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Vitamin B12 status and neurological function in older people

LISA M MILES

Thesis submitted in accordance with the requirements for the degree of

Doctor of Public Health of the University of London

MAY 2016

Department of Population Health

Faculty of Epidemiology and Population Health

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

No funding received

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LISA MILES

23 MAY 2016

Abstract

Ageing is associated with a decline in vitamin B12 status. Overt vitamin B12 deficiency can lead to neurologic disturbances but the public health impact for neurological disease of moderately low vitamin B12 status in older people is unclear. This study aimed to assess the relationship between vitamin B12 status and neurologic function in older people by systematically reviewing existing evidence and conducting secondary analyses on an existing high quality dataset.

A systematic review of observational studies showed limited evidence of an association of vitamin B12 status with neurological function in older people. The possibility of an association between vitamin B12 status and neurologic function was further explored in cross-sectional analyses of baseline data from the Older People and Enhanced Neurological Function (OPEN) study, which investigated the effectiveness of vitamin B12 supplementation on electrophysiological indices of neurological function in asymptomatic older people with moderately low vitamin B12 status. This secondary analysis did not show any association between any measure of vitamin B12 status with electrophysiological indices or clinical markers of neurologic function.

A systematic review of intervention studies suggested no benefits of vitamin B12 supplementation on neurologic function in asymptomatic older people; but it remained possible that improvement is only apparent in people with the lowest vitamin B12 status. This hypothesis was explored in further secondary analyses of OPEN data: there were no differences in the neurologic response to vitamin B12 supplementation according to baseline or change in vitamin B12 status.

The available evidence indicates that concerns over the neurologic impact of moderately low vitamin B12 status in otherwise healthy older people may be unwarranted. Evidence is insufficient to support population screening for moderate vitamin B12 deficiency or population-wide recommendations for vitamin B12 supplementation in healthy asymptomatic older people, even among those with the lowest vitamin B12 status.

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Abbreviations

2MCA	2-methylcitrate
AD	Abductor hallucis
ADM	Abductor digit minimi
BNF	British Nutrition Foundation
CMAP	Compound muscle action potential
CMCT	Central motor conduction time
CNS	Central nervous system
CSF	Cerebrospinal fluid
DM	Diabetes mellitus
DrPH	Doctorate in Public Health
EBPHP	Evidence-based public health policy
EEG	Electroencephalography
EGF	Epidermal growth factor
GABA	Gamma-aminobutyric acid
Hcy/hcy	Homocysteine
HoloTC/holoTC	Holotranscobalamin
IL-6	Interleukin-6
LMPD	Leadership, management and personal development
LRNI	Lower reference nutrient intake
LSHTM	London School of Hygiene and Tropical Medicine
MCM	L-methylmalonyl CoA mutase
MEP	Motor evoked potential
MeS	Methionine synthase
MMA	Methyl malonic acid
MMSE	Mini-mental state examination
MRC	Medical Research Council
NCV	Nerve conduction velocity
NDNS	National Diet and Nutrition Survey
NF	Electrophysiological measure of nerve function

NHANES	National Health and Nutrition Examination Survey
NMDA	N-methyl-D-aspartate
NP	Clinically assessed signs of neuropathy
nRCT	Non-randomised controlled trial
NS	Self-report neurological symptoms.
OHAP	Oxford Healthy Aging Project
OPA	Organisational policy analysis
OPEN	Older people and enhanced neurological function
PN	Peripheral neuropathy
PNS	Peripheral nervous system
RCT	Randomised controlled trial
RDA	Recommended daily allowance
RNI	Reference nutrient intake
ROC	Receiver operating characteristic
SAM	S-adenosylmethionine
SAP	Sensory action potential
SCD	Subacute combined degeneration of the cord
SIGN	Scottish Intercollegiate Guidelines Network
SNAP	Sensory nerve action potential
TC	Transcobalamin
TMS	Transcranial magnetic stimulation
TNF	Tumour necrosis factor
WHO	World Health Organization

Acknowledgements

In particular, I would like to thank my supervisor, Dr Alan Dangour (London School of Hygiene and Tropical Medicine) for his academic guidance throughout my DrPH. Because of his support, I have learned an incredible amount throughout this process and gained research skills that I hope to put into practice in my future career.

I am also grateful for academic guidance on my research thesis from my Advisory Committee members listed below:

Dr Elizabeth Allen, London School of Hygiene and Tropical Medicine

Dr Robert Clarke, University of Oxford

Prof Kerry Mills, King's College London

I appreciate the work conducted by all investigators from the original OPEN study, as well as the OPEN study participants, without which large components of my research would not have been possible. I also acknowledge the Food Standards Agency (N05072) and the Department of Health as funders of the original OPEN study.

Finally, I would like to offer special thanks to my husband, my mother and my late father who have all given me tremendous support and encouragement in countless ways throughout this journey.



Lisa Miles

DrPH Integration statement

I decided to pursue a Doctorate in Public Health (DrPH) because I wanted to gain experience that would help me deal with the application of research to achieve public health gains, and develop skills to prepare me for management and leadership positions. Having already worked in the field of public health nutrition for several years, I decided to progress the DrPH on a part-time basis alongside working in several roles across the governmental and non-governmental health sector.

In 2009-2010 I completed the compulsory taught component of the DrPH, namely modules in 'Leadership, Management and Personal Development (LMPD)' and 'Evidence-based Public Health Policy (EBPHP)'. LMPD introduced organisational management and leadership concepts and facilitated personal development of individual leadership and management skills. EBPHP explored the relationship between research and policy making and focused on key skills required for shaping policy and practice, involving accessing, understanding, developing, disseminating and facilitating the use of the evidence base for better public health outcomes. Whilst completing the taught component of the course, I was also working as a Senior Nutrition Scientist at the British Nutrition Foundation (BNF) and then subsequently as a nutritionist at the Department of Health, focusing on maternal and infant nutrition policy. I was able to draw on many aspects of the taught course in my daily work, particularly around leadership and management at BNF as I was responsible for managing and training junior colleagues, and around use of evidence in policy making at the Department of Health, where a large part of my role was working as a member of the Secretariat for the UK's Scientific Advisory Committee on Nutrition. This committee advises government agencies and departments on nutrition and related health issues.

My knowledge of the process of policy making as well as what makes good management and leadership certainly expanded through the taught component of the DrPH, and I was able to put these new skills to use in the second component of the DrPH, the Organisational Policy Analysis (OPA). I conducted my OPA at small charity called Coeliac

UK, dedicated to the support of people with coeliac disease. I was employed full-time at the charity as the Deputy Head of Diet and Health on a fixed term contract, and conducted my OPA alongside this role. Careful observation of the organisation allowed me to prepare an OPA project protocol and ethics application (from September to December 2011), which was in line with my interests but also of value to Coeliac UK as my host organisation.

My OPA was an analysis of the use of research by Coeliac UK, with a particular focus on the research it funds. In conducting the OPA, I was able to draw on skills learned from both compulsory modules and build on these further. The OPA critically assessed: 1) how the organisation uses research, which was relevant to the relationship between research and policy making covered in EBPHP; this was extended further to an analysis of research funding priorities; and 2) how the structure of the organisation affects the charity's abilities to meet its strategic aims on research, which drew on organisation and management theories from LMPD. In doing this I was able to gain experience in applying policy science and organisational management theories to the critical analysis of a real world organisational case study.

I collected data for my OPA from January to July 2012, namely through observation, documentation review and conducting semi-structured interviews. I therefore gained valuable skills in qualitative data collection and synthesis. Through these activities I was able to develop recommendations to increase the effectiveness of the organisation in terms of funding of and using scientific evidence in order to deliver its strategic aims. In particular, the findings of my OPA were subsequently used to inform Coeliac UK's new research strategy (see Appendices).

I left Coeliac UK at the end of 2012 to pursue the research component of my DrPH alongside working part-time for Cancer Research UK. For the final part of the DrPH I returned to my background of public health nutrition and was able to take up the opportunity of working on an existing dataset at LSHTM from the Older People and Enhanced Neurological Function (OPEN) study to develop my research questions. The OPEN study is an individually randomised double-blind placebo-controlled trial which

aimed to assess whether dietary supplementation with vitamin B12 will improve electrophysiological indices of neurological function in older people.

Frank vitamin B12 deficiency can typically present with neurological disturbances, but the functional significance of 'subclinical' low B12 status in older people is not clear, particularly in terms of neurological manifestations. This is of public health concern because low vitamin B12 status is relatively common in old age. I was keen to use the opportunity to conduct original research in an area of public health nutrition that I had no specific previous experience of, so I could gain new knowledge. Vitamin B12 status and its relationship with neurological function in older people is an interesting area of public health nutrition that I had never studied before and is explored in my research thesis presented here.

Firstly, it was important to review existing evidence on vitamin B12 status and neurological function. During 2013, I conducted two systematic reviews: 1) of observational studies and 2) of vitamin B12 intervention studies, to assess evidence of an association between vitamin B12 status and neurological function, and effectiveness of vitamin B12 interventions on neurological function respectively. The first of these is published in the British Journal of Nutrition (Chapter 2) and the second report is presented in Chapter 4. My skills in systematic review have therefore been further built upon, and this again draws on methods taught in the EBPHP module of the course. I also presented the results of the systematic review of observational studies at the 2014 LSHTM student poster day.

I presented my literature review findings and research protocol for further analyses using OPEN data, at my DrPH review in early 2014. As a high quality existing dataset, the OPEN study provides evidence that addresses many inadequacies of the existing studies on vitamin B12 and neurological function. The primary trial results for the OPEN study have been published by others, and I was able to develop two research questions to further explore the relationship between vitamin B12 status and neurological function in older people in secondary analyses: 1) to assess whether any cross-sectional associations exist between vitamin B12 and neurological function in the baseline dataset

and 2) to conduct a longitudinal analysis of the trial data to investigate whether the effectiveness of the intervention alters according to baseline or change in B12 status.

In preparation for the work on statistical analysis, I attended three MSc modules on statistics at the School: Statistics for epidemiology and population health (2013); Statistical methods for epidemiology (2014); and Advanced statistical methods for epidemiology (2015). Each of these helped to refresh and develop my understanding of statistical concepts and methods. I started work in earnest on statistical analyses of the OPEN dataset in late Spring 2015, following a year of maternity leave.

By progressing secondary analyses on an existing dataset, I have been able to hone my skills in data management and detailed statistical analysis. I overcame many challenges associated with working on existing data generated from several sources and in multiple files and climbed a learning curve in terms of statistical analysis. I am now competent in using STATA statistical software and have performed several exploratory and regression analyses. Furthermore, I have further developed my understanding of epidemiological concepts and now have experience of writing up results from an original research study for publication. Further manuscripts prepared for publication are presented in Chapters 3 and 5.

Finally, the research component of the DrPH has enabled me to integrate and synthesise scientific evidence from the existing evidence-base alongside new study findings, in order to present a cohesive discussion of the state of knowledge in my chosen area of public health nutrition. The act of 'pulling everything together' is a valuable exercise which I have found satisfying, and will no doubt be a useful experience when consulting scientific evidence to inform policy making in the future.

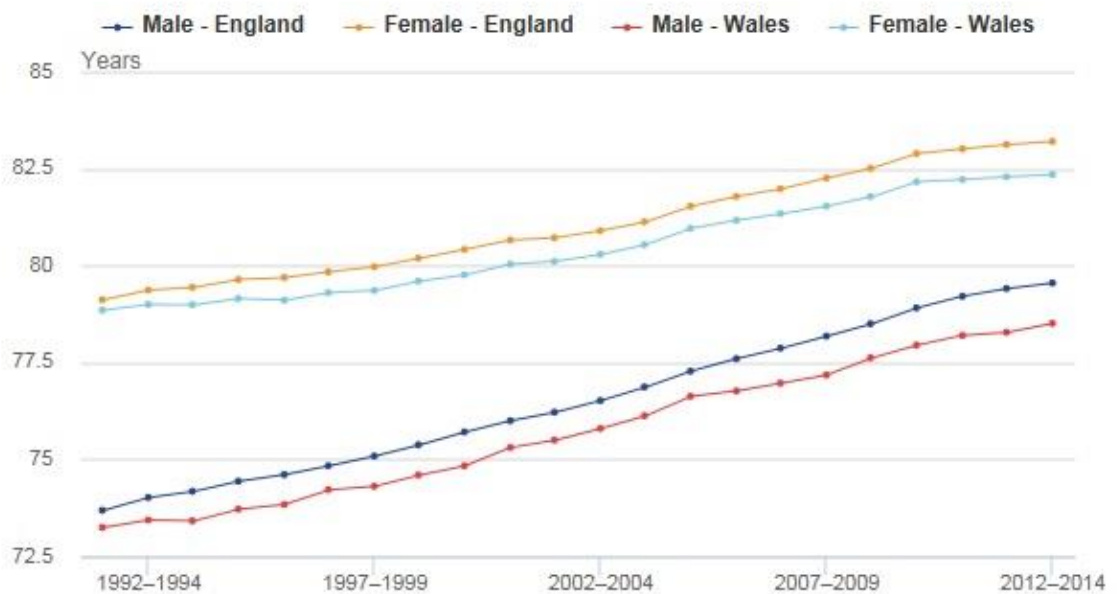
The DrPH programme at LSHTM is an excellent format of study designed to train future leaders in public health. It is a challenging programme with many different components, but certainly rewarding. I feel I have further developed many skills to complement my experience gained in the work place and gained a wealth of knowledge that I hope to put into practice in my future career.

Chapter 1: Background

1.1 Introduction

The number and proportion of older people in populations around the world is increasing, and the pace of increase is remarkable. If trends continue, most babies born since 2000 in several Western countries, including the UK, will celebrate their 100th birthdays¹. Figure 1 shows the rapid increase in life expectancy at birth seen in England and Wales. These changes are driven largely by increasing survival in older age². Over the last 30 years there has been an upward trend in life expectancy at older ages in England³. By 2025 about one-third of Europe's population will be aged 60 years and over, and there will be a particularly rapid increase in the number of people aged 80 years and older⁴.

Figure 1: Life expectancy at birth in England and Wales from 1991-93 to 2012-14⁵.



