does not provide evidence to suggest that oral supplementation with vitamin B12 has beneficial effects on neurological function in individuals with marginal vitamin B12 status. While prevention of vitamin B12 deficiency remains important, especially in older people, the available evidence does not support population-wide screening for moderate vitamin B12 deficiency in the absence of anaemia or neurological symptoms, nor population-wide recommendations for vitamin B12 supplementation in healthy asymptomatic older people. Treatment for neurological impairment attributed to vitamin B12 deficiency should be managed as appropriate by clinicians, rather than handled at the population level.

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LMM and ADD designed the study. LMM conducted the statistical analyses, wrote the first draft of the manuscript and had primary responsibility for final content. EA provided statistical support for the analyses. KM conducted all neurological function tests. All authors read and approved the final manuscript.

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**Table 1: Mean and standard deviation (SD) values by quartile of serum vitamin B12<sup>1</sup>, holotranscobalamin (holoTC)<sup>2</sup>, homocysteine (tHcy)<sup>3</sup> and a composite indicator (cB12) in the study population (n=91) of older people in the intervention arm of the OPEN study at baseline (0 months) and follow-up (12 months)**

Baseline quartile	Baseline vitamin B12 (pmol/L)		Follow up vitamin B12 (pmol/L)		Baseline holoTC (pmol/L)		Follow up holoTC (pmol/L)		Baseline tHcy (µmol/L)		Follow up tHcy (µmol/L)		Baseline cB12		Follow up cB12	
	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
<b>Q1</b>	19	167.2 (18.8)	17	638.9 (221.6)	21	31.1 (5.8)	17	185.1 (83.8)	18	12.6 (0.9)	16	11.3 (1.0)	18	-0.7 (0.2)	15	1.1 (0.4)
<b>Q2</b>	23	211.5 (8.8)	21	614.5 (244.5)	16	43.3 (2.0)	14	187.4 (93.6)	23	14.9 (0.7)	21	12.0 (1.4)	19	-0.3 (0.1)	17	1.3 (0.4)
<b>Q3</b>	20	251.6 (13.6)	20	613.6 (99.3)	17	57.7 (5.3)	17	254.1 (152.5)	21	17.6 (1.0)	19	14.8 (2.1)	22	-0.1 (0.1)	21	1.2 (0.5)
<b>Q4</b>	19	302.2 (26.4)	16	711.8 (203.8)	24	79.8 (14.8)	23	302.2 (221.3)	18	23.5 (4.6)	17	19.1 (5.5)	19	0.2 (0.2)	18	1.4 (0.5)

<sup>1</sup>Microbiologic assay

<sup>2</sup>Axis-Shield radioimmunoassay

<sup>3</sup>Abbott IMx analyzer; Abbott Laboratories

**Table 2: Linear regression analyses to assess relationship between baseline vitamin B12 status and change in nerve conduction in response to supplementation<sup>1</sup>**

