**Vitamin B-12 status and neurologic function in older people: a cross-sectional analysis of baseline trial data from the OPEN study**

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**Running head** Vitamin B-12 status and neurologic function

**Trial registration** www.isrctn.comISRCTN54195799

**Abbreviations**

ADM: Abductor digiti minimi

AD: Abductor hallucis

CMAP: Compound muscle action potential

CMCT: Central motor conduction time

HoloTC: Holotranscobalamin

Hcy: Homocysteine

MEP: Motor evoked potentials

MMA: Methyl malonic acid

OPEN: Older People and Enhanced Neurological Function study

SAP: Sensory action potentials

**Abstract**

**Background**

Ageing 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 neurological disease of moderately low vitamin B-12 status in older people is unclear. Evidence from observational studies is limited by heterogeneity in definition of vitamin B-12 status and imprecise measures of nerve function.

**Objective**

This study aims to determine whether vitamin B-12 status is associated with electrophysiological indices of peripheral or central neurologic function in asymptomatic older people with moderately low vitamin B-12 status.

**Design**

We report cross-sectional analysis of baseline data from the Older People and Enhanced Neurological Function study conducted in the South East of England. This trial investigated the effectiveness of vitamin B-12 supplementation on electrophysiological indices of neurological function in asymptomatic older people (mean age 80y) with moderately low vitamin B-12 status (serum vitamin B-12 concentrations ≥107 and <210 pmol/L without anaemia, n=201). Vitamin B-12 status was assessed using total vitamin B-12, holotranscobalamin and a composite marker, cB-12. Electrophysiological 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 neurological 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 generalisable to a less healthy older population with more severe vitamin B-12 deficiency.

**Keywords**

Neurologic, older people, vitamin B-12, peripheral and central nerve conduction.

**Introduction**

Ageing is associated with a decline in vitamin B-12 status, and vitamin B-12 deficiency is relatively common in older people ([1-3](#_ENREF_1)). In the UK, 5% of adults aged 65–74 years and 10% adults aged ≥75 years have low vitamin B-12 levels (defined as vitamin B-12 <150 pmol/L) or metabolically significant vitamin B-12 deficiency (defined as vitamin B-12 <200 pmol/L and homocysteine level >20 mmol/L) ([1](#_ENREF_1)). As intakes of vitamin B-12 are mostly adequate ([4](#_ENREF_4)), poor status in older people is largely attributable to age-related malabsorption of vitamin B-12 ([5](#_ENREF_5)).

Vitamin B-12 is required for the initiation and maintenance of myelination of the nervous system ([6](#_ENREF_6)). The classical 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](#_ENREF_6), [7](#_ENREF_7)). Neurologic disturbances associated with B-12 deficiency can affect peripheral motor and sensory systems and include ataxia, gait disturbance, symmetric paresthesias, numbness, impaired vibration or position sensation, abnormal balance, reflexes and weakness ([3](#_ENREF_3), [6](#_ENREF_6), [7](#_ENREF_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. Neurological signs and symptoms associated with moderately low vitamin B-12 status can be non-specific and are often undetected because they are attributed to 'old age’, yet can have an important impact on physical function. A recent systematic review ([8](#_ENREF_8)) evaluated the association of vitamin B-12 status with neurological function and clinically relevant neurological outcomes in older people. Evidence from observational studies was limited and the heterogeneity and quality of the available studies precluded definitive conclusions. Few studies used electrophysiological measures of nerve conduction, which are the most sensitive and objective measure of neurological function relevant to vitamin B-12 status. Many studies were constrained by bias; and few reported composite measures of vitamin B-12 status that include both a biomarker of circulating vitamin B-12 and a functional biomarker (methyl malonic acid or homocysteine), as now recommended ([9](#_ENREF_9)).