Source: Office for National Statistics

Ageing populations offer substantial opportunities: older people contribute to society in many ways, for example through informal care of family members and as an experienced workforce that can benefit local communities or society more generally. Yet, making the

most of older people in society is enhanced if their extra years are spent in good health, and this presents a public health challenge. Healthy ageing is defined by the World Health Organization (WHO) as the process of developing and maintaining the functional ability that enables well-being in older age. Functional ability comprises the health-related attributes that enable people to be and to do what they have reason to value. Maintaining functional ability in older age reduces the need for dependence on carers and health services, and the costs associated with them. Maintaining independence in older age can also improve quality of life as well as economic and social productivity, and importantly reduce frequency of hospital admissions^{2, 4, 6}.

23% of the global burden of disease arises in older people. Age-related disorders increase in prevalence in parallel with the numbers of people aged over 60 years. Chronic non-communicable disease accounts for most of this burden including cardiovascular disease, cancer, chronic respiratory diseases, musculoskeletal diseases and mental and neurological disorders in rank order globally; although mental and neurological disorders make a greater contribution in high-income countries⁷. Together, these disorders contribute greatly to loss of functional ability in older age. Specifically, it has been estimated that neurologic and psychiatric disorders account for 28% of all years of life lived with a disability^{8, 9}.

Health in later life reflects the interaction of a wide range of factors: some intrinsic to the individual such as biological and genetic factors; and other factors related to interaction with the environment such as behavioural and social factors. Nutrition is one of several lifestyle factors that can affect healthy ageing and this has been subject to considerable research^{2, 6}. Although energy needs decrease with age, the demand for essential nutrients remains, highlighting the importance of a nutrient dense diet. However, the ageing process is associated with physiological changes that affect nutrition status (Box 1).

Box 1: Age-related physiologic changes that can affect nutrition^{2, 4, 10}

- Changes to senses of taste and smell
- Poor oral/dental health and reduced saliva production
- Side effects of medication eg constipation, reduced appetite
- Gastric atrophy and reduced gastric secretions
- Impaired vision, hearing and/or mobility affecting ability to shop and prepare food
- Chronic disease including dementia and depression

National survey data shows that older people living in the UK, particularly in institutions, have low intakes of a range of vitamins and minerals. Adult aged 65 years and over living in institutions are amongst specific population groups identified as most at risk of poor dietary variety, low nutrient intake and biochemical status¹¹. Men and women aged 65 years and over have biochemical evidence of low vitamin D and iron status for example^{11, 12}.

Vitamin B12 is a nutrient whose status is affected by physiologic changes seen with ageing. A decline in vitamin B12 status is associated with ageing and this is largely due to impaired absorption rather than reduced intakes (see Sections 1.4 and 1.8). There are several age-related contributory factors including a decrease in gastric acidity, presence of atrophic gastritis, compromised functional and structural integrity of the vitamin B12 binding proteins and lack of liver vitamin B12 stores¹³.

Vitamin B12 deficiency is one of many known causes of peripheral neuropathy (others include diabetes, alcohol misuse, renal or liver disease, HIV and some malignancies¹⁴) but up to a third of patients are without a known cause (cryptogenic sensory peripheral neuropathy)¹⁵. Estimates indicate that 8% of people aged over 55 years have peripheral neuropathy and this figure rises with increasing age^{16, 17}. Clinical recognition of neurological impairment in older people is challenging. It is easy to miss neurological impairments because they are often assumed to be part of the natural ageing process or a result of 'wear and tear'. However, these assumptions cause potential opportunities

for prevention or treatment to be missed. Aging is not an invariable and sufficient cause of peripheral neuropathy but could be the result of preventable disease^{18, 19}.

This thesis focuses on the relationship between vitamin B12 and the nervous system in older people. Though vitamin B12 also has a key role in blood cell formation, adequate vitamin B12 is also important for neurologic function (Section 1.5.2). This thesis has a specific focus on central and peripheral nerve function as existing research on these outcomes is limited. Cognitive function has been researched and reviewed extensively elsewhere²⁰⁻²³.

1.1.1 Aims and research questions

This study aims to assess the relationship between vitamin B12 status and neurologic function in older people. This is done firstly by systematically reviewing existing evidence, and secondly, by conducting secondary analyses using data from the OPEN Study. The present study aims to address the following two research questions in systematic reviews of the literature:

- 1a) Is there any evidence in the available literature that vitamin B12 status is associated with neurological function in older people?
- 1b) Is there any evidence in the available literature that vitamin B12 supplementation improves neurological function in older people?

The findings of the systematic reviews are then used to identify gaps in the evidence-base. Secondary analyses (using data from the OPEN study) are then conducted to address these evidence gaps. Based on the findings of the systematic reviews, the following research questions for the secondary analyses were developed:

- 2a) Is vitamin B12 status associated with electrophysiological indices of peripheral or central neurologic function in asymptomatic older people with moderately low vitamin B12 status?
- 2b) Does baseline vitamin B12 status or change in vitamin B12 status alter the effectiveness of dietary supplementation with vitamin B12 on

electrophysiological indices of neurologic function in asymptomatic older people with moderately low vitamin B12 status?

In order to provide further context to the study, the remainder of Chapter 1 provides background information relevant to the study of vitamin B12 and neurologic function, including data on vitamin B12 status, measurement of vitamin B12 status and neurologic function and the potential mechanisms by which vitamin B12 status affects neurologic function.

1.2 Vitamin B12 function

Vitamin B12, or cobalamin, is a water-soluble vitamin with a key role in blood cell formation and normal functioning of the brain and nervous system. Vitamin B12 functions as a cofactor for two coenzymes that are active in human metabolism: methionine synthase and L-methylmalonyl CoA mutase. Together with folate, vitamin B12 is required for methyl group transfer during protein metabolism, DNA synthesis and the methylation of DNA and other substrates. Specifically, methionine synthase requires methyl-cobalamin as for a cofactor for the methyl transfer from methyltetrahydrofolate to homocysteine (hcy) to form methionine and tetrahydrofolate. L-methylmalonyl CoA mutase requires adenosylcobalamin to convert L-methylmalonyl CoA to succinyl-CoA in an isomerisation reaction^{13, 24}.

1.3 Dietary vitamin B12

The original source of vitamin B12 in the diet is from microorganisms (bacteria and algae) that synthesise it. Vitamin B12 then enters the human food chain through incorporation into foods of animal origin. Therefore, vitamin B12 is found in the diet in all foods of animal origin and certain seaweed (Table 1)²⁵.

Diet-related vitamin B12 deficiency is usually restricted to vegans or strict vegetarians. Hydroxocobalamin and, in particular, cyanocobalamin are synthetic forms used in vitamin supplements and in the fortification of food²⁶. The UK reference nutrient intake (RNI) for vitamin B12 for adult men and women (including those aged 50+ years) is 1.5 µg/day, and the lower reference nutrient intake (LRNI) is 1.0 µg/day²⁷. The US

Recommended Daily Allowance (RDA) for vitamin B12 is 2.4 µg/day for adults, though adults aged >50 years are advised to meet their RDA mainly by consuming food fortified with vitamin B12 or a vitamin B12-containing supplement¹³. The European Food Safety Authority has recently proposed an Adequate Intake for cobalamin at 4 µg/day for adults based on data on different biomarkers of vitamin B12 status and in consideration of observed mean intakes across Europe, which range between 4.2 and 8.6 µg/day in adults²⁸. Vitamin B12 has low toxicity and no safe upper limit has been identified²⁶.

Table 1: Food sources of vitamin B12²⁹

Food	Amount of vitamin B12 per 100g edible portion (µg)
Semi-skimmed milk	0.4
Whole milk yoghurt (plain)	0.2
Egg (boiled)	1.1
Cheddar cheese	2.4
Beef topside (cooked)	3
Lamb leg (cooked)	2
Cod (cooked)	2

1.4 Absorption of vitamin B12

There are two pathways by which vitamin B12 is absorbed: a) via intrinsic factor and b) by passive diffusion. The intrinsic factor–mediated pathway requires vitamin B12 to be released from dietary protein in the stomach by the action of acid and pepsin. The vitamin B12 then binds to R proteins secreted by the salivary glands and gastric mucosa. R proteins are then degraded in the alkaline environment of the small intestine, releasing vitamin B12 to bind with intrinsic factor. The resulting complex of intrinsic factor-vitamin B12 is then taken up by receptors in the ileum and absorbed by phagocytosis. Absorption via intrinsic factor has a limited capacity of 3µg at one meal. If larger doses of vitamin B12 are consumed, absorption by passive diffusion can occur;

the rate of absorption is 1% of the ingested amount of vitamin B12^{13, 24}. Although the total amount of vitamin B12 absorption increases with increasing dose, the percentage absorption decreases²⁴. All circulating vitamin B12 is bound to plasma binding proteins, transcobalamin I, II or III. Vitamin B12 enters cells mainly in the form bound to TCII, known as holotranscobalamin (holoTC). The liver is the main storage site for vitamin B12 in the body. Body storage is relatively high at about 1-5mg so deficiency from diminished intake or absorption may not manifest for several years after the depletion of stores³⁰.

A decline in vitamin B12 status is associated with ageing^{24, 31} (Section 1.8) and this is largely attributed to absorption issues. There are several contributory factors associated with ageing including a decrease in gastric acidity, presence of atrophic gastritis, compromised functional and structural integrity of the vitamin B12 binding proteins and lack of liver vitamin B12 stores¹³. Lack of stomach acid (a physiological state called hypochlorhydria) is a major issue: there is an age-related reduction in the ability of parietal cells to secrete hydrochloric acid. It is thought that up to 25% of older people can have some degree of hypochlorhydria as a result of atrophic gastritis. It is proposed that the absence of acid in the stomach prevents the release of protein-bound vitamin B12 in food but does not have such an effect on free vitamin B12 found in supplements or fortified foods²⁵. Bacterial overgrowth associated with atrophic gastritis can also reduce absorption of vitamin B12.

1.5 Vitamin B12 deficiency

Dietary vitamin B12 deficiency is rare in healthy populations^{32, 33}. However, food-cobalamin malabsorption can account for up to 60-70% confirmed cases of vitamin B12 deficiency in older patients³³. Food-cobalamin malabsorption is most commonly caused by gastric atrophy, which is present in 40% of patients aged over 80 years, and is characterised by the inability to release cobalamin from food or intestinal transport proteins (Section 1.4).

A known cause of severe clinical vitamin B12 deficiency is pernicious anaemia, which is an autoimmune gastritis where autoantibodies cause loss of parietal cells in the

stomach. This progressive destruction leads to loss of intrinsic factor and so limits intrinsic factor–mediated vitamin B12 absorption.

The clinical manifestations of vitamin B12 deficiency can be haematologic, neurologic or both³². Neurological symptoms can occur in the absence of anaemia in 20-30% of cases³⁴; it has been suggested that the occurrence of neurological manifestations is inversely related to the degree of anaemia^{13, 24, 35}. Cognitive and gastrointestinal disturbances can also occur^{24, 32, 33}. Standard treatment of vitamin B12 deficiency in the UK is to begin parenteral treatment with intramuscular hydroxocobalamin. Initial treatment for patients without neurological involvement is 1000 µg intramuscularly three times a week for two weeks (or alternate days for up to three weeks in patients with neurological involvement).

1.5.1 Impact on haematology

Megaloblastic anaemia is the classical manifestation of vitamin B12 deficiency. Haematological investigations may show an increased mean cell volume (reflecting macrocytosis), low haemoglobin, hypersegmentation of neutrophils and presence of oval macrocytes^{30, 33, 36}. A patient affected by vitamin B12 deficiency may complain of fatigue and/or weakness as a result of the anaemia. The haematological effects of vitamin B12 deficiency are often indistinguishable from folate deficiency; the underlying mechanism for both deficiencies is interference with DNA synthesis. The haematological complications of vitamin B12 deficiency are reversible by treatment¹³.

1.5.2 Impact on the nervous system

The nervous system is organised into the central nervous system (CNS) and the peripheral nervous system (PNS) (Figure 2). At the cellular level, neurons allow electric signals to travel between the central and peripheral systems in both directions (Figure 3). The afferent neurons carry sensory signals from the periphery to the spinal cord then brain. The efferent neurons carry motor signals from the brain to the periphery.

Figure 2: Organisation of the central and peripheral nervous system (reproduced from Tortora, 1995, p439)³⁷