Change in outcome	Baseline B12 (pmol/L)	Baseline holoTC (pmol/L)	Baseline tHcy (μmol/L)	Baseline cB12
<b>Motor tibial CMAP amplitude (mV)</b>	n 73 <sup>2</sup>	70 <sup>2</sup>	72 <sup>2</sup>	70 <sup>2</sup>
	B -0.00 (-0.01 - 0.00)	0.01 (-0.01 – 0.03)	0.04 (-0.03 – 0.15)	-0.12 (-1.21 – 1.01)
	p 0.45	0.42	0.32	0.84
<b>Motor common peroneal CMAP amplitude (mV)</b>	n 71 <sup>3</sup>	69 <sup>4</sup>	70 <sup>3</sup>	69 <sup>4</sup>
	B -0.01 (-0.01 - -0.00)	0.00 (-0.01 – 0.01)	-0.00 (-0.06 – 0.08)	-0.02 (-0.81 – 0.83)
	p 0.02	0.94	0.97	0.97
<b>Motor tibial conduction velocity (m/s)</b>	n 72	69	71	69
	B -0.02 (-0.04- -0.01)	-0.02 (-0.06 – 0.03)	0.11 (-0.09 – 0.27)	-2.12 (-4.40 – 0.34)
	p 0.01	0.40	0.21	0.08
<b>Motor common peroneal conduction velocity (m/s)</b>	n 71	69	70	69
	B 0.00 (-0.01 – 0.02)	0.04 (0.00 – 0.09)	-0.11 (-0.25 – 0.14)	2.05 (-0.28 – 4.21)
	p 0.75	0.04	0.25	0.07
<b>Sensory sural SAP amplitude (μV)</b>	n 59 <sup>5</sup>	58 <sup>6</sup>	59 <sup>7</sup>	58 <sup>6</sup>
	B -0.01 (-0.04 – 0.00)	-0.01 (-0.04 – 0.03)	0.09 (-0.04 – 0.21)	-1.03 (-3.09- 0.59)
	p 0.23	0.64	0.15	0.27
<b>Sensory superficial peroneal SAP amplitude (μV)</b>	n 49 <sup>8</sup>	48 <sup>9</sup>	49 <sup>10</sup>	48 <sup>9</sup>
	B -0.00 (-0.01 – 0.02)	-0.00 (-0.07 – 0.03)	0.07 (-0.26 – 0.37)	-0.81 (-4.50 – 1.84)
	p 0.85	0.85	0.66	0.61
<b>Sensory sural conduction velocity (m/s)</b>	n 59	58	59	58
	B 0.02 (0.00 – 0.03)	0.03 (-0.02 – 0.07)	0.03 (-0.16 – 0.30)	1.81 (-0.96 – 4.35)
	p 0.05	0.18	0.82	0.17
<b>Sensory superficial peroneal conduction velocity (m/s)</b>	n 49	48	49	48
	B -0.00 (-0.03 – 0.03)	-0.02 (-0.11 – 0.07)	0.17 (-0.15 – 0.62)	-1.58 (-7.07 – 3.25)
	p 0.83	0.62	0.38	0.54
<b>ADM CMCT (ms)</b>	n 72	69	71	69
	B 0.00 (-0.00 – 0.01) <sup>11</sup>	0.01 (-0.01 – 0.03) <sup>11</sup>	-0.00 (-0.08 – 0.09) <sup>11</sup>	0.53 (-0.75 – 1.43) <sup>11</sup>
	p 0.77 <sup>11</sup>	0.21 <sup>11</sup>	0.98 <sup>11</sup>	0.31 <sup>11</sup>
<b>AH CMCT (ms)</b>	n 66	63	65	63
	B 0.00 (-0.01 – 0.02)	0.03 (-0.01 – 0.06)	-0.07 (-0.20 – 0.08)	2.19 (0.10 – 4.04)
	p 0.77	0.12	0.35	0.03
<b>Mean abductor digiti motor (ADM) MEP amplitude (mV)</b>	n 74	71	73	71
	B 0.00 (-0.00 – 0.01) <sup>11</sup>	0.00 (-0.01 – 0.01) <sup>11</sup>	-0.07 (-0.13 - -0.03) <sup>11</sup>	0.72 (0.04 – 1.29) <sup>11</sup>
	p 0.17 <sup>11</sup>	0.33 <sup>11</sup>	0.00 <sup>11</sup>	0.03 <sup>11</sup>

<sup>1</sup>Adjusted for baseline measure of the neurologic outcome, baseline age, baseline sex and change in skin temperature (foot), unless otherwise stated.

<sup>2</sup>Three subjects with 0 values for tibial CMAP amplitude at baseline or follow-up excluded.

<sup>3</sup>Five subjects with 0 values for common peroneal CMAP amplitude at baseline or follow-up excluded.

<sup>4</sup> Four subjects with 0 values for common peroneal CMAP amplitude at baseline or follow-up excluded.

<sup>5</sup> Seventeen subjects with 0 values for sural SAP amplitude at baseline or follow-up excluded; 8 of these had detectable sural SAP amplitude at baseline and no detectable (0) sural SAP amplitude at follow-up; and 3 of these had undetectable (0) sural SAP amplitude at baseline and detectable sural SAP amplitude at follow-up.

<sup>6</sup> Fifteen subjects with 0 values for sural SAP amplitude at baseline or follow-up excluded; 7 of these had detectable sural SAP amplitude at baseline and no detectable (0) sural SAP amplitude at follow-up; and 3 of these had undetectable (0) sural SAP amplitude at baseline and detectable sural SAP amplitude at follow-up.