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

**Participants and Methods**

*Recruitment and procedures*

This study is a secondary analysis of cross-sectional baseline data from the OPEN study, the protocol of which has been published ([10](#_ENREF_10)) (www.isrctn.com; ISRCTN54195799). The OPEN study was a randomised double-blind placebo-controlled trial that reported no benefits of dietary supplementation with oral vitamin B-12 for 12 months on electrophysiological indices of neurological function in older people with moderate B-12 deficiency ([11](#_ENREF_11)).

Participants aged ≥75 years were recruited from 7 general practices in South East England. Individuals with diabetes, dementia, epilepsy, alcohol addiction, pacemakers or other implanted metallic devices, residents of nursing homes, or a previous diagnosis of pernicious anaemia 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 months were also excluded, as were potential participants with significant cognitive impairment. Individuals with moderate vitamin B-12 deficiency who did not have anaemia (serum vitamin B-12 concentrations ≥107 and <210 pmol/L [Beckman Coulter assay] and haemoglobin 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 the OPEN study were used in this secondary analysis (the participant flowchart is available in the online supplemental material). 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 neurophysiological 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. Height and weight were measured to allow calculation of Body Mass Index. Blood samples were analysed for serum concentrations of vitamin B-12 (microbiologic assay; CV range: 5-7%); holotranscobalamin (HoloTC; Axis-Shield radioimmunoassay; CV range: 5-8%; Axis-Shield plc), total homocysteine (Hcy; Abbott IMx analyzer; CV range: 2-3%; Abbott Laboratories), and folate (chloramphenicol-resistant microbiologic assay; CV range: 5-8%) in a single laboratory in Trinity College Dublin. The Beckman Coulter method ([12](#_ENREF_12)) was used to assess vitamin B-12 status to screen participants for study eligibility. A microbiological assay was used at study baseline to assess the vitamin B-12 status of study participants. A full blood count was analysed for haematological markers including haemoglobin, haematocrit and mean corpuscular volume.

A single expert physician (KM) 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. As nerve conduction in peripheral nerves is sensitive to temperature of the limbs ([13](#_ENREF_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 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 fibres 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](#_ENREF_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 centred over the vertex to excite the hand area of the left motor cortex. A standard technique ([15](#_ENREF_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, by using a double cone coil, the leg area of 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 was performed on any participant. Any participants shown to have significant neurologic deficit 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: four SAP amplitudes in the sural, superficial peroneal, median and ulnar nerves as an index of nerve fibre number; four sensory conduction velocities in the sural, superficial peroneal, median and ulnar nerves to indicate degree of myelination; four distal CMAP amplitudes in the tibial, common peroneal, median and ulnar nerves which reflects the number of motor axons accessed by an electrical stimulus, which in turn reflects muscle strength ([16](#_ENREF_16), [17](#_ENREF_17)); and four motor conduction velocities in the tibial, common peroneal, median and ulnar nerves to indicate degree of myelination. Reduced sensory or motor conduction velocity is a sign of demyelination ([18](#_ENREF_18)). The remaining three outcomes assessed central nerve conduction: mean right ADM MEP amplitude and central motor conduction time to the right AH and ADM (the latter two were grouped in the analyses). The single physician (KM) also assessed four clinical measures of neurologic function at baseline: 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 combined use of at least one biomarker of circulating vitamin B-12 (serum vitamin B-12 or holoTC) together with one functional biomarker [methyl malonic acid (MMA) or Hcy] ([9](#_ENREF_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 one indicator ([19](#_ENREF_19)). cB-12 can be derived using equations that allow for an incomplete set of indicators, i.e. based on two or three of these markers ([19](#_ENREF_19)). In the present study, three 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 (08/H0305/18) and the London School of Hygiene and Tropical Medicine Ethics Committee (no. 5298). The secondary analyses presented here were approved by the London School of Hygiene & Tropical Medicine Ethics Committee (no. 7176).