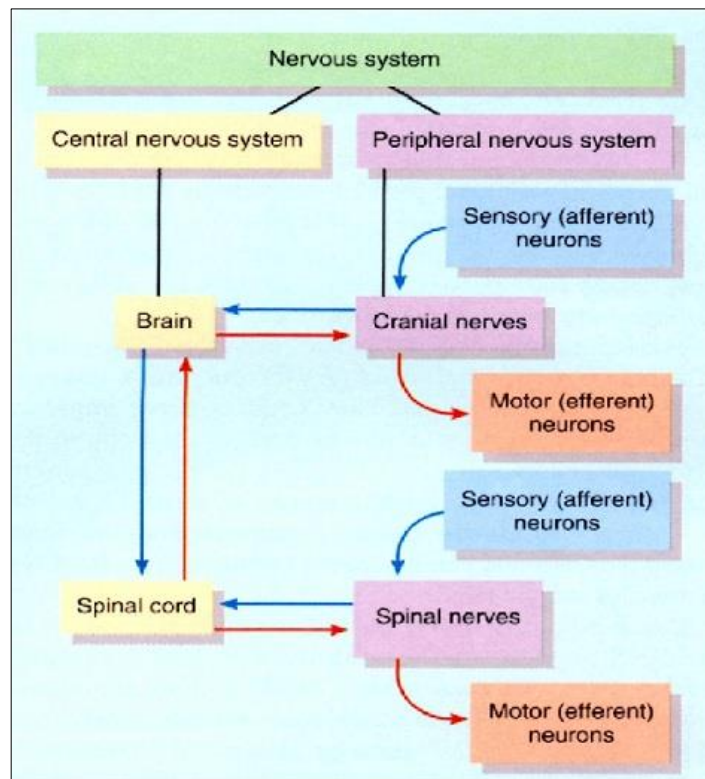
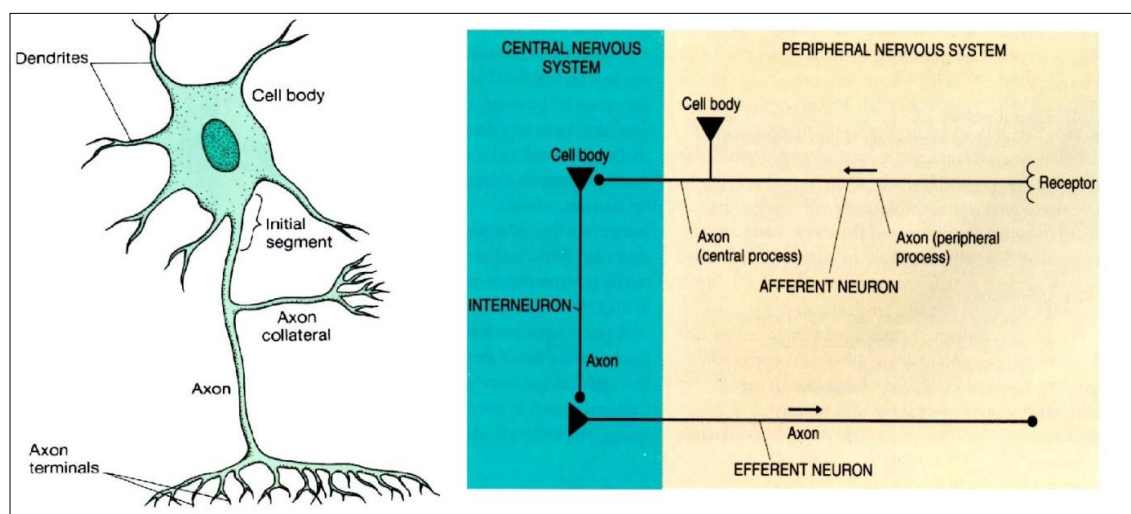
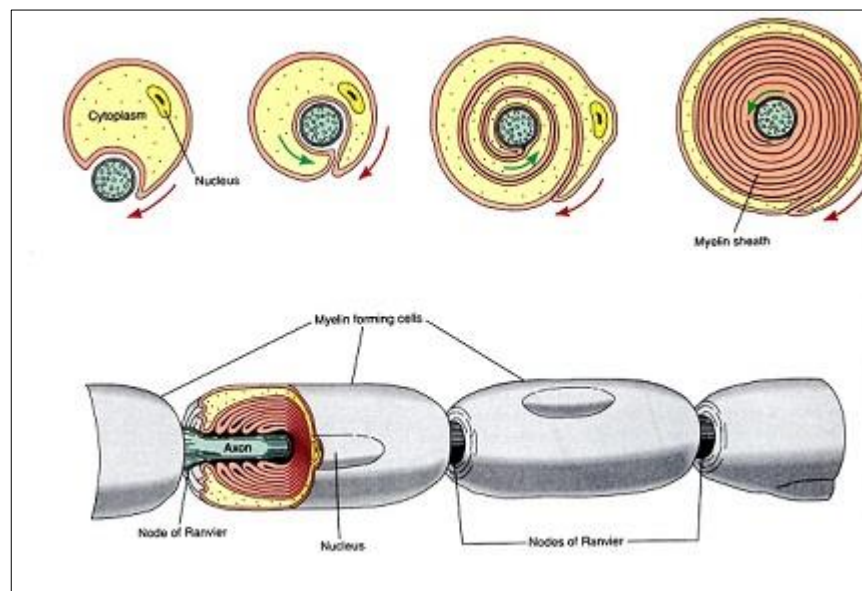


Figure 3: Diagrammatic representation of a neuron and the neuronal connections in the central nervous system and peripheral nervous system (adapted from Vander, 1994, p181 and 183)³⁸.



The axons of neurons constitute nerve fibres and these vary in length from a few mm (in the brain) to a metre (in nerves reaching from the spinal cord to the feet). Axons can be myelinated, that is surrounded by a fatty myelin sheath which is deposited around the axons by Schwann cells (Figure 4). Myelin acts as an insulator to decrease ion flow through the membranes. However, ions flow quickly through the junctions between two Schwann cells (nodes of Ranvier). This structure causes depolarisation to jump across intervals, thereby increasing the velocity of signals along the nerve. Nerve conduction in myelinated nerve fibres is at a much greater velocity than in unmyelinated nerve fibres³⁹

Figure 4: Myelination of axons (reproduced from Vander, 1994, p182)³⁸



Vitamin B12 is required for the initial myelination of the nervous system, and its maintenance^{27, 32}. In vitamin B12 deficiency pathological investigations reveal demyelination in the spinal cord, peripheral nerves and/or the white matter of the brain³² and an abnormal increase in astrocytes in the brain due to damage to nearby neurons (astrogliosis)⁴⁰.

The full neurological syndrome associated with vitamin B12 deficiency is called subacute combined degeneration of the cord (SCD) due to demyelination of the posterior columns and PNS, giving rise to ataxia (a lack of voluntary coordination of muscle movements) and impaired cutaneous sensation respectively. Other neurologic symptoms associated

with vitamin B12 deficiency include symmetric paresthesias, numbness, gait problems, impaired vibration or position sensation and weakness^{24, 32}. The reversibility of neurological complications associated with vitamin B12 deficiency is not clear and may depend on their duration¹³.

1.6 Mechanisms by which vitamin B12 affects neurologic function

1.6.1 Role of vitamin B12 metabolites

Early theories of the mechanisms to explain the neurological manifestations of vitamin B12 deficiency focussed on impaired activity of the two vitamin B12-dependent enzymes: methionine synthase (MeS) and L-methylmalonyl CoA mutase (MCM). MeS requires methyl-cobalamin as a cofactor for the methyl transfer from methyltetrahydrofolate to hcy to form methionine and tetrahydrofolate. Impairment of this function in the case of vitamin B12 deficiency leads to an elevation in hcy. MCM requires adenosylcobalamin to convert L-methylmalonyl CoA to succinyl-CoA in an isomerisation reaction. Impairment of this function with vitamin B12 deficiency leads to an elevation in MMA.

There have been various reports that the metabolites MMA and hcy, which are elevated as a result of impaired enzyme activity, act as neurotoxins, whose accumulation may be responsible for the onset of SCD⁴¹. However, hypotheses (discussed further below) rely heavily on animal and cell culture work; there is a paucity of mechanistic studies conducted in humans.

1.6.1.1 Impaired function of MeS and elevated hcy

It has been proposed that vitamin B12-dependent neurological effects may be due to a defect in MeS. The hypothesis was developed because monkeys treated with nitrous oxide (which blocks MeS) develop neurological disease similar to that observed in vitamin B12 deficient humans⁴².

Vitamin B12 regulates, together with 5-methyl-tetrahydrofolate, the remethylation of hcy to L-methionine and the subsequent formation of S-adenosylmethionine (SAM).

SAM is a methyl donor essential to most biological methylation reactions, which can be genomic or non-genomic⁴³. In the brain, SAM-dependent methylations are extensive⁴⁴. As a result, inadequacy in vitamin B12 can lead to impaired DNA synthesis and transcription, impaired genomic methylation and epigenetic mechanisms and hcy-mediated DNA damage⁴⁵ as well as impaired methylation of myelin, neurotransmitters and phospholipids in the CNS, which may contribute to demyelination^{41, 44, 45}. It has also been proposed that hcy may also have direct neurotoxic effects in the CNS via NMDA (N-methyl-D-aspartate) receptor activation⁴⁶ or oxidative damage⁴⁴. However, there is a lack of supportive evidence to suggest the concentrations of hcy in human brain or cerebrospinal fluid reach neurotoxic levels⁴⁴.

1.6.1.2 Impaired function of MCM and elevated methyl malonic acid

Impairment of the function of MCM in vitamin B12 deficiency causes accumulation of methylmalonyl CoA in the mitochondria, an elevation in methyl malonic acid (MMA), acidosis and elevated MMA excretion. The accumulation of mitochondrial methylmalonyl CoA depletes the CoA pool available for other mitochondrial enzymes and metabolites, and the increased propionyl CoA can be incorporated into long chain fatty acids in place of acetyl CoA. It has been proposed that there is a resultant accumulation of unusual fatty acids in myelin affecting the integrity of the myelin structure, which causes demyelination^{41, 42}, but the evidence for this is not resolved^{42, 45}.

1.6.2 Other proposed mechanisms

Some clinical and experimental findings defy the biochemical/enzyme impairment theories described in Section 1.6.1. In particular, inherited deficits in MCM in children and MeS knockout rats do not show typical SCD lesions, and patients with homocysteinemia for any reason other than vitamin B12 deficiency do not show SCD-like neurological manifestations⁴¹. This has led to further research on other proposed mechanisms.

Scalabrino and Peracchi⁴⁷ have demonstrated that the severity of neuropathological features in the white matter of the spinal cord of TGX rats¹ (an experimental rat model of vitamin B12 deficiency) does not correlate with progressive accumulation of hcy and MMA in the spinal cord or serum, thereby suggesting that these metabolites are unlikely to cause SCD-like lesions. Scalabrino's research group has conducted extensive research using experimental rat models to propose new mechanisms responsible for the pathogenesis of SCD⁴¹. It has been known for a long time that various cytokines and neurotrophic growth factors are synthesised and released by CNS cells, mainly glial cells. Mechanisms have been proposed that focus on an imbalance in growth factors and cytokines in the CNS seen with vitamin B12 deficiency.

In vivo evidence from the CNS and cerebrospinal fluid (CSF) of rats has demonstrated an overproduction of myelinolytic tumour necrosis factor (TNF) α and reduced synthesis of two neurotrophic agents, epidermal growth factor (EGF) and interleukin-6 (IL-6). TNF α is implicated in the pathogenesis of some neurological diseases and EGF positively regulates the proliferation and/or differentiation of neurons and/or glia. IL-6 is a multi-functional cytokine. The imbalance between TNF α and EGF induced by vitamin B12 deficiency has been verified in the sera of patients with pernicious anaemia and the CSF of SCD patients.

The cytokine and growth factor abnormalities, as well as the morphological lesions and biochemical abnormalities, in TGX rats are substantially corrected by chronic vitamin B12 treatment; this suggests that they are all specifically linked to vitamin B12 deficiency⁴⁷.

Demonstration of these new mechanisms does not exclude the possibility that the classical biochemical enzyme mechanisms coexist. Further research is needed to reconcile these new and classic theories. Further, research is ongoing to determine whether cytokine/growth factor mediated pathology extends to the PNS. Much of this

¹ This experimental rat model mimics the pathogenesis of pernicious anaemia using gastrectomised rats and reproduces typical SCD lesions and reduced nerve conduction velocity. Hallmarks are also intramyelinic and interstitial oedema of the white matter of the CNS.

research focuses on the role of normal cellular prion protein (PrP^c), which is involved in maintenance of myelinated fibres in both the CNS and PNS, and its regulation by the cytokines and growth factors shown to be linked to vitamin B12 deficiency^{48, 49}. At the moment it is not possible to determine whether the imbalance in cytokine/growth factors is a cause or consequence of vitamin B12 deficiency in humans, but it does appear to be necessary for the development of SCD in the experimental rat model^{41, 50}.

1.7 Measuring vitamin B12 status

1.7.1 Serum/plasma vitamin B12

There is currently no 'gold standard' test for the diagnosis of vitamin B12 deficiency, though a serum vitamin B12 assay is usually the standard initial diagnostic test in routine clinical practice³⁶. It is low cost and widely available. The concentration of vitamin B12 in the serum or plasma reflects both vitamin B12 intake and the status of body stores and quantitates inactive (holohaptocorrin) and active (holotranscobalamin) forms of vitamin B12^{30, 36}. However, as deficiency develops, serum vitamin B12 may be maintained at the expense of body stores, so an adequate vitamin B12 plasma/serum does not necessarily indicate adequate vitamin B12 status¹³. It is relatively common for false negatives and false positives to be reported with use of serum vitamin B12, which may be a result of both inactive and active forms being measured³².

Various assays for vitamin B12 are available. Historically, the microbiological assay for vitamin B12 was standard⁵¹ and it has been suggested that this assay can be reliably established in most laboratories⁵². Now, the most common assays in clinical laboratories are based on intrinsic factor binding of vitamin B12 and immune-chemiluminescence based assays^{36, 53}. Comparability has therefore become an issue because the microbiological assay is a functional measurement and the intrinsic factor and immune-chemiluminescence based assays are substance quantity measurements⁵¹.

Furthermore, particular issues have been reported when intrinsic factor is used as the assay binding protein in competitive binding methods. Anti-intrinsic factor antibodies

need to be removed from samples; otherwise there is a risk of false normal results to be reported, especially in patients with pernicious anaemia^{32, 54, 55}.

A review of the published biomarkers and cut-offs for the diagnosis of vitamin B12 deficiency has highlighted that a wide range of laboratory assays have been used to measure vitamin B12⁵³. Standardisation of assays and development of an international reference standard across laboratories would be ideal. Otherwise, each laboratory should have its own reference range identified locally. However, serum vitamin B12 levels can be affected by diet, use of supplements/medication, pregnancy and age; therefore establishment of local reference ranges can be difficult³⁶. Robust studies that have accounted for these variables are possible but rare⁵⁶.

Inconsistency in how vitamin B12 deficiency is defined is not only hindered by the various assays used, but also a lack of consensus on cut-off points. Several thresholds to indicate low vitamin B12 status and/or deficiency have been used, ranging from 100pmol/L to 350 pmol/L⁵³ and there has been much debate about how stringent a threshold to use^{24, 30}. The WHO has recognised the lack of a universally accepted cut-off to define vitamin B12 deficiency and developed a consensus statement to define a cut-off that should be used for assessing the nutritional status of populations: plasma vitamin B12 <150 pmol/l (note no specific guidance on use of assay was given)⁵⁷. This cut-off is based on vitamin B12 concentrations below which plasma MMA becomes elevated. Similarly, the British Society of Haematology advise that a serum vitamin B12 cut-off level of either 148 pmol/L or one derived from a local reference range be evidence of vitamin B12 deficiency, when present alongside clinical symptoms³⁶. Again, the British Society of Haematology cut-off is not provided alongside guidance on the use of a specific assay so issues of comparability across assays has not been taken into account.