<sup>7</sup> Sixteen subjects with 0 values for sural SAP amplitude at baseline or follow-up excluded; 7 of these had detectable sural SAP amplitude at baseline and no detectable (0) sural SAP amplitude at follow-up; and 3 of these had undetectable (0) sural SAP amplitude at baseline and detectable sural SAP amplitude at follow-up.

<sup>8</sup> Twenty-seven subjects with 0 values for superficial peroneal SAP amplitude at baseline or follow-up excluded; 11 of these had detectable superficial peroneal SAP amplitude at baseline and no detectable (0) superficial peroneal SAP amplitude at follow-up; and 10 of these had undetectable (0) superficial peroneal SAP amplitude at baseline and detectable superficial peroneal SAP amplitude at follow-up.

<sup>9</sup> Twenty-five subjects with 0 values for superficial peroneal SAP amplitude at baseline or follow-up excluded; 10 of these had detectable superficial peroneal SAP amplitude at baseline and no detectable (0) superficial peroneal SAP amplitude at follow-up; and 9 of these had undetectable (0) superficial peroneal SAP amplitude at baseline and detectable superficial peroneal SAP amplitude at follow-up.

<sup>10</sup> Twenty-six subjects with 0 values for superficial peroneal SAP amplitude at baseline or follow-up excluded; 10 of these had detectable superficial peroneal SAP amplitude at baseline and no detectable (0) superficial peroneal SAP amplitude at follow-up; and 10 of these had undetectable (0) superficial peroneal SAP amplitude at baseline and detectable superficial peroneal SAP amplitude at follow-up.

<sup>11</sup> Adjusted for baseline measure of the neurologic outcome, baseline age, baseline sex and change in skin temperature (hand).

**Table 3: Linear regression analyses to assess relationship between change in vitamin B12 status and change in nerve conduction in response to supplementation<sup>1</sup>**

Change in outcome	Change in B12	Change in holoTC	Change in tHcy	Change in cB12
<b>Motor tibial CMAP amplitude (mV)</b>	n 71 <sup>2</sup>	68 <sup>2</sup>	70 <sup>2</sup>	68 <sup>2</sup>
	β 0.00 (-0.00 – 0.00)	0.00 (-0.00 – 0.01)	-0.07 (-0.22 – 0.08)	0.71 (-0.36 – 1.91)
	p 0.41	0.38	0.34	0.22
<b>Motor common peroneal CMAP amplitude (mV)</b>	n 69 <sup>3</sup>	67 <sup>4</sup>	68 <sup>3</sup>	67 <sup>4</sup>
	β 0.00 (-0.00 – 0.00)	0.00 (-0.00 – 0.00)	0.10 (-0.12 – 0.34)	0.52 (-0.14 – 1.39)
	p 0.98	0.31	0.41	0.18
<b>Motor tibial conduction velocity (m/s)</b>	n 70	67	69	67
	β 0.00 (-0.00 – 0.00)	0.00 (-0.00 – 0.01)	-0.07 (-0.59 – 0.43)	0.72 (-1.50 – 3.35)
	p 0.98	0.52	0.77	0.57
<b>Motor common peroneal conduction velocity (m/s)</b>	n 69	67	68	67
	β -0.00 (-0.00 – 0.00)	-0.00 (-0.01 – 0.00)	0.10 (-0.39 – 0.78)	0.24 (-1.70 – 2.46)
	p 0.81	0.35	0.75	0.82
<b>Sensory sural SAP amplitude (μV)</b>	n 58 <sup>5</sup>	57 <sup>6</sup>	58 <sup>7</sup>	57 <sup>6</sup>
	β -0.00 (-0.00 – 0.00)	0.00 (-0.01 – 0.01)	-0.13 (-0.56 – 0.22)	0.23 (-1.62 – 2.59)
	p 0.93	0.98	0.51	0.82
<b>Sensory superficial peroneal SAP amplitude (μV)</b>	n 49 <sup>8</sup>	48 <sup>9</sup>	49 <sup>10</sup>	48 <sup>9</sup>
	β 0.00 (-0.00 – 0.01)	0.00 (-0.01 – 0.02)	-0.37 (-1.08 – -0.00)	2.45 (-0.52 – 7.40)
	p 0.66	0.69	0.15	0.19
<b>Sensory sural conduction velocity (m/s)</b>	n 58	57	58	57
	β 0.00 (-0.00 – 0.01)	0.00 (-0.01 – 0.01)	0.29 (-0.21 – 0.82)	0.94 (-2.43 – 3.23)
	p 0.05	0.64	0.25	0.51
<b>Sensory superficial peroneal conduction velocity (m/s)</b>	n 49	48	49	48
	β -0.01 (-0.02 – 0.01)	-0.01 (-0.03 – 0.01)	-0.18 (-0.82 – 0.74)	-1.64 (-7.42 – 3.01)
	p 0.37	0.32	0.65	0.54
<b>ADM CMCT (ms)</b>	n 70	67	69	67
	β -0.00 (-0.00 - 0.00) <sup>11</sup>	-0.00 (-0.00 - 0.00) <sup>11</sup>	0.02 (-0.19 – 0.17) <sup>11</sup>	-0.19 (-1.01 – 0.90) <sup>11</sup>
	p 0.15 <sup>11</sup>	0.35 <sup>11</sup>	0.81 <sup>11</sup>	0.68 <sup>11</sup>
<b>AH CMCT (ms)</b>	n 64	61	63	61
	β 0.00 (-0.00 - 0.00)	0.00 (-0.01 – 0.01)	0.18 (-0.22 – 0.66)	0.42 (-1.53 – 2.02)
	p 0.41	0.84	0.42	0.64
<b>Mean abductor digiti motor (ADM) MEP amplitude (mV)</b>	n 72	69	71	69
	β 0.00 (-0.00 - 0.00) <sup>11</sup>	0.00 (-0.00 - 0.00) <sup>11</sup>	-0.03 (-0.13 – 0.07) <sup>11</sup>	0.50 (-0.27 – 1.23) <sup>11</sup>
	p 0.36 <sup>11</sup>	0.72 <sup>11</sup>	0.52 <sup>11</sup>	0.19 <sup>11</sup>