*Statistical analysis*

All statistical analyses were conducted using STATA (version 14, StataCorp, Texas USA). Descriptive statistics for all exposures, outcomes and known or potential confounders have been generated. Scatter plots were used to visually explore the nature of any potential associations present. The functional form of any potential relationships was also explored by producing lowess smoother curves ([20](#_ENREF_20)). Three measures of vitamin B-12 status and two nerve conduction outcomes had >10% missing data, but preliminary analysis suggested that there was no reason to assume that these were not missing at random, so all analyses were on 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](#_ENREF_21)). Nerve conduction outcomes were grouped (as defined above) according to the component of nerve function they reflect, in order to minimise multiple testing of several outcomes. All multivariate models were boot-strapped to allow for non-normal distributions and results were presented as appropriate effect sizes with bias corrected 95% confidence intervals. Clinical marker outcomes were analysed separately using logistic regression; results were presented as odds ratios with 95% confidence intervals. Because the analyses involved multiple comparisons, p-values have been 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 relationship between vitamin B-12 status and neurological function and so have been adjusted for in analyses. In addition, the following variables: alcohol frequency (daily, >once a week, approximately once a fortnight, rarely/never); haemoglobin (g/l); haematocrit (%); mean corpuscular volume (fl); and use of statins (yes/no) or proton-pump inhibitors (yes/no) 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 here combine outcomes in upper and lower limbs and so inclusion of 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 due to concerns over collinearity.

Sensitivity and subgroup analyses were conducted to test the robustness of the findings. Sensitivity analyses were performed excluding participants with clinical (previously decompressed nerves) or neurophysiological evidence [a median nerve sensory conduction velocity <40 m/s and (an ulnar sensory conduction velocity at least 10 m/s faster and/or a median distal motor latency of >4.5 m/s)] of carpal tunnel syndrome as this syndrome is known to affect median sensory and motor nerve conduction parameters. Subgroup analyses were done to explore whether any association between vitamin B-12 and neurologic function differs by age or folate status, by testing for interaction 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 years and 47% of the population were male (**Table 1)**. At study baseline, 88% of recruited participants had vitamin B-12 status below the median value (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) (personal communication Dr Anne Molloy, 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. OPEN participants display sural nerve SAP amplitudes in line [3.1µV ±1.2([22](#_ENREF_22))] with available reference ranges for sural nerve SAP amplitudes for older people. Further, the clinical markers of neurologic function show that neurologic function was sub-optimal amongst participants. In particular, 66% of participants had absent right great toe vibration sense and 28% 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 direction of effects were 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.

Sensitivity analysis, excluding 31 participants with carpal tunnel syndrome, did not alter these conclusions (**Supplemental 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 electrophysiological 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 consistent across all neurological outcomes. There was also no evidence of an interaction between folate and vitamin B-12 status, for any electrophysiological measure of nerve function.

*Comparison with other studies*

Few other studies have assessed neurologic function using nerve conduction tests. In a longitudinal study ([23](#_ENREF_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 two cross-sectional studies were mixed. Vitamin B-12 deficient individuals in one study had lower CMAP and nerve conduction velocity (measured between popliteal fossa and ankle) ([24](#_ENREF_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](#_ENREF_25)). Nevertheless, a recent pre- and post-treatment study in asymptomatic older adults with serum vitamin B-12 <120 pmol/l 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 months after a single dose of intramuscular treatment of 10 mg vitamin B-12, 100mg pyridoxine and 100 mg thiamine ([26](#_ENREF_26)). Heterogeneity in assays and cut-offs used to assess vitamin B-12 status ([27](#_ENREF_27)) constrains fair comparison between similar studies. The present study was the first to assess vitamin B-12 status as measured by cB-12 and its relationship with neurologic function, and offers a high quality dataset in which to investigate the relationship between vitamin B-12 status and neurologic function. The results presented here are consistent with a conclusion of no association of moderately low vitamin B-12 status with nerve function.