1.7.2 Holotranscobalamin

New immunoassays for holotranscobalamin (holoTC) are now available; measuring holoTC has the advantage of measuring the active fraction of vitamin B12 by measuring the vitamin B12 saturation of the binding protein transcobalamin. Studies have shown

the holoTC assay to provide some improvement in specificity for detecting elevated MMA over assays of total serum cobalamin^{32, 58}. Using MMA as the reference standard, it has been suggested that holoTC has a modestly superior diagnostic accuracy for the detection of vitamin B12 deficiency, when compared to conventional serum vitamin B12⁵⁹. Using red cell cobalamin (as an index of vitamin B12 stores) as the reference standard, it has also been shown that holoTC performs significantly better than all other indicators in older people⁵⁶. However, these findings have limitations as it is not clear whether MMA or red cell cobalamin are suitable gold standard tests in themselves; elevated MMA in particular is associated with renal failure in older people. Nevertheless, it has been suggested that holoTC is a suitable, and even preferred, assay for assessment of vitamin B12 status^{36, 56}. It has been reported that the uncertainty range for holoTC is smaller than for assays of vitamin B12^{36, 56}. Consequently, some authors view holoTC abnormality as the earliest possible marker of vitamin B12 deficiency because it occurs in patients with normal concentrations of all other biomarkers, and so may be appropriate for the subclinical situation^{52, 60}.

Like serum vitamin B12, use of holoTC also suffers from lack of consensus in cut-offs. In a recent review, cut-offs for holoTC ranged between 20 and 50 pmol/L⁵³. HoloTC assays are also less widely available and consequently factors that influence holoTC have not been widely studied⁶⁰: holoTC was used in less than a third of studies assessing vitamin B12 status published between 1992-2014⁵³.

1.7.3 MMA and hcy

Blood levels of these metabolites are also valuable indicators of vitamin B12 status, as they are components of the vitamin B12 metabolic pathway. The levels of hcy and/or MMA are elevated in the majority of patients with clinical vitamin B12 deficiency. These metabolites can be used in combination with measures of vitamin B12 to confirm deficiency or marginal status.

The concentration of MMA rises when the supply of vitamin B12 is low because of impaired function of the enzyme L-methylmalonyl coA mutase. However, elevated MMA is not entirely specific to low vitamin B12 status because it can also be elevated as a

result of renal failure³². Yet, because elevated MMA represents a metabolic change as a result of vitamin B12 deficiency it is often used as an indicator of vitamin B12 status¹³. Vitamin B12 is also a cofactor for the enzyme methionine synthase; impaired function of this enzyme when vitamin B12 supply is low leads to elevated hcy. However, a lack of vitamin B6 or folate also results in elevated hcy concentration and so hcy has poor specificity as an indicator of vitamin B12 status. Renal insufficiency can also be a cause of elevated hcy⁶¹, often with aging, so is an important consideration when using these metabolites to assess vitamin B12 status.

1.7.4 Composite markers to assess vitamin B12 status

Several studies have used a combination of measures of vitamin B12 to confirm deficiency or marginal status, further expanding the number of ways in which vitamin B12 status can be assessed. Issues around the use of a composite marker for vitamin B12 have been explored in detail at a roundtable held in 2010 to assess measurement procedure issues for the monitoring of folate and vitamin B12 status by the US National Health and Nutrition Examination Survey (NHANES)⁶². The roundtable discussed the relative merits of two biomarkers of circulating vitamin B12: serum vitamin B12 and holoTC and two functional biomarkers of vitamin B12: the metabolites hcy and MMA. Overall, the roundtable regarded information from both types of biomarker concurrently as preferable to either alone, because of problems with each biomarker's sensitivity and specificity. The roundtable agreed the need for including at least one biomarker of circulating vitamin B12 (serum vitamin B12 or holoTC) and one functional biomarker (MMA or hcy) in NHANES^{60, 62}.

The roundtable also noted that, despite slightly better sensitivity and specificity over vitamin B12, the full range of alternative influences on holoTC concentrations has not been adequately explored⁶⁰. Therefore, if a choice must be made between serum vitamin B12 and holoTC, the roundtable felt that because holoTC measurement is new and would benefit from performance studies, it was agreed that serum vitamin B12 was preferable as the measure of circulating vitamin B12⁶².

Plasma hcy has a comparable sensitivity to MMA for vitamin B12 deficiency and both are excellent for monitoring response to vitamin B12 supplementation⁶³. However, use of hcy is limited because it is particularly susceptible to changes in folate status. Indeed, a greater proportion of hcy variation is attributed to folate than vitamin B12 status in countries without folate fortification⁶⁰. The roundtable therefore agreed that if a choice must be made between use of hcy and MMA, MMA is preferable because it increases with vitamin B12 inadequacy but hcy increases with both folate and vitamin B12 inadequacies⁶².

1.7.4.1 Novel composite marker, cB12

A novel approach has been developed to use a composite marker of vitamin B12, called cB12, that combines measures of vitamin B12, holoTC, hcy and MMA into one indicator using the following equation⁶⁴:

$$cB12 = \left[\log_{10} \left(\frac{holoTC \cdot B12}{MMA \cdot Hcy} \right)_{Test} - \log_{10} \left(\frac{holoTC \cdot B12}{MMA \cdot Hcy} \right)_{Ref} \right] - \frac{3.79}{1 + \left(\frac{age}{230} \right)^{2.6}}$$

The model mathematically connects all the markers and presents four independent measurements as a single point on a 'diagnostic surface'. The first element of the above equation represents the logarithmic ratio of test measurements, and the second element is a reference combination at the stipulated age. The final component is an adjustment for aging.

cB12 is more reliable when based on all four markers because of the larger amount of information encoded, but can be derived using equations that allow incomplete indicators, i.e. based on two or three markers. When using three markers, having hcy missing is more reliable than having any of the other three markers missing⁶⁴. Adjustment of cB12 is required if serum folate is <10 nmol/L and hcy is measured. cB12 values are classified as follows: >1.5=elevated vitamin B12; -0.5-1.5=adequate vitamin B12; -1.5 – -0.5=low vitamin B12; -2.5 - -1.5=possible vitamin B12 deficient; and <-2.5=probable vitamin B12 deficient. These cut-points are based on statistical criteria and physiological indicators based on haemoglobin and cognitive score, and are relevant for

adults only. A key advantage of using cB12 to measure vitamin B12 status is its use of both circulating and functional markers of vitamin B12.

However, cB12 has been used in only four⁶⁵⁻⁶⁸ studies to date. One of these is a small pre-treatment and post-treatment study⁶⁵ of older subjects excluded from taking part in a larger cluster RCT⁶⁹ looking at nerve conduction and cognitive function (because their vitamin B12 level was <120 pmol/l). In relation to cB12, this study reported that, in response to intramuscular treatment of 10 mg cyanocobalamin, 100mg pyridoxine and 100 mg thiamin for 4 months, subjects with baseline serum folate above the median had statistically significantly less improvement in cB12 than those with baseline folate below the median. Risch *et al.*⁶⁶ measured several indicators of vitamin B12 status, including cB12², and its association with age, gender and kidney function in a healthy Swiss population aged ≥60 years. cB12 was independently associated with renal function but not age. As discussed in Section 1.7.3, elevated MMA and hcy are also associated with impaired renal function. The remaining two studies were conducted in cancer patients and subjects with mild cognitive impairment. Vashi *et al.*⁶⁸ used ROC (receiver operating characteristic) curves to evaluate the diagnostic accuracy of vitamin B12, MMA and hcy using cB12 as the 'gold standard' in cancer patients. Kobe *et al.*⁶⁷ conducted secondary cross-sectional analyses using cB12 and memory performance and hippocampal structure in a subjects with mild cognitive impairment. Associations between lower serum vitamin B12 and poorer memory performance, recognition scores and lower delayed-recall scores were largely unchanged when cB12 was used as a measure of vitamin B12 status instead. Overall, use of cB12 as a measure of vitamin B12 status is in its early stages and to date has not been specifically validated against neurologic outcomes.

1.8 Vitamin B12 status in the UK

This section presents data on vitamin B12 intakes and status in the UK, based on the National Diet and Nutrition Survey (NDNS). This is a survey of the nutritional status of the UK population; it is now a rolling programme updated annually.

² In this study cB12 was calculated using an earlier version of the cB12 equation without the age adjustment factor. 70. Fedosov, S.N., *Metabolic signs of vitamin B(12) deficiency in humans: computational model and its implications for diagnostics*. *Metabolism*, 2010. **59**(8): p. 1124-38.

1.8.1 Intakes

The most recent NDNS reports vitamin B12 intakes across all age groups for years 1-4 of the rolling programme (data collected from 2008/2009 – 2011/12)¹². Vitamin B12 intakes (measured by 4 day food diaries) were adequate across adults and older adults (mean daily intake was 6.4µg for adults aged ≥65 years). Only 1% of adults aged ≥65 years had vitamin B12 intakes below the LRNI³.

1.8.2 Biological measures of status

Results from years 1-4 of the NDNS rolling programme¹² report mean serum vitamin B12 and % below 150 pmol/l as shown in Table 2. The assay used was a competitive immunoassay using direct chemiluminescence (ADVIA Centaur vitamin B12 assay). This survey shows some evidence of low vitamin B12 status in women and older men and women.

Table 2: Vitamin B12 status in National Diet and Nutrition Survey, by age and sex

	Men		Women		Total	
	19-64y	≥65y	19-64y	≥65y	19-64y	≥65y
Mean (SD) serum vitamin B12 (pmol/l)	265 (96.1)	254 (95.3)	265 (97.6)	283 (109.8)	265 (96.8)	270 (104.6)
% below 150 pmol/l	2.5	5.9	7.1	5.9	4.9	5.9

An earlier NDNS published in 1998 focussed on adults ≥65 years⁷¹. Serum vitamin B12 (measured using a radioimmunoassay using human intrinsic factor as a binder) data showed that 8% and 7% of men and 5% and 10% of women (free-living and living in institutions respectively) had serum vitamin B12 <118 pmol/l. This older survey clearly shows some evidence of low vitamin B12 status in older adults.

Clarke *et al.*³¹ studied the prevalence of vitamin B12 deficiency in an elderly population in the UK. In this study conventional vitamin B12 deficiency was defined as vitamin B12<150pmol/l and metabolically significant vitamin B12 deficiency as vitamin B12<200

³ LRNI is the amount of a nutrient that is enough for only the small number of people who have low requirements (2.5%).

pmol/l and hcy>20µmol/l. The results of three population based studies of older people [The Oxford Healthy Aging Project (OHAP)⁷², the NDNS of people aged 65 years and older⁷¹ and the Medical Research Council (MRC) nutrition study⁷³] were used, from which 1549 subjects with complete data were included in analyses. Each study used a different vitamin B12 assay: the OHAP used a competitive protein-binding radioimmunoassay on an ACS Centaur using an automated chemiluminescence system; the NDNS used a radioimmunoassay using human intrinsic factor as a binder; and the MRC nutrition study used a Becton Dickinson simultrac kit. The variability between assays limits the application of a universal cut-off value to define vitamin B12 status. However, the use of the 'metabolically significant vitamin B12 deficiency definition' attempts to compensate for this by including hcy status in the definition; hcy correlates more closely between individuals. Overall, 5% of people aged 65-74 years and 10% people aged ≥75 years had low vitamin B12 levels or metabolically significant vitamin B12 deficiency. In all three studies, the prevalence of vitamin B12 deficiency increased with age.

Further afield, there is evidence of a high prevalence (up to 15%) of elevated MMA and associated low/low-normal vitamin B12 in older population groups across the US and Europe⁷⁴. Less is known about vitamin B12 status in developing countries.

1.9 Measuring neurological function

1.9.1 Clinical assessment

The neurological effects of vitamin B12 status can be measured in many ways. Some methods rely simply on symptoms reported by a subject/patient. A subject might be asked questions about pain, feelings of altered sensation (eg tingling/burning known as paresthesia) or numbness (reduced sense of touch known as hypoesthesia), unsteady gait or weakness.

Clinicians can conduct a number of examinations to detect neurological impairment. Common tests and signs are described in Box 2. These can be used in studies as clinical

markers of neurological function, but these methods are not free from subjectivity of the clinician conducting the examinations and can also be influenced by the patient.

Box 2: Common clinical signs used to assess neurological function

Proprioception/position sense is the sense of the relative position of neighbouring parts of the body and strength of effort being employed by movement. In the Romberg's test, the patient is stood up and asked to close his eyes. A loss of balance is interpreted as a positive **Romberg's sign**.

Vibration sensation is the sense of vibration sometimes tested by placing a vibrating tuning fork on the skin and asking the patient whether they can detect any vibration.

Ataxia is the lack of voluntary coordination of muscles. **Finger-nose** and **heel-knee-shin tests** can be used to assess a patient's ability to judge the position of a target.

Monofilament testing is used to detect peripheral neuropathy in the feet. The monofilament is placed on the foot to assess the loss of protective sensation.

The **plantar response/reflex** is elicited when the sole of the foot is stimulated with a blunt instrument. When normal this causes a downward response of the great toe. When an upward response of the great toe is seen this is called the **Babinski sign**.

Ankle/knee jerk reflexes are sought in response to taps on the Achilles tendon or patellar ligament with a reflex hammer. Absence of a reflex response (hyporeflexia) is a sign of nerve pathology.

1.9.2 Nerve conduction studies

1.9.2.1 Nerve conduction in the peripheral nervous system

Nerve conduction studies provide objective information about different types of neuropathy in the PNS: axonal degeneration and demyelination. They can also tell us about the anatomical distribution of neuropathies and whether they are sensory or motor^{75, 76}.

In nerve conduction studies, the nerve is stimulated by placing a negative cathode and adjacent anode over the nerve and then generating an electrical pulse between them (a supramaximal stimulus is used to ensure that all nerve fibres within the nerve are activated). Signals are then recorded from an electrode placed over another distant part of the nerve (for sensory nerves) or over the innervated muscle (for motor nerves). The

recording electrode registers a signal with a distinctive shape; there is an initial upward (negative) deflection followed by a downward deflection. The recorded signals are called the compound muscle action potentials (CMAPs) for motor nerves (Figure 5) or sensory (nerve) action potentials (SNAPs or SAPs) for sensory nerves (Figure 6)⁷⁷. Sensory nerve responses are three times smaller than motor responses so several signals need to be averaged when recording to be able to produce reliable results.