<sup>1</sup> Adjusted for baseline measure of neurologic outcome, baseline B12/holoTC/tHcy/cB12 status, baseline age, baseline sex and change in skin temperature (foot) unless otherwise stated.

<sup>2</sup> Three subjects with 0 values for tibial CMAP at baseline or follow-up excluded.

<sup>3</sup> Five subjects with 0 values for common peroneal CMAP at baseline or follow-up excluded.

<sup>4</sup>Four subjects with 0 values for common peroneal CMAP at baseline or follow-up excluded.

<sup>5</sup>Sixteen subjects with 0 values for sural SAP at baseline or follow-up excluded; 7 of these had detectable sural SAP amplitude at baseline and no detectable (0) sural SAP amplitude at follow-up; and 3 of these had undetectable (0) sural SAP amplitude at baseline and detectable sural SAP amplitude at follow-up.

<sup>6</sup>Fourteen subjects with 0 values for sural SAP at baseline or follow-up excluded; 6 of these had detectable sural SAP amplitude at baseline and no detectable (0) sural SAP amplitude at follow-up; and 3 of these had undetectable (0) sural SAP amplitude at baseline and detectable sural SAP amplitude at follow-up.

<sup>7</sup>Fifteen subjects with 0 values for sural SAP at baseline or follow-up excluded; 6 of these had detectable sural SAP amplitude at baseline and no detectable (0) sural SAP amplitude at follow-up; and 3 of these had undetectable (0) sural SAP amplitude at baseline and detectable sural SAP amplitude at follow-up.