*Strengths and weaknesses*

A strength of the study was the use of several measures of vitamin B-12 status. Of particular note, holoTC (which measures the active fraction of B-12) has been proposed as appropriate to use in the subclinical situation ([28](#_ENREF_28), [29](#_ENREF_29)). Further, 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 B-12 status (Hcy in this case). However, cB-12 is more reliable when based on four markers, which was not possible in the current study because MMA was not measured. When using three markers, having MMA missing is less reliable than having any of the other three markers missing ([19](#_ENREF_19)). Furthermore, renal function, which is known to affect Hcy ([30](#_ENREF_30)) , was not measured in OPEN. In fact, cB-12 has recently been reported to be independently associated with renal function ([31](#_ENREF_31)). There is uncertainty about the most appropriate measures or cut-offs to assess vitamin B-12 status. It has been suggested that age and sex-specific reference cut-offs may be needed ([27](#_ENREF_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, while moderately deficient, were too replete in vitamin B-12 to be able to detect any associations between vitamin B-12 status and neurological 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 UK. Participants also had relatively high levels of educational achievement, suggesting that the sample was not fully representative of older people in the UK. The results of this study are unlikely to be generalisable to a less healthy older population with more severe vitamin B-12 deficiency.

An important strength of the current study was the use of nerve conduction tests to measure neurologic function. Nerve conduction tests provided objective measures of neurologic function by using state-of-the-art methods, and all testing was conducted by a single neurophysiologist which eliminated inter-observer variability. A wide range of neurologic outcomes were 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](#_ENREF_32)) and the available age-specific reference ranges suggest OPEN participants had little or only mild neurological impairment.

Risk of bias from confounding was minimised 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. At 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 neurological 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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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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**References**

1. Clarke R, Grimley Evans J, Schneede J, Nexo E, Bates C, Fletcher A, Prentice A, Johnston C, Ueland PM, Refsum H, et al. Vitamin B12 and folate deficiency in later life. Age Ageing 2004;33(1):34-41.

2. Finch S, Doyle W, Lowe C. The National Diet and Nutrition Survey: people aged 65 years and over. 1998;Vol 1: Report of the Diet and Nutrition Survey.

3. Baik HW, Russell RM. Vitamin B12 deficiency in the elderly. Annual review of nutrition 1999;19:357-77. doi: 10.1146/annurev.nutr.19.1.357.

4. Bates B, Lennox A, Prentice A, Bates C, Page P, Nicholson S, Swan G. National Diet and Nutrition Survey. Headline results from Years 1, 2, 3 and 4 (combined) of the Rolling Programme (2008/2009 – 2011/12). 2014.

5. Institute of Medicine. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Washington DC: National Academy of Sciences., 1998.

6. Stabler SP. Vitamin B12 deficiency. N Engl J Med 2013;368(2):149-60.

7. Hunt A, Harrington D, Robinson S. Vitamin B12 deficiency. BMJ (Clinical research ed) 2014;349:g5226. doi: 10.1136/bmj.g5226.

8. Miles LM, Mills K, Clarke R, Dangour AD. Is there an association of vitamin B12 status with neurological function in older people? A systematic review. The British journal of nutrition 2015;114(4):503-8. doi: 10.1017/s0007114515002226.

9. Yetley EA, Pfeiffer CM, Phinney KW, Bailey RL, Blackmore S, Bock JL, Brody LC, Carmel R, Curtin LR, Durazo-Arvizu RA, et al. Biomarkers of vitamin B-12 status in NHANES: a roundtable summary. Am J Clin Nutr 2011;94(1):313S-21S. doi: 10.3945/ajcn.111.013243.

10. Dangour AD, Allen E, Clarke R, Elbourne D, Fasey N, Fletcher AE, Letley L, Richards M, Whyte K, Mills K, et al. A randomised controlled trial investigating the effect of vitamin B12 supplementation on neurological function in healthy older people: the Older People and Enhanced Neurological function (OPEN) study protocol [ISRCTN54195799]. Nutr J 2011;10:22. doi: <http://dx.doi.org/10.1186/1475-2891-10-22>.