Figure 5: Motor nerve conduction study and sample of compound muscle action potential recording (adapted from Misulis, 2003, p135⁷⁸)

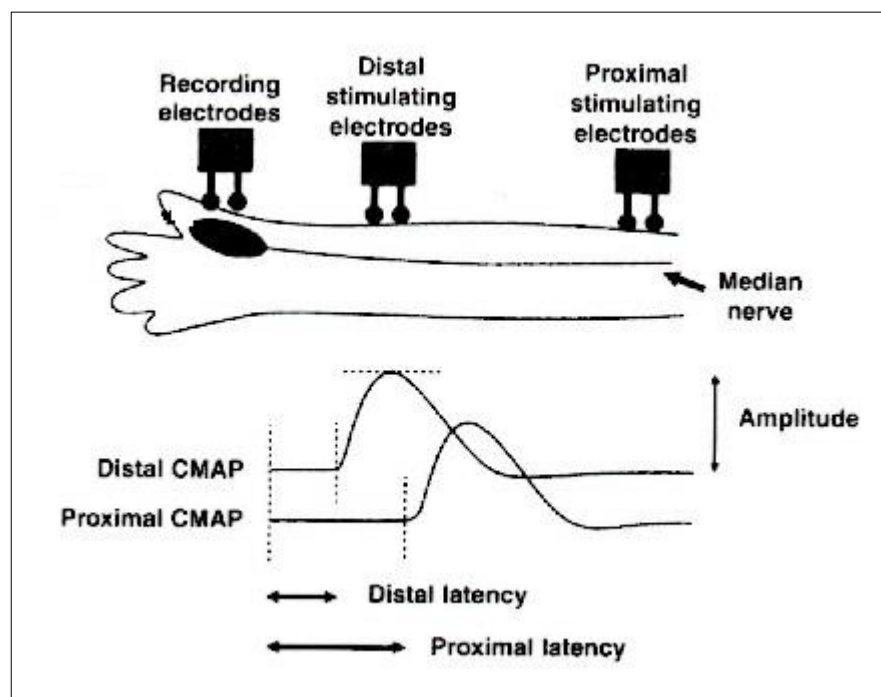
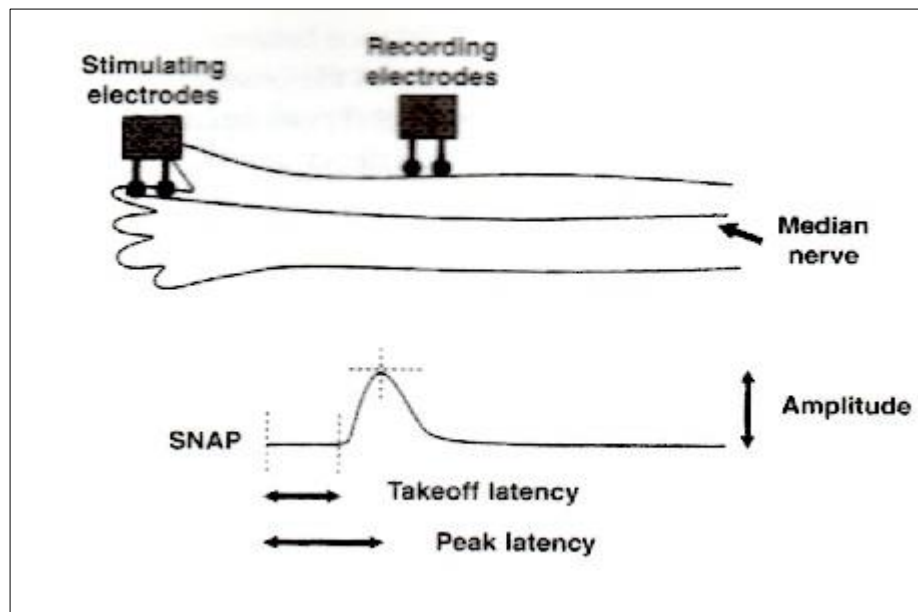


Figure 6: Sensory nerve conduction study and sample of sensory nerve action potential recording (adapted from Misulis, 2003, p139⁷⁸)



The signal amplitude (max height of the negative deflection), the area (under the negative deflection) and the signal latency time (time from stimulus onset to onset of negative deflection) are all used to interpret function of the nerve. The latency can be used to calculate nerve conduction velocity (NCV). To ensure that the conduction velocity only reflects the conduction in the nerve alone (i.e. not including conduction in the muscle or at the neuromuscular junction) the stimulus is applied at two points along the nerve; the difference in latencies reflects the NCV between the two points of stimulation on the nerve⁷⁷.

Axonal degeneration is characterised by reduced signal amplitude as there are fewer functional nerve fibres to carry the action potential that is picked up by the recording electrode. Demyelination is characterised by reduced conduction velocities⁷⁷. Vitamin B12 has a role in myelination of nerves so NCV is of particular interest when investigating the relationship between vitamin B12 status and neurological function.

1.9.2.2 Nerve conduction in the central nervous system

Methods have also been developed to investigate neurological function in the CNS. Motor evoked potentials (MEP) are signals recorded from muscles following direct

stimulation of the motor cortex of the brain. Stimulation can be induced by transcranial magnetic stimulation (TMS) or using an electrical stimulator. TMS involves inducing a rapidly changing magnetic field in the brain by passing a massive current through a coil of wire held over the scalp⁷⁹, and is painless for the subject. This initiates motor nerve signals and a muscle twitch.

Central conduction can be assessed from the delay, or latency, between the stimulus and the evoked response. Signals running from the brain to muscles not only involve the CNS but also involve the PNS. This causes variations in the delay between stimulus and response, largely because conduction velocity in peripheral nerves is sensitive to temperature of the limbs⁷⁹. It is possible to isolate measurement of nerve conduction in the central nervous system alone by calculating the central motor conduction time (CMCT), which is a measure of nerve conduction from the motor cortex of the brain to the spinal cord.

There are two approaches to measuring CMCT. The first is an F-wave study which involves stimulating a peripheral motor nerve at the muscle to elicit a signal (F-wave) that travels to the spinal cord (opposite to usual direction) and back to the muscle⁸⁰. The F wave latency is then divided by two and subtracted from the latency of the signals (MEPs) running from the cortex to the muscle. Alternatively MEPs are recorded following stimulus at the cervical/lumbar region of the spinal cord. CMCT can be calculated as the difference between the latency to the muscle after cortical stimulation and latency to the muscle after cervical/lumbar stimulation⁸¹.

There are other measures used to assess function of the CNS. The cortical silent period is an electrical silence lasting 40-300ms in a contracting muscle following a TMS-induced MEP. The cortical silent period is useful for investigating some neurological disorders and is thought to reflect function of the inhibitory neurotransmitter GABA (gamma aminobutyric acid)⁸². The corticomotor threshold is defined as the lowest stimulation intensity able to evoke a MEP of minimal size and is usually assessed in the small hand muscle⁸². A raised threshold could represent inexcitability of spinal motoneurons or motor cortex neurons and is implicated in various neurological disorders⁸⁰.

1.10 Uncertainties in the neurological effects of low vitamin B12 status.

It is known that low vitamin B12 status affects many older people but the functional significance of 'subclinical' low vitamin B12 status (absence of symptoms) in older people is not clear. In particular, the public health significance of low vitamin B12 status on neurological manifestations remains unknown. Neurological signs and symptoms caused by low vitamin B12 status may be non-specific and often attributed to 'old age'. Such symptoms can nevertheless have an important impact on functional ability in terms of physical function, social independence, mobility and ability to conduct activities of daily life. For example, researchers have shown a link between peripheral neuropathy and impaired balance and falls. Older people prone to falls may then limit their physical activity further adding to loss of functional ability. Peripheral neuropathy has also been associated with lower health-related quality of life, foot deformity and pain and skin ulceration^{18, 19, 83}.

Accordingly, the neurologic impact of low vitamin B12 status in older people could contribute significantly to the burden of loss of functional ability in older age, with public health consequences at a population level. Currently, very little is known about the neurologic implications of low vitamin B12 status outside the clinical setting where the focus is on direct examination or treatment of patients. Impaired neurological function is a recognised consequence of frank vitamin B12 deficiency but it is not known what needs to be done to identify and treat those at risk. It has been suggested that routine vitamin B12 supplementation is needed in older people in light of the high prevalence of mild, preclinical deficiency⁶¹ but it is important to gather evidence on whether those at risk will benefit. In order to optimise assessment and monitoring of interventions the WHO has identified a research need to establish objective criteria to assess early neurological impairment caused by vitamin B12 deficiency, ideally using neurophysiologic measurements⁵⁷. Early detection of 'subclinical' vitamin B12 status could lead to a reduction in vitamin B12-neurological impairment and consequent impaired functional ability by preventing progression to a more serious clinical deficiency state.

Maintenance of functional ability in later life has become a major public health concern, a key area of ageing research and a target for the development of effective policies and programmes^{2, 4}. To help answer the uncertainties outlined above, this thesis aims to improve our understanding of the contribution of vitamin B12-neurological impairment to the public health challenge of ageing. Determining whether subclinical low vitamin B12 status is a problem and should be treated requires evidence on the number of people likely to develop neurological impairment and the efficacy of treatment³¹. The research questions outlined in Section 1.1.1 focus on the neurological effects of low vitamin B12 status in older people and will be addressed in the subsequent chapters of this thesis.

1.10.1 Structure of the thesis

The thesis has been presented in research paper style and includes three manuscripts prepared for publication in peer-reviewed journals, alongside additional material in linking chapters. Each manuscript prepared for publication and included as a thesis chapter is preceded by a cover sheet on publication details and a preface describing how the manuscript fits into the thesis as a whole.

Chapter 2 (addressing research question 1a) is a systematic review of observational studies that has been published in the British Journal of Nutrition. Chapter 3 (addressing research question 2a) is a secondary analysis of baseline data from the OPEN study prepared and submitted for publication. Chapter 4 reports the findings of a systematic review of intervention studies (addressing research question 1b). Chapter 5 is a manuscript prepared for submission to a peer-reviewed journal, reporting further secondary analyses using data from the OPEN study (addressing research question 2b). Finally, Chapter 6 discusses and integrates the findings from each of the research questions to derive overall conclusions and draw policy implications for the study findings. Chapter 6 also outlines the strengths and limitations of all the work conducted and suggests directions for further research.

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Chapter 2: Is there an association of vitamin B12 status with neurological function in older people? A systematic review

Preface

As described in Chapter 1, this study aims to improve our understanding of the contribution of vitamin B12-neurological impairment to the public health challenge of ageing. By assessing the relationship between low vitamin B12 status and neurologic function in older people, it is hoped that findings will help determine whether subclinical low vitamin B12 status in older people is a public health problem. To date, the epidemiological evidence of the association of vitamin B12 status with neurological function has not been systematically reviewed. Chapter 2 presents a systematic review which aims to evaluate the association of low vitamin B12 status with neurological outcomes relevant to central and peripheral nerve function in older people. The research question addressed here is:

- 1a) Is there any evidence in the available literature that vitamin B12 status is associated with neurological function in older people?

My role in this research was to develop the protocol for the review, conduct the systematic search of the literature, select articles for inclusion, complete data extraction and quality assessment of included studies, interpret results of the review and write the manuscript.

This systematic review has already been published in the British Journal of Nutrition so the manuscript is presented here alongside supplemental materials available on the journal website, namely A) Medline search strategy B) Study characteristics used to assess risk of bias and Supplemental tables S1 (Study characteristics and main findings of studies based on subjects from the general population) and S2 (Study characteristics and main findings of studies based on subjects with low vitamin B12 status).



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Systematic Review

Is there an association of vitamin B₁₂ status with neurological function in older people? A systematic review

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(Submitted 7 August 2014 – Final revision received 6 February 2015 – Accepted 20 May 2015)

Abstract

Low vitamin B₁₂ status is common in older people; however, its public health significance in terms of neurological manifestations remains unclear. The present systematic review evaluated the association of vitamin B₁₂ status with neurological function and clinically relevant neurological outcomes in adults aged 50+ years. A systematic search of nine bibliographic databases (up to March 2013) identified twelve published articles describing two longitudinal and ten cross-sectional analyses. The included study populations ranged in size (n 28–2287) and mean/median age (range 65–81 years). Studies reported various neurological outcomes: nerve function; clinically measured signs and symptoms of nerve function; self-reported neurological symptoms. Studies were assessed for risk of bias, and results were synthesised qualitatively. Among the general population groups of older people, one longitudinal study reported no association, and four of seven cross-sectional studies reported limited evidence of an association of vitamin B₁₂ status with some, but not all, neurological outcomes. Among groups with clinical and/or biochemical evidence of low vitamin B₁₂ status, one longitudinal study reported an association of vitamin B₁₂ status with some, but not all, neurological outcomes and three cross-sectional analyses reported no association. Overall, there is limited evidence from observational studies to suggest an association of vitamin B₁₂ status with neurological function in older people. The heterogeneity and quality of the evidence base preclude more definitive conclusions, and further high-quality research is needed to better inform understanding of public health significance in terms of neurological function of vitamin B₁₂ status in older people.

Key words: Vitamin B₁₂; Neurological function; Nerve conduction; Older people

Ageing is associated with a decline in vitamin B₁₂ status^(1,2), and there is widespread evidence of low vitamin B₁₂ status in older people^(1,3). In the UK, 5% of adults aged 65–74 years and 10% adults aged ≥75 years have low vitamin B₁₂ levels (defined as vitamin B₁₂ <150 pmol/l) or metabolically significant vitamin B₁₂ deficiency (defined as vitamin B₁₂ <200 pmol/l and homocysteine level >20 μmol/l)⁽¹⁾. However, intakes of vitamin B₁₂ in adults are mostly adequate and in the most recent National Diet and Nutrition Survey of the UK, only 1% of adults aged 65–74 years and ≥75 years had vitamin B₁₂ intakes below the lower reference nutrient intakes⁽⁴⁾. Several factors relating to vitamin B₁₂ absorption may contribute to poor status in older people, including a decrease in gastric acidity, the presence of atrophic gastritis, compromised functional and structural integrity of vitamin B₁₂-binding proteins, and lack of liver vitamin B₁₂ stores⁽⁵⁾.

Indeed, food-cobalamin malabsorption can account for up to 60–70% of confirmed cases of vitamin B₁₂ deficiency in older people⁽⁶⁾.