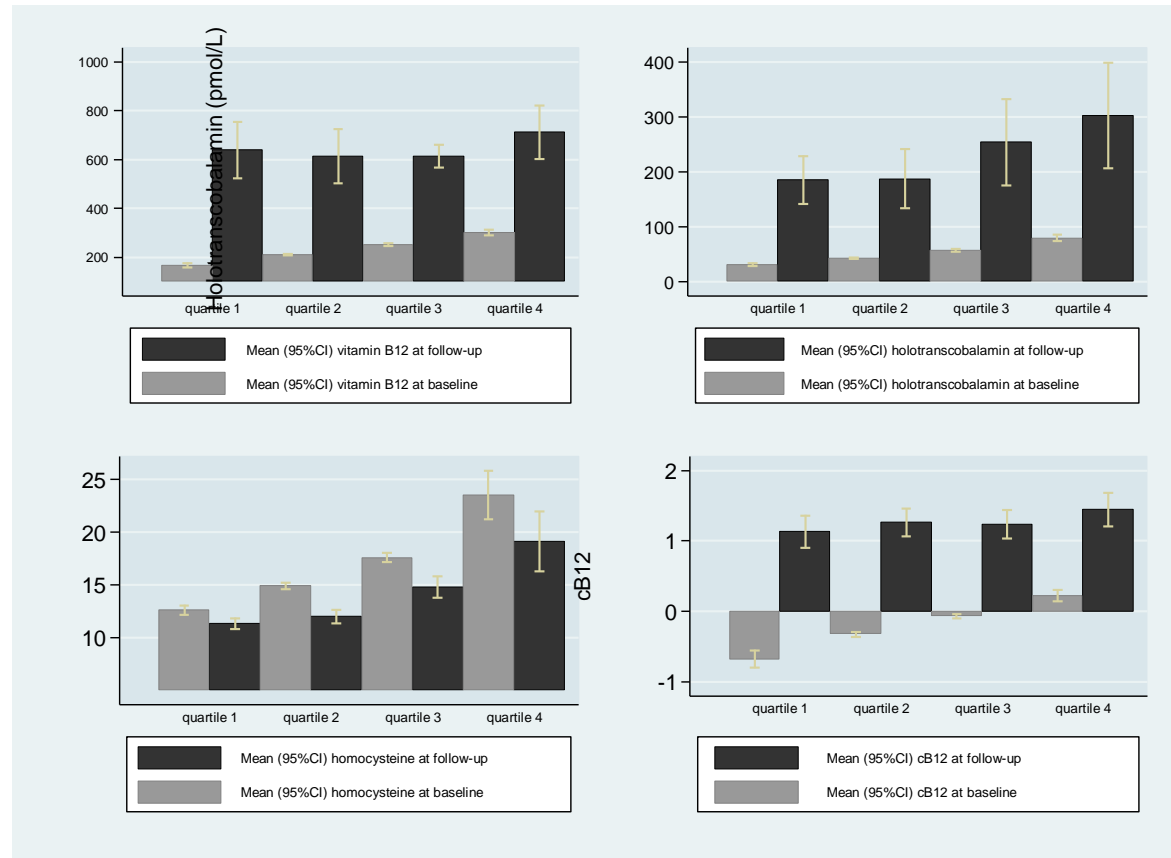
<sup>8</sup>Twenty-five subjects with 0 values for superficial peroneal SAP at baseline or follow-up excluded; 10 of these had detectable superficial peroneal SAP amplitude at baseline and no detectable (0) superficial peroneal SAP amplitude at follow-up; and 9 of these had undetectable (0) superficial peroneal SAP amplitude at baseline and detectable superficial peroneal SAP amplitude at follow-up.

<sup>9</sup>Twenty-three subjects with 0 values for superficial peroneal SAP at baseline or follow-up excluded; 9 of these had detectable superficial peroneal SAP amplitude at baseline and no detectable (0) superficial peroneal SAP amplitude at follow-up; and 8 of these had undetectable (0) superficial peroneal SAP amplitude at baseline and detectable superficial peroneal SAP amplitude at follow-up.

<sup>10</sup>Twenty-four subjects with 0 values for superficial peroneal SAP at baseline or follow-up excluded; 9 of these had detectable superficial peroneal SAP amplitude at baseline and no detectable (0) superficial peroneal SAP amplitude at follow-up; and 9 of these had undetectable (0) superficial peroneal SAP amplitude at baseline and detectable superficial peroneal SAP amplitude at follow-up.

<sup>11</sup>Adjusted for baseline measure of neurologic outcome, baseline B12/holoTC/tHcy/cB12 status, baseline age, baseline sex and change in skin temperature (hand).

**Figure 1: Vitamin B12 status by quartile of serum vitamin B12<sup>1</sup>, holotranscobalamin<sup>2</sup>, homocysteine<sup>3</sup> and a composite indicator (cB12) in the study population (n=91) of older people in the intervention arm of the OPEN study at baseline (0 months) and follow-up (12 months)**



<sup>1</sup>Microbiologic assay

<sup>2</sup>Axis-Shield radioimmunoassay

<sup>3</sup>Abbott IMx analyzer; Abbott Laboratories