11. Dangour AD, Allen E, Clarke R, Elbourne D, Fletcher AE, Letley L, Richards M, Whyte K, Uauy R, Mills K. Effects of vitamin B-12 supplementation on neurologic and cognitive function in older people: a randomized controlled trial. Am J Clin Nutr 2015;102(3):639-47. doi: 10.3945/ajcn.115.110775.

12. Akbas N, Schryver PG, Algeciras-Schimnich A, Baumann NA, Block DR, Budd JR, Gaston SJ, Klee GG. Evaluation of Beckman Coulter DxI 800 immunoassay system using clinically oriented performance goals. Clin Biochem 2014;47(16-17):158-63. doi: 10.1016/j.clinbiochem.2014.08.005.

13. Holmes O. Human neurophysiology : a student text. London; New York: Chapman & Hall Medical, 1993.

14. Mills KR. Magnetic stimulation of the human nervous system. Oxford; New York: Oxford University Press, 1999.

15. Mills KR, Nithi KA. Corticomotor threshold to magnetic stimulation: normal values and repeatability. Muscle Nerve 1997;20(5):570-6.

16. Fine EJ, Soria E, Paroski MW, Petryk D, Thomasula L. The neurophysiological profile of vitamin B12 deficiency. Muscle and Nerve 1990;13(2):158-64.

17. Watanabe T, Kaji R, Oka N, Bara W, Kimura J. Ultra-high dose methylcobalamin promotes nerve regeneration in experimental acrylamide neuropathy. J Neurol Sci 1994;122(2):140-3.

18. Carpenter R, Reddi B. Neurophysiology: a conceptual approach. 2012.

19. Fedosov SN, Brito A, Miller JW, Green R, Allen LH. Combined indicator of vitamin B12 status: modification for missing biomarkers and folate status and recommendations for revised cut-points. Clin Chem Lab Med 2015;53(8):1215-25. doi: 10.1515/cclm-2014-0818.

20. Cleveland WS. LOWESS: A program for smoothing scatterplots by robust locally weighted regression. The American Statistician, 1981;35(1):54.

21. Statacorp. Stata Multivariate statistics reference manual. Texas, USA: Stata Press, 2009.

22. Burke D, Skuse NF, Lethlean AK. Sensory conduction of the sural nerve in polyneuropathy. J Neurol Neurosurg Psychiatry 1974;37(6):647-52.

23. Leishear K, Ferrucci L, Lauretani F, Boudreau RM, Studenski SA, Rosano C, Abbate R, Gori AM, Corsi AM, Di Iorio A, et al. Vitamin B12 and homocysteine levels and 6-year change in peripheral nerve function and neurological signs. Journals of Gerontology Series A: Biological Sciences & Medical Sciences 2012;67(5):537-43.

24. Leishear K, Boudreau RM, Studenski SA, Ferrucci L, Rosano C, De Rekeneire N, Houston DK, Kritchevsky SB, Schwartz AV, Vinik AI, et al. Relationship between vitamin B12 and sensory and motor peripheral nerve function in older adults. J Am Geriatr Soc 2012;60(6):1057-63. doi: <http://dx.doi.org/10.1111/j.1532-5415.2012.03998.x>.

25. Sucharita S, Thomas T, Antony B, Vaz M. Vitamin B12 supplementation improves heart rate variability in healthy elderly Indian subjects. Autonomic Neuroscience: Basic and Clinical 2012;168(1-2):66-71. doi: <http://dx.doi.org/10.1016/j.autneu.2011.12.002>.

26. Brito A, Verdugo R, Hertrampf E, Miller JW, Green R, Fedosov SN, Shahab-Ferdows S, Sanchez H, Albala C, Castillo JL, et al. Vitamin B-12 treatment of asymptomatic, deficient, elderly Chileans improves conductivity in myelinated peripheral nerves, but high serum folate impairs vitamin B-12 status response assessed by the combined indicator of vitamin B-12 status. Am J Clin Nutr 2016;103(1):250-7. doi: 10.3945/ajcn.115.116509.