The clinical manifestations of vitamin B₁₂ deficiency can be haematological, neurological or both⁽⁷⁾; neurological symptoms can occur in the absence of anaemia in 20–30% of cases⁽⁸⁾. Pathological investigations in vitamin B₁₂ deficiency reveal demyelination in the spinal cord, peripheral nerves and/or the white matter of the brain⁽⁷⁾ and an abnormal increase in astrocytes in the brain due to damage to nearby neurons⁽⁹⁾. It has been proposed that the mechanism by which vitamin B₁₂ deficiency affects neurological function involves impairment of vitamin B₁₂-dependent enzyme functions. Impaired function of methionine synthase leads to elevated homocysteine levels, and impaired function of L-methylmalonyl-CoA mutase leads to elevated methyl

Abbreviation: SIGN, Scottish Intercollegiate Guidelines Network.

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malonic acid concentrations, each resulting in impaired methylation reactions and changes to fatty acid incorporation in myelin^(10,11). However, there is recent evidence to suggest that mechanisms involving an imbalance in cytokines and growth factors in the central nervous system may be important; it is not yet clear whether these findings can be extended to the peripheral nervous system^(12,13). Symptoms of peripheral neuropathy associated with vitamin B₁₂ deficiency commonly include symmetric paresthesias, numbness or gait problems. Other neurological defects include impaired vibration sense, impaired position and cutaneous sensation, ataxia, and weakness^(2,7). The relationship between vitamin B₁₂ status and cognitive function has been reviewed extensively elsewhere^(14–17).

The functional and public health significance of low vitamin B₁₂ status in older people is currently unclear. In the clinical setting, impaired neurological function is a known marker of frank vitamin B₁₂ deficiency. However, whether neurological impairment is associated with low vitamin B₁₂ status at a population level is unknown. To date, the epidemiological evidence of the association of vitamin B₁₂ status with neurological function has not been systematically reviewed. The present systematic review aims to evaluate the association of low vitamin B₁₂ status with neurological outcomes relevant to central and peripheral nerve function in older people. It is hoped that findings will inform public health and nutrition guidance to be provided on the functional relevance of vitamin B₁₂ status in later life.

Experimental methods

Methods of analysis and inclusion criteria were specified in advance and documented (protocol available on request from the authors). The present systematic review is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.

Search strategy

A systematic search of bibliographic databases was conducted on 28 March 2013. All observational study designs assessing the association of vitamin B₁₂ status with neurological outcomes in older people were included except case reports/series, narrative reviews, editorials and conference reports. Included subject groups were older people with a median or mean age ≥ 50 years and could be resident in institutions or the community. Studies of subject groups with known existing medical conditions affecting neurological function (including alcoholism, HIV, diabetes-associated neuropathy or motor neurone disease), vitamin B₁₂ status (including bariatric surgery) or metabolites of vitamin B₁₂ (including renal insufficiency) were excluded. Included exposures were serum/plasma vitamin B₁₂, transcobalamin (a binding protein for vitamin B₁₂), holotranscobalamin (the form of vitamin B₁₂ able to cross into the cells), methyl malonic acid (a metabolite of vitamin B₁₂) and dietary vitamin B₁₂ (but not multiple vitamin and mineral supplements). Included outcomes were peripheral sensory or motor nerve function/conduction, central motor conduction; peripheral neuropathy; clinical

signs and symptoms of neurological (but not cognitive) function (somatosensory disorders, knee and ankle jerk/reflexes, joint, position and vibration sense, ataxia, and proprioception); and self-reported neurological (but not cognitive) symptoms (pain, altered sensation, unsteadiness, prickly feelings, weakness, numbness, and difficulty walking).

Search terms were developed for nine medical and public health databases using both MeSH terms and text terms where possible: MEDLINE (1946 to March week 3, 2013; see online Supplementary data); Global Health (1910 to February 2013); EMBASE (1974 to 27 March 2013); PsycInfo (1806 to March week 3, 2013); CINAHL Plus (1937 to March 2013); Cochrane Library (inception to March 2013); ClinicalTrials.gov (inception to March 2013); Scopus (1823 to 28 March 2013); TRIP database (inception to March 2013). Papers not translated into English and grey literature were excluded. The reference lists of included studies were hand-searched for any further articles relevant to the review.

Assessment of eligibility of studies and data extraction

Articles were assessed for inclusion first using titles and abstracts, and then full copies of articles considered potentially relevant were reviewed. Eligibility assessment was carried out by two independent reviewers (L. M. M. and A. D. D.). Disagreements were resolved by discussion between the two reviewers; if no agreement could be reached, a third party was available to arbitrate.

Once the included list of papers was finalised, one reviewer (L. M. M.) extracted data and assessed risk of bias in each paper; another author (A. D. D.) checked the extracted data. Disagreements about extracted data were resolved by discussion between the two reviewers. Forms for data extraction and assessment of risk of bias were developed based on checklists developed by the Scottish Intercollegiate Guidelines Network (SIGN)⁽¹⁸⁾. These checklists relate to study characteristics that are likely to have a significant influence on the validity of the reported results. Data were extracted into forms, defined according to the SIGN guidance, and risk of bias was assessed for each study based on the research question, selection of subjects (including age and population type), exposure and outcome assessment, comparisons made and effect sizes/summary measures, follow-up time (where appropriate), adjustment for confounders, and statistical analysis. Each of these study characteristics was judged as 'well covered', 'adequately addressed', 'poorly addressed', 'not addressed', 'not reported' or 'not applicable'. Neurological outcomes were categorised as measures of nerve function (nerve conduction studies), clinically assessed signs and symptoms of peripheral neuropathy or self-reported symptoms. The latter outcomes were judged to be 'poorly addressed' because self-reported symptoms are subjective and open to bias from the subject. Studies mostly achieving 'well covered' or 'adequately addressed' were judged to have a low risk of bias; others were judged to have a risk of bias. The assessment of risk of bias was completed at the study level (see online Supplementary data) and was used in the qualitative synthesis of results to place greater emphasis

on studies of higher quality. No study was excluded based on the assessment of risk of bias.

All study characteristics, effect sizes and summary measures were extracted from studies and tabulated to allow comparison. Care was taken to identify cases where multiple articles were published from the same study. When a report referred to data on relevant exposures and outcomes that could be included in the review, or when potentially relevant exposures or outcomes were not reported, efforts were made to contact the author for further information. The authors of eight articles were contacted by email and four authors responded. Of these, two authors provided further information that has been included in the results and two authors did not provide any additional information.

Results were synthesised in a qualitative manner. Studies were reviewed to assess whether findings varied according to the study population (general population or subjects with evidence of vitamin B₁₂ deficiency), category of neurological outcome, definition of low vitamin B₁₂ status, adjustment for confounders or age. Combining data by meta-analysis was not possible as a result of the heterogeneity of the available evidence. To identify risk of bias from selective reporting, for each included article, the reviewers checked whether all exposures and outcomes measured were also reported in the results section. No formal testing of publication bias was possible.

Results

Study selection

The search identified 982 records. Of these, 835 were excluded as not relevant following title and abstract review. Full-text articles for the remaining 147 records were sought,

though one was not retrievable⁽¹⁹⁾. Furthermore, two additional articles identified through hand-searching did not meet the inclusion criteria. A total of 148 full-text articles were examined in detail; 136 did not meet the pre-defined inclusion criteria. Full-text articles were most commonly excluded because they were not original reports from observational studies, neurological outcomes focused only on cognition, and/or the mean/median age of the study subjects was ≤ 50 years. Finally, twelve articles were included in the review (Fig. 1).

Study characteristics

Twelve reports from ten studies (two longitudinal and ten cross-sectional analyses) met the inclusion criteria. Of these, six were conducted in Europe^(20–27), with the remaining studies conducted in the USA⁽²⁸⁾, Asia^(29,30) and Australia⁽³¹⁾. Of the European articles, three were from Denmark with overlapping samples of participants from the same study^(20–22). There was heterogeneity in the types of population from which subjects were drawn; eight articles^(23,24,26–31) were based on general population groups of older people, though two of these recruited from hospitals (see online Supplementary Table S1), and four articles^(20–22,25) (from two studies) were based on subjects with clinical and/or biochemical evidence of low vitamin B₁₂ status (see online Supplementary Table S2). The studies included in the review involved participants aged 65–81 years. There was heterogeneity between studies in the definition of vitamin B₁₂ status and in the neurological outcomes reported. Most studies reported on more than one category of neurological outcome. Five studies measured electrophysiological measures of nerve function,

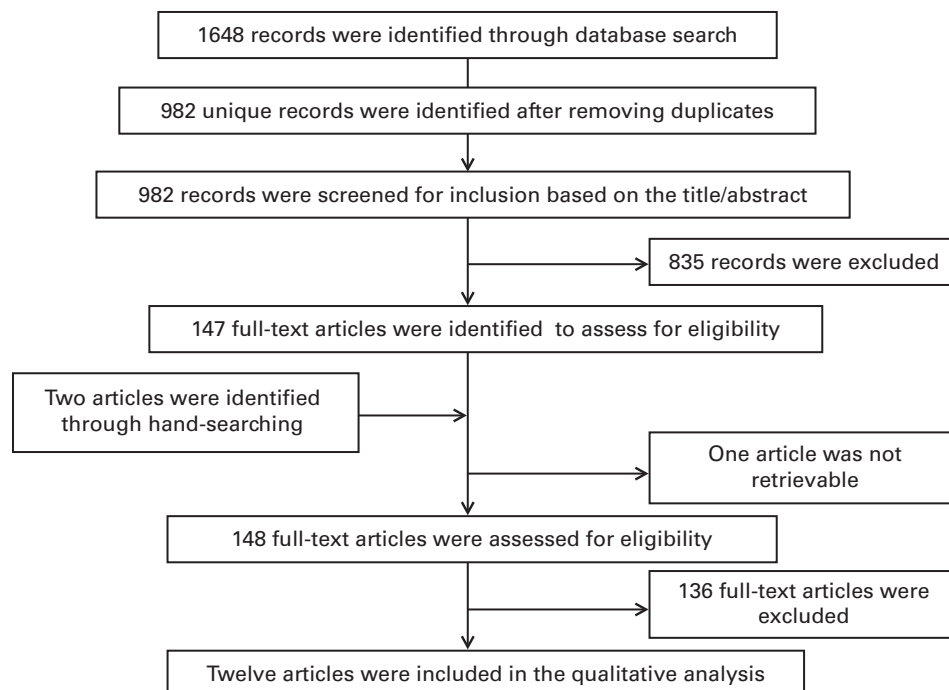


Fig. 1. Study selection process for the systematic review of the association of vitamin B₁₂ status with neurological function in older people.

ten studies reported clinically measured signs and symptoms of neuropathy, and eight studies collected self-reported neurological symptoms.

Studies based on subjects from the general population

One longitudinal⁽²³⁾ and seven cross-sectional^(24,26–31) analyses were based on general population groups of older people (see online Supplementary Table S1). One longitudinal study⁽²³⁾ with a follow-up of 3 and 6 years and low risk of bias provided no evidence of an association of vitamin B₁₂ status or change in vitamin B₁₂ status with electrophysiological measures of nerve function or peripheral neuropathy. One cross-sectional analysis⁽²⁸⁾ with a low risk of bias suggested an association of low vitamin B₁₂ status with some, but not all, electrophysiological measures of nerve function. One small cross-sectional analysis⁽²⁹⁾ with an identified risk of bias found no association of vitamin B₁₂ status and electrophysiological measures of nerve function. Five further cross-sectional studies with low^(24,26) or identified^(27,30,31) risk of bias reported clinically measured signs and symptoms of neuropathy and self-reported neurological symptoms, and provided mixed results. Three analyses^(24,27,30) identified a statistically significant association of vitamin B₁₂ status with some, but not all, clinical signs of neuropathy or self-reported symptoms, the remaining two cross-sectional studies^(26,31) reported no associations with any outcome. Four studies based on subjects from the general population adjusted for at least age and sex in the analyses; there was no clear pattern in findings according to adjustment for confounders.

Studies based on subjects with low vitamin B₁₂ status

One longitudinal analysis⁽²²⁾ (mean follow-up 1.0–3.9 years) and three cross-sectional analyses^(20,21,25) were based on subjects with clinical and/or biochemical evidence of low vitamin B₁₂ status (see online Supplementary Table S2). One small cross-sectional study⁽²⁵⁾ with an identified risk of bias found no association of vitamin B₁₂ status with electrophysiological measures of nerve function (no adjustment for confounders). The remaining three articles from one Danish study had identified risk of bias, reported neurological signs and symptoms as outcomes, and adjusted for age and sex. The cross-sectional analyses^(20,21) from the Danish study did not identify any association of vitamin B₁₂ status with neurological symptoms; the results from the longitudinal analysis were mixed.

The next two paragraphs refer to all the results, not just studies based on subjects with low vitamin B₁₂ status.

Overall, there was no clear pattern in findings according to age of subjects or adjustment for confounders. Of the five studies that reported positive associations between vitamin B₁₂ status and neurological function, three used composite definitions of low vitamin B₁₂ status involving plasma/serum vitamin B₁₂ plus elevated methyl malonic acid or homocysteine levels.

The exercise to identify selective reporting bias showed that two cross-sectional studies^(27,30) did not report results for all neurological outcomes measured. In addition, one longitudinal analysis of participants with low vitamin B₁₂ status⁽²²⁾

measured dietary vitamin B₁₂ intake, but did not report any results for associations with neurological outcomes. Overall, this suggests that selective reporting of outcomes within some studies may have affected risk of bias in the cumulative evidence.

Discussion

Overall, there is limited evidence from observational studies of older people living in the general population to suggest an association of vitamin B₁₂ status with neurological function. Few studies are available, and the majority are cross-sectional in design, limiting any causality inference. The single longitudinal analysis using a general population group found no association between vitamin B₁₂ status and nerve conduction⁽²³⁾. The limited evidence for an association comes from four of seven cross-sectional analyses^(24,27,28,30) that identified positive associations of vitamin B₁₂ and neurological function for some, but not all, outcomes. There is a similar lack of evidence of an association from studies of subjects with low vitamin B₁₂ status. None of the three cross-sectional analyses (from two studies)^(20,21,25) reported any association of vitamin B₁₂ status with neurological function, and in the single longitudinal analysis⁽²²⁾, results were mixed.

This is the first systematic review investigating the association of vitamin B₁₂ status with neurological function in older people. Systematic reviews specifically aim to minimise bias resulting from partial identification, evaluation and reporting of the available evidence base. The search strategy used in the present systematic review was comprehensive, study eligibility assessment was carried out by two independent reviewers, and each included study was assessed for risk of bias. The literature search was limited to English language and published literature, and may have missed relevant studies published in other languages and in the grey literature such as conference proceedings. The heterogeneity of the available evidence precluded the conduct of meta-analysis.