27. Aparicio-Ugarriza R, Palacios G, Alder M, Gonzalez-Gross M. A review of the cut-off points for the diagnosis of vitamin B12 deficiency in the general population. Clin Chem Lab Med 2015;53(8):1149-59. doi: 10.1515/cclm-2014-0784.

28. Green R. Indicators for assessing folate and vitamin B-12 status and for monitoring the efficacy of intervention strategies. Am J Clin Nutr 2011;94(2):666S-72S. doi: 10.3945/ajcn.110.009613.

29. Carmel R. Biomarkers of cobalamin (vitamin B-12) status in the epidemiologic setting: a critical overview of context, applications, and performance characteristics of cobalamin, methylmalonic acid, and holotranscobalamin II. Am J Clin Nutr 2011;94(1):348S-58S. doi: 10.3945/ajcn.111.013441.

30. Carmel R. Current concepts in cobalamin deficiency. Annual review of medicine 2000;51:357-75. doi: 10.1146/annurev.med.51.1.357.

31. Risch M, Meier DW, Sakem B, Medina Escobar P, Risch C, Nydegger U, Risch L. Vitamin B12 and folate levels in healthy Swiss senior citizens: a prospective study evaluating reference intervals and decision limits. BMC geriatrics 2015;15:82. doi: 10.1186/s12877-015-0060-x.

32. Liveson JA MD. Laboratory reference for clinical neurophysiology. New York: Oxford university Press, 1992.

**Tables**

**Table 1: Demographic characteristics and blood biochemical measures of study participants**

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| --- | --- | --- |
|  | Total n |  |
| Demographics |  |  |
| Age (years)1 | 201 | 80.0 (3.6) |
| Sex male, n (%) | 201 | 94 (47) |
| Age left education (years) 1 | 201 | 18.1 (6.0) |
| Educational achievement None, n (%) Basic or clerical, n (%) Advanced or university, n (%) Other, n (%) | 198 | 54 (27)34 (17)52 (26)58 (29) |
| Body mass index2 <18.5, n (%) 18.5 – 24.9, n (%) 25.0 – 29.9, n (%) ≥30, n (%) | 201 | 26.8 (24.0, 29.3)1 (0)69 (34)87 (43)44 (22) |
| Statins use, n (%) | 162 | 67 (41) |
| Proton-pump inhibitor use, n (%) | 162 | 53 (33) |
| Frequency of alcohol consumption Daily, n (%) >once/week, n (%) approx. once/fortnight, n (%) rarely/never, n (%) | 195 | 68 (35)63 (32)19 (10)45 (23) |
| Frequency of meat consumption >once/week, n (%) | 191 | 139 (73) |
| Blood biochemical measures |  |  |
| Vitamin B-12 (pmol/L)2,3  | 165 | 225.5 (196.0, 269.6) |
| Holotranscobalamin (pmol/L) 2 | 159 | 49.3 (38.8, 64.8) |
| Homocysteine (µmol/L) 2 | 162 | 16.2 (13.8, 19.5) |
| Folate (nmol/L) 2 | 164 | 17.6 (4.8, 25.4) |
| cB-121  | 159 | -0.2 (0.4) |
| Haematocrit (%)1 | 177 | 40.8 (3.1) |
| Haemoglobin (g/L) 1 | 177 | 139.3 (12.0) |
| Mean corpuscular volume (fL) 1 | 177 | 88.6 (4.3) |

1Mean (SD)

2Median (IQR)

3Vitamin B-12 status assessed using a microbiological assay.