Studies assessed a heterogeneous range of neurological outcomes. The most sensitive and objective measures of neurological function relevant to vitamin B₁₂ status are electrophysiological measures of nerve conduction. However, many of the included studies were reliant only on self-reported symptoms and/or signs and symptoms of neuropathy, which are open to bias from the subject or clinician. It is possible that outcome ascertainment is a limitation of the review. There was also clinical heterogeneity in study populations (general population or vitamin B₁₂ deficient) and in how low vitamin B₁₂ status was defined. Debate continues about the most appropriate method for assessing vitamin B₁₂ status in terms of the thresholds used to define low status or deficiency and the use of various biomarkers. Recent developments suggest the need to measure vitamin B₁₂ status using both a biomarker of circulating vitamin B₁₂ and a functional biomarker such as methyl malonic acid or homocysteine⁽³²⁾. This approach of using a composite measure was used in three of the included studies, all of which reported some positive associations. Close attention to the most appropriate definition of low vitamin B₁₂ status is warranted in future research.

Several studies identified in the review were subject to risk of bias. Some studies did not provide adequate information about response rates when recruiting to the study or information about non-respondents, and five studies did not adjust for confounders (age and sex) in the analyses. In addition, selective reporting of outcomes within some studies may have affected risk of bias in the cumulative evidence. Overall, the lack of longitudinal studies, heterogeneity of the evidence, and risk of bias made it difficult to reach any firm conclusions.

Overall, there is limited evidence from observational studies to suggest an association of vitamin B₁₂ status with neurological function in older people. Further high-quality research is needed to better inform understanding of the clinical significance (in terms of neurological function) of vitamin B₁₂ status in older people. Well-designed observational studies that comply with the latest recommendations on measuring vitamin B₁₂ status⁽³²⁾ and measure neurological function by nerve conduction would be most valuable. It is also important that any studies measuring associations also identify and adjust appropriately for confounders. Such evidence is required in order to provide robust conclusions on whether measures of vitamin B₁₂ status are clinically relevant markers of central and peripheral nerve function in older people.

Supplementary material

To view supplementary material for this article, please visit <http://dx.doi.org/10.1017/S0007114515002226>

Acknowledgements

The authors acknowledge funding from the Food Standards Agency (NO5072) and the Department of Health that supported in part the preparation of the manuscript. The research received no other grant from any funding agency, commercial or not-for-profit sectors.

The authors' contributions are as follows: L. M. M. developed the protocol for the present review, selected the articles for inclusion in the review, completed the data extraction, interpreted the results of the review, and wrote the manuscript; A. D. D. critically reviewed the protocol for the review, selected the articles for inclusion in the review, checked the data extraction, and critically reviewed the manuscript; K. M. reviewed the search strategy and critically reviewed the manuscript; R. C. critically reviewed the manuscript.

The authors declare that they have no conflicts of interest.

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Supplementary materials

- A. Medline search strategy
- B. Study characteristics used to assess risk of bias (based on adapted Scottish Intercollegiate Guidelines Network checklist)

A. Medline Search Strategy

Database: Ovid MEDLINE(R) <1946 to March Week 3 2013>

-
- 1 Vitamin B 12/ (16932)
 - 2 Transcobalamins/ (929)
 - 3 Vitamin B 12 Deficiency/ (4745)
 - 4 vitamin b12.ab,ti. (11012)
 - 5 cobalamin.ab,ti. (2945)
 - 6 cyanocobalamin.ab,ti. (1016)
 - 7 hydroxocobalamin.ab,ti. (512)
 - 8 holotranscobalamin.ab,ti. (124)
 - 9 (vitamin b12 adj4 (supplement or supplementation)).ab,ti. (242)
 - 10 (dietary adj4 (cobalamin or vitamin b12)).ab,ti. (169)
 - 11 (vitamin b12 adj4 (deficient or deficiency or status)).ab,ti. (2328)
 - 12 (cobalamin adj4 (deficient or deficiency or status)).ab,ti. (809)
 - 13 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 (23474)
 - 14 Geriatrics/ (25951)
 - 15 Middle Aged/ (3098034)
 - 16 Aged/ (2159247)
 - 17 Aging/ (177196)
 - 18 "Aged, 80 and over"/ (554589)
 - 19 Frail Elderly/ (6054)
 - 20 elderly.ab,ti. (150170)
 - 21 geriatric.ab,ti. (24101)
 - 22 veteran.ab,ti. (2218)
 - 23 "senil*".ab,ti. (13776)
 - 24 Neurologic Examination/ (23007)
 - 25 Nervous System/ (18420)
 - 26 Peripheral Nervous System/ (2786)
 - 27 Paresthesia/ (4930)
 - 28 Hypesthesia/ (2112)
 - 29 Hyperesthesia/ (794)
 - 30 Hyperalgesia/ (6580)
 - 31 Somatosensory Disorders/ (888)
 - 32 Reflex/ (29643)
 - 33 Sensation/ (12176)
 - 34 Proprioception/ (5817)
 - 35 Touch/ (12279)
 - 36 Ataxia/ (6102)
 - 37 Peripheral Nerves/ (19809)
 - 38 Neural Conduction/ (24964)
 - 39 Motor Neurons/ (33288)
 - 40 Motor Activity/ (70355)
 - 41 (neur* adj4 function*).ab,ti. (62314)
 - 42 (nerv* adj4 function*).ab,ti. (18456)
 - 43 paresthesia.ab,ti. (2748)
 - 44 numbness.ab,ti. (4575)
 - 45 hyp#sthesia.ab,ti. (705)
 - 46 hyperalgesia.ab,ti. (7821)
 - 47 (sensory adj4 (impair* or coordinat* or perform* or funct*)).ab,ti. (9706)

48 (neur* adj4 (examin* or test*)).ab,ti. (59739)
 49 ataxia.ab,ti. (22229)
 50 proprioception.ab,ti. (2255)
 51 (motor adj4 (funct* or skill* or neur* or activit* or nerv* or conduct* or impair* or coordinat*
 or
 perform*)).ab,ti. (83854)
 52 (nerv* adj4 (conduct* or funct* or perform*)).ab,ti. (32451)
 53 (neur* adj conduct*).ab,ti. (501)
 54 somatosensory disorders.ab,ti. (14)
 55 touch.ab,ti. (12006)
 56 Neurophysiology/ (5808)
 57 (old adj4 (people or person or m#n or wom#n or femal* or mal* or adult)).ab,ti. (281586)
 58 (frail adj4 (people or person or m#n or wom#n or femal* or mal* or adult)).ab,ti. (935)
 59 (senior adj4 (people or person or m#n or wom#n or femal* or mal* or adult)).ab,ti. (623)
 60 (elderly adj4 (people or person or m#n or wom#n or femal* or mal* or adult)).ab,ti. (31643)
 61 (aged adj4 (people or person or m#n or wom#n or femal* or mal* or adult)).ab,ti. (94672)
 62 (mature adj4 (people or person or m#n or wom#n or femal* or mal* or adult)).ab,ti. (6430)
 63 (advanced age adj4 (people or person or m#n or wom#n or femal* or mal* or adult)).ab,ti. (884)
 64 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 57 or 58 or 59 or 60 or 61 or 62 or 63
 (3912034)
 65 Action Potentials/ (71309)
 66 Electromyography/ (63955)
 67 Sensory Thresholds/ (14561)
 68 Evoked Potentials, Motor/ (5579)
 69 Kinesthesia/ (2853)
 70 (nerv* adj4 (examin* or test*)).ab,ti. (9593)
 71 compound muscle action potential.ab,ti. (1015)
 72 F-wave.ab,ti. (970)
 73 corticomotor threshold.ab,ti. (13)
 74 evoked potential.ab,ti. (9488)
 75 (monofilament adj4 (test* or examin*)).ab,ti. (255)
 76 (CMAP or SNAP or MEP or NCS or NCV or TMS or CMCT).ab,ti. (19770)
 77 transcranial magnetic stimulation.ab,ti. (6891)
 78 "central motor conduction stud*".ab,ti. (15)
 79 ((jerk or reflex) adj4 (knee or ankle)).ab,ti. (496)
 80 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40
 or 41 or 42 or
 43 or 44 or 45 or 46 or 47 or 48 or 49 or 50 or 51 or 52 or 53 or 54 or 55 or 56 or 65 or 66 or 67 or 68
 or 69 or 70 or
 71 or 72 or 73 or 74 or 75 or 76 or 77 or 78 or 79 (596558)
 81 13 and 64 and 80 (381)
 82 limit 81 to english language (284)
 83 Transcranial Magnetic Stimulation/ (5498)
 84 80 or 83 (596991)
 85 13 and 64 and 84 (381)
 86 limit 85 to english language (284)

B. Study characteristics used to assess risk of bias (based on adapted Scottish Intercollegiate Guidelines Network checklist)

i. Longitudinal studies

Study characteristics	Hvas et al (2001) ²²	Leishear et al (2012) ²³
Focussed research question	Adequately addressed	Well covered
Groups being studied are selected from source populations that are comparable	Poorly addressed	Well covered
Study indicates how many people were asked to take part in study	Adequately addressed	Well covered
Percentage drop-out before study completed	7%	18% at 3 y 38% at 6y
Comparison is made between full participants and those lost to follow-up, by exposure status	Not reported	Not reported
Outcomes are clearly defined	Adequately addressed (neurological markers based on clinical examination) Poorly addressed (self-reported neurological symptom score)	Well covered
Measurement of exposure is reliable/validated	Adequately addressed	Adequately addressed
Measurement of outcome is reliable/validated	Poorly addressed	Well covered
Exposure level is assessed more than once	Well covered	Well covered
Confounding taken into account	Adequately addressed	Well covered

ii. Cross-sectional studies

Author (year)	Focussed research question	Subjects representative of larger population	Study indicates how many people were asked to take part in study	Information provided on non-respondents	Outcomes clearly defined	Measurement of exposure reliable/ validated	Measurement of outcome reliable/validated	Confounding taken into account
Bjorkegren and Svardsudd (2003)²⁶	Well covered	Well covered	Well covered	Adequately addressed	Adequately addressed (neurological markers based on clinical examination) Poorly addressed (self-reported neurological symptoms)	Adequately addressed	Adequately addressed (neurological markers based on clinical examination) Poorly addressed (self-reported neurological symptoms)	Adequately addressed
Gadoth et al (2005)²⁷	Adequately addressed	Adequately addressed	Poorly addressed	Not reported	Adequately addressed (neurological markers based on clinical examination) Poorly addressed (self-reported neurological symptoms)	Well covered	Adequately addressed (neurological markers based on clinical examination) Poorly addressed (self-reported neurological symptoms)	Not addressed
Hin et al (2006)²⁴	Well covered	Well covered	Well covered	Poorly addressed	Adequately addressed (neurological markers based on clinical examination) Poorly addressed (self-reported neurological symptoms)	Adequately addressed	Adequately addressed (neurological markers based on clinical examination) Poorly addressed (self-reported neurological symptoms)	Adequately addressed
Hvas et al (2001)²¹	Well covered	Poorly addressed	Adequately addressed	Poorly addressed	Adequately addressed (neurological markers based on clinical examination) Poorly addressed (self-reported neurological symptoms)	Adequately addressed	Adequately addressed (neurological markers based on clinical examination) Poorly addressed (self-reported neurological symptoms)	Adequately addressed
Hvas and Nexø (2005)²⁰	Poorly addressed	Poorly addressed	Not addressed	Not addressed	Adequately addressed (neurological markers based on clinical examination) Poorly addressed (self-reported neurological symptoms)	Adequately addressed	Adequately addressed (neurological markers based on clinical examination) Poorly addressed (self-reported neurological symptoms)	Adequately addressed
Leishear et al (2012)²⁸	Well covered	Adequately addressed	Not addressed	Not addressed	Well addressed (NCS) Adequately addressed (neurological markers based on clinical examination) Poorly addressed (self-reported neurological symptoms)	Well covered	Well addressed (NCS) Adequately addressed (neurological markers based on clinical examination) Poorly addressed (self-reported neurological symptoms)	Well covered
Metz et al (1996)³¹	Adequately addressed	Not addressed	Not reported	Not reported	Adequately addressed	Adequately addressed	Poorly addressed	Not addressed
Sucharita et al (2012)²⁹	Adequately addressed	Poorly addressed	Adequately addressed	Not addressed	Well covered	Adequately addressed	Well covered	Not addressed

Turgut et al (2006)²⁵	Poorly addressed	Not addressed	Not addressed	Not reported	Well covered	Poorly addressed	Well covered	Not addressed
Wang et al (2009)³⁰	Poorly addressed	Not addressed	Not reported	Not reported	Poorly addressed (NCS only in B12 deficient subjects)	Adequately addressed	Well addressed (NCS) Adequately addressed (neurological markers based on clinical examination) Poorly addressed (self-reported neurological symptoms)	Not addressed

NCS, Nerve Conduction Studies

Supplementary Table S1: Study characteristics and main findings of studies based on subjects from the general population