**Table 2: Neurological function of study participants1**

|  |  |  |
| --- | --- | --- |
|  | Total n |  |
| Neurological function |  |  |
| SAP amplitudes (µV)2,3 Median  Ulnar Sural Superficial peroneal | 200200199199 | 8.2 (5.6, 11.9)6.6 (4.1, 9.1)3.8 (1.6, 6.3)2.9 (1.1, 5.4) |
| Sensory nerve conduction velocities (m/s)4 Median  Ulnar Sural Superficial peroneal | 194192172159 | 45.1 (5.5)44.7 (4.6)40.4 (5.3)41.1 (5.6) |
| CMAP amplitudes (mV)2 Median  Ulnar Tibial Common peroneal | 200200199199 | 3.8 (2.7, 5.0)9.7 (8.5, 11.2)4.6 (2.1, 7.3)2.4 (1.1, 3.6) |
| Motor nerve conduction velocities (m/s)4 Median  Ulnar Tibial Common peroneal | 200200193189 | 51.3 (5.2)54.5 (5.2)40.0 (5.1)42.8 (4.3) |
| Central motor conduction Left hemisphere ADM CMCT (ms)4 Left hemisphere AH CMCT (ms)4 Left hemisphere mean ADM MEP amplitude (mV)2 | 198182200 | 5.5 (1.3)13.6 (3.4)3.4 (2.1, 4.4) |
| Clinical markers |  |  |
| Absent right knee jerk, n (%) | 201 | 21 (10) |
| Absent right ankle jerk, n (%) | 201 | 57 (28) |
| Absent right great toe position sense, n (%) | 201 | 14 (7) |
| Absent right great toe vibration sense, n (%) | 201 | 132 (66) |
|  |  |  |

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

2Median (IQR)

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

4Mean (SD)

**Table 3: Association between vitamin B-12 status and nerve conduction outcomes1.**

|  |  |
| --- | --- |
|  |  |
|  | Adjusted2 coefficients(95% CI) | B-12 (pmol/l) | HoloTC (pmol/l) | cB-12 |
| Sensory SAP amplitudes (µV)3 | n | 164 | 158 | 158 |
|  | Median  | -0.01 (-0.02, 0.00) | -0.02 (-0.05, 0.02)4 | -1.05 (-3.19, 1.06) 4 |
|  |  |  |  |  |
|  | Ulnar | -0.01 (-0.02, -0.00) | -0.01 (-0.04, 0.02)4 | -0.72 (-2.10, 0.52) 4 |
|  |  |  |  |  |
|  | Sural | -0.00 (-0.01, 0.01) | -0.01 (-0.04, 0.02) 4 | -0.17 (-1.72, 1.42) 4 |
|  | Superficial peroneal | 0.00 (-0.01, 0.01) | -0.01 (-0.03, 0.02) 4 | 0.26 (-1.11, 1.45) 4 |
|  |  | p=0.12 | p=0.874 | p=0.604 |
| 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=0.28 | p=0.12 | p=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=0.02 | p=0.49 | p=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=0.80 | p=0.66 | p=0.86 |
| Central motor conduction (ms) | n | 147 | 142 | 142 |
|  | ADM CMCT | 0.00 (-0.00, 0.01) | -0.00 (-0.01, 0.01) | 0.20 (-0.29, 0.67) |
|  | AH CMCT | 0.00 (-0.01, 0.01) | -0.00 (-0.03, 0.03) | -0.09 (-1.90, 1.80) |
|  |  | p=0.41 | p=0.72 | p=0.66 |
|  | n | 164 | 158 | 158 |
|  | Mean ADM MEP amplitude (mV) | -0.00 (-0.00, 0.00) 5 | -0.00 (-0.01, 0.01) 5 | 0.05 (-0.53, 0.59) 5 |
|  |  | p=0.995 | p=0.925 | p=0.865 |

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

2Multivariate regression analyses adjusted for age, sex and skin temperature (foot), unless otherwise stated.

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

4Mean corpuscular volume confounded the relationship between holoTC and cB-12 with SAP amplitudes; these models have therefore been adjusted for age, sex, skin temperature (foot) and mean corpuscular volume.

5Adjusted for age, sex, skin temperature (hand)