Author (year)	Subject characteristics	Exposures	Outcomes	Effect sizes
Leishear et al (2012)²³	General population in Italy (n=678). Mean age = 72.2 ±6.2y	Serum B12 Change in serum B12 at 3y	NF: CMAP and NCV measured between fibular head and ankle NP: Abnormal touch/vibration sensitivity, impaired sense of ankle position, abnormal deep tendon reflexes, positive Babinski sign and positive Romberg sign NS: Pain and sensation in feet	NF ^a : - No stat sig differences in CMAP at baseline, 3y or 6 y (p=0.43, p=0.99, p=0.64) or NCV at baseline, 3y or 6 y (p=0.90, p=0.46, p=0.12) by change in B12 at 3y - In multivariate mixed models*, no stat sig association between change in B12 at 3y and change in CMAPs (low to normal B12, p=0.84, normal to low B12 p=0.55) or NCV (low to normal B12, p=0.88, normal to low B12 p=0.20) NS ^a : - No association between baseline B12 and symptoms, cross-sectionally or longitudinally (6y)**: Low B12 (<260pmol/l) and feeling numbness OR=1.30 (95% CI 0.81-2.07); Low B12 and feeling coldness OR=1.18 (95%CI 0.81-1.72).
Bjorkegren and Svardsudd (2003)²⁶	General population in Sweden (n=161), mean age=77y.	Serum B12 Serum MMA	NP: Vibration perception threshold (carpal, tibial, tarsal), Romberg's sign, reflex response (biceps, brachioradial, triceps, patellar, Achilles) NS: Numbness, paresthesia, walking difficulties, fumblingness and impaired touch perception	NP: - No stat sig associations between serum B12 and vibration sense, Romberg sign or reflex response*** - No stat sig associations between serum MMA and vibration sense, Romberg sign or reflex response*** NS: - No stat sig association between serum B12 or serum MMA and neurological symptoms***
Gadoth et al (2005)²⁷	Older people living in institutions/visiting day care centres, based in Israel. Mean age=79.5y. Subjects with B12 deficiency (n=113) and controls (n=212)	P-B12 MMA	NP: Sensory neuropathy based on symmetrical glove and stocking hypoesthesia, decreased vibration sensation and hyporeflexia Neurological impairment score based on sensory neuropathy and MMSE, myelopathy and history of confusional state	NP: - Subjects with B12 deficiency (P-MMA ≥0.24 μmol/l and B12≤147 pmol/l) were 3.0 (95%CI 1.6-5.4, p<0.001) times more likely to have sensory neuropathy and 2.1 (95% CI 1.3-3.3, p=0.002) times more likely to have decreased vibration sensation than controls; subjects with DM excluded. - Subjects with B12 deficiency had a stat sig higher neurological impairment score than controls (46.7% versus 19.5%, p<0.0001)
Hin et al (2006)²⁴	General population in the UK, mean age=81.4y. Patients with low serum B12 (n=125) and normal serum B12 (n=875)	Serum B12 Serum HoloTC Serum MMA	NP: Knee/ankle tendon jerks, joint position sense (great toe) and plantar response NS: unsteadiness, altered sensation, falls and pins and needles PN present if clinical sign score and symptom score both >2	NP: - Stat sig association between low holoTC (OR=1.49, 95% CI 1.00-2.21 lowest v highest quartile) and elevated MMA (OR=1.76, 95% CI 1.18-2.62, highest v lowest quartile) with risk of missing ankle tendon jerks**** NS: - No stat sig difference in neurological symptoms between patients with low (serum B12<133pmol/l, Beckman assay) versus normal (serum B12≥133pmol/l, Beckman assay) B12 status. - No stat sig associations between HoloTC, Serum B12 or MMA (as continuous variables) and PN****

Leishear et al (2012)²⁸	General population in the USA; n=2287; mean age 76.5y	Serum B12 Serum MMA 2MCA Hcy Crystathione	NF: CMAP and NCV (between popliteal fossa and ankle) NP: Monofilament testing and average vibration threshold (great toe) NS: Numbness, prickly feelings and aching/burning pain	NF: - Compared to reference group , deficient B12 subjects (B12 <260pmol/l and MMA>0.27 µmol/l and MMA>2MCA) were stat sig more likely to have a lower mean NCV (p=0.006)***** - No significant differences found between deficient B12 status and reference group in CMAP***** - In multivariate analyses, compared to reference group, B12 deficient subjects had lower NCV (β=-1.16, p=0.01)***** - In multivariate analyses, no significant differences were found between deficient B12 status and reference group in CMAP***** NP: - Compared to reference group , deficient B12 subjects were stat sig more likely to be unable to detect 1.4g monofilament (p=0.01) and have a greater (worse) mean vibration threshold (p=0.003)***** - No significant differences found between deficient B12 status and reference group in ability to detect standard 10g monofilament and % unable to detect vibration***** - In multivariate analyses, compared to reference group, B12 deficient subjects were (OR=1.5, 95% CI 1.06-2.13) more likely to be unable to detect 1.4g monofilament***** - In multivariate analyses, no significant differences were found between deficient B12 status and reference group in mean vibration threshold***** NS: - No significant differences found between deficient B12 status and reference group in PN symptoms***** - No significant differences found between deficient B12 status and reference group in % of subjects reporting numbness or aching/burning pain. - Using the alternative defn of low serum B12 (B12 <260pmol/l only), univariate and multivariate associations were similar
Metz et al (1996)³¹	Hospital patients based in Australia. Mean age in patients with normal B12 = 80.7y (n=51) subnormal B12= 79.8y (n=43). 70 completed neurological examinations	Serum B12 HoloTCII	NP: Neurological score based on vibration sense, joint position sense, cutaneous sensation, knee jerk reflex, plantar response and Romberg sign	NP: - No stat sig difference in mean neurological score between subnormal B12 (<150 pmol/l) group (7.2 ±0.7) or normal B12 (≥150 pmol/l) group (6.6±0.4), p=0.457 - No stat sig correlation between HoloTCII and neurological score (no effect sizes given)
Sucharita et al (2012)²⁹	General population in India, mean age =67±7y (B12 deplete, n=29), 62±4y (B12 replete, n=18)	Serum B12	NF: Nerve conduction assessment on median nerve (sensory and motor)	NF: - No stat sig difference in median motor NCV in B12 deplete (serum B12 <148pmol/l) (55.4±2.7 m/s) versus B12 replete (serum B12 ≥148pmol/l) (55.7±4.2 m/s) patients. - No stat sig difference in median sensory NCV in B12 deplete (58.4±4.8 m/s) versus B12 replete (57.7±4.8 m/s) patients. - Distal latencies for median motor nerve and amplitudes for median sensory

				nerve were also comparable between the two vitamin B12 status groups (no effect sizes given)
Wang et al (2009)³⁰	Neurology dept patients, based in China. Mean age = 77.1±7.5y. B12 deficient patients n=163, normal B12 patients n=664.	Serum B12	NF ^b : NCV, Sensory, brainstem auditory and visual evoked potentials and EEG NP ^c : Ankle/knee tendon jerks Joint position sense of great toe Plantar responses NS ^c : Unsteady walking in the dark, paresthesia, altered sensation in feet on walking	NF: - Amongst B12 deficient patients, there is no stat sig association of B12 status with any NF measure (no effect sizes given). NP: - B12 deficient (serum B12 <189 pmol/l and Hcy >15µmol/l) patients (34.36%) stat sig more likely to have hypopallesthesia compared to normal B12 patients (22.29%), p<0.01. - No stat sig differences in absent knee/ankle tendon jerks in B12 deficient versus normal B12 patients NS: - B12 deficient patients (26.99%) stat sig more likely to experience unsteadily walking in the night compared to normal B12 group (19.43%), p<0.05. - No stat sig differences in altered sensation in feet on walking or paresthesia in B12 deficient versus normal B12 patients

2MCA, 2-methylcitrate; BMI, body mass index; CMAP, compound muscle action potential; defn; definition; DM, diabetes mellitus; EEG, electroencephalography; Hcy, homocysteine; HoloTC, holotranscobalamin; MMA, methyl malonic acid; MMSE, mini-mental state examination; n, sample size; NCV, nerve conduction velocity; NF, electrophysiological measure of nerve function; NP, clinically assessed signs of neuropathy; NS, self-reported neurological symptoms; P-B12, plasma vitamin B12; P-MMA, plasma methyl malonic acid; PN, peripheral neuropathy; stat sig, statistically significant; y, years.

Footnotes:

*Adjusted for age, sex, clinic site, DM.

**Numbness adjusted for age, sex, site, diabetes, BMI and coldness adjusted for age, sex, site, diabetes, alcohol, physical activity

*** Adjusted for age, sex and creatinine

****Adjusted for age, sex, smoking

*****Univariate analyses

*****Multivariate analyses, adjusted for age, sex, race, clinic site, DM, height, alcohol use and MMSE score

*****Multivariate analyses, adjusted for age, sex, race, clinic site, DM, height, weight, alcohol use, ankle-brachial index and systolic blood pressure

*****Multivariate analyses, adjusted for age, sex, race, clinic site, DM, height, weight, ankle-brachial index, MMSE score, high cystatin-C, 3MS score and vibration variance

*****Multivariate analyses, adjusted for age, sex, race, clinic site, DM, height, ankle-brachial index, cholesterol level and high cystatin-C

a odds ratios and p values shown for Leishear et al (2012)²³ obtained from additional information from the author

b measured in B12 deficient subjects only

c measured in all study subjects

Supplementary Table S2: Study characteristics and main findings of studies based on subjects with low vitamin B₁₂ status

Author (year)	Subject characteristics	Exposures	Outcomes	Effect sizes
Hvas et al (2001)²²	Patients with elevated MMA >0.28µmol/l recruited 1995-98 based in Denmark (n=432). Mean age=72y	P-B12: follow-up (mean 1.0-3.9y) P-MMA: baseline and follow-up	NP: Neurological disability score based on vibration sense, joint position sense, cutaneous sensation, Romberg sign, gait, finger-nose test and heel-knee-shin test NS: Patient interview	NP: - Significant but weak association between baseline P-MMA and neurological disability score (R=0.10, p=0.05, log transformed)* - No associations between either P-MMA or P-B12 at follow-up and neurological disability score (or component measures) (p=0.64,p=0.39) * - No association between change in P-MMA and neurological disability score * NS: - No associations between either P-MMA or P-B12 at follow-up and neurological symptoms (p=0.73,p=0.57)* - No association between change in P-MMA and neurological symptoms * - No association between baseline P-MMA and neurological symptoms(p=0.56)*
Hvas and Nexø (2005)²⁰	Patients with elevated MMA (P-MMA >0.28µmol/l if recruited 1995-98, ≥0.40µmol/l if recruited 1998-2000), based in Denmark. Mean age=75y, n=534	P-B12 HoloTC TC TC saturation (holoTC/TC) MMA	NP: Vibration sensation and neurological disability score based on muscle strength, reflexes and sensory loss NS: Neurological symptom score	NP: - No stat sig association between HoloTC (<40pmol/l versus ≥40pmol/l), TC saturation (<4% versus ≥4%), P-B12 (<200pmol/l versus ≥200pmol/l) or MMA (≥0.29 µmol/l versus <0.29 µmol/l) and neurological disability score [(ORs= 1.20 (0.80-1.82), 1.04 (0.69-1.57), 0.73 (0.45-1.18) and 0.92 (0.61-1.38) respectively]* NS: - No stat sig association between HoloTC, TC saturation, P-B12 or MMA and neurological symptoms [ORs= 1.21 (0.80-1.81), 1.40 (0.94-2.10), 1.35 (0.86-2.12) and 1.09 (0.72-1.64) respectively]*
Hvas et al (2001)^{21 c}	Patients with elevated MMA (0.40-2.00 µmol/l), based in Denmark. Median age=74y (placebo, n=70), 75y (treatment, n=70)	P-MMA	NP: Vibration sensation and neurological disability score based on clinical examination of finger-nose test, heel-knee-shin test, dysdiadochokinesis, Romberg test and gait. NS: Score based on absence/presence of muscle weakness, sensory disturbances or autonomic symptoms	NP: - No stat sig association between P-MMA and reduced vibration sensation [upper (p=0.36) or lower (p=0.31) extremities]* - No stat sig association between P-MMA and neurological disability score (p=0.89), finger-nose test (p=0.07), knee-heel-shin test (p=0.97), dysdiadochokinesis (p=0.25), Romberg sign (p=0.38) and abnormal gait (p=0.38)* NS: - No stat sig association between P-MMA and neurologic symptoms (p=0.29)*
Turgut et al (2006)²⁵	Patients with megaloblastic anaemia / B12 deficiency (serum B12 <147 pmol/l), based in Turkey (n=28), median age=66y.	Serum B12	NF: CMAPs (median, ulnar, tibial and common peroneal nerves) and SNAPs (median , ulnar and dorsal sural nerves); sural antidromic sensory nerve conduction studies and tibial somatosensory evoked potentials	NF: - No stat sig correlation between all NF measures and serum vitamin B12 (no effect sizes given)

CMAP, compound muscle action potential; HoloTC, holotranscobalamin; MMA, methyl malonic acid; n, sample size; NF, electrophysiological measure of nerve function; NP, clinically-assessed signs of neuropathy; NS, self-reported neurological symptoms; P-B12, plasma vitamin B12; P-MMA, plasma methyl malonic acid; SNAP, sensory nerve action potential; stat sig, statistically significant; TC, transcobalamin; y, years.

Footnotes:

*Adjusted for age and sex

Chapter 3: Vitamin B12 status and neurologic function in older people: a cross-sectional analysis of baseline trial data from the OPEN study

Preface

As described in Chapter 2, the systematic review of observational studies identified several sources of heterogeneity and risks of bias in the available evidence, which have been useful to inform further research. There was considerable heterogeneity between studies in the definition of vitamin B12 status and in the neurological outcomes reported; few studies used a composite measure of vitamin B12 status and only five of the included studies used electrophysiological measures of nerve conduction to assess neurologic function; and many existing studies failed to identify and adjust appropriately for confounders.

The OPEN study afforded an opportunity to test whether there was a cross-sectional association between vitamin B12 status and neurologic function in a high quality dataset with data on electrophysiological indices of peripheral and central neurologic function. By identifying limitations in the currently available evidence, this component of this study (presented here in Chapter 3) could be designed to address these evidence gaps. A composite indicator of vitamin B12 status (cB12) was included in the analyses and an extensive exercise was conducted to identify potential confounders.

Chapter 3 presents a secondary analysis of baseline data from the OPEN study to address the following research question:

- 2a) Is vitamin B12 status associated with electrophysiological indices of peripheral or central neurologic function in asymptomatic older people with moderately low vitamin B12 status?

My role in this research was to design the study, conduct the statistical analyses, interpret findings and write the manuscript.

This report has been prepared for publication and has already been submitted to a peer-reviewed journal. The manuscript is presented here alongside supplemental materials submitted to the journal for online supporting material, namely:

- Supplemental table 1: Multivariate regression models to assess association between vitamin B12 status and nerve conduction outcomes (unadjusted analyses)
- Supplemental table 2: Logistic regression models to assess association between vitamin B12 status and clinical markers of nerve function
- Supplemental table 3: Sensitivity analyses showing multivariate regression models to assess association between vitamin B12 status and nerve conduction outcomes in subjects with carpal tunnel syndrome excluded
- Supplemental table 4: Subgroup analyses showing multivariate regression models to assess interaction between vitamin B12 status and age for nerve conduction outcomes.



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Student	Lisa Miles
Principal Supervisor	Alan Dangour
Thesis Title	Vitamin B12 and neurological function in older people

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?			
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Where is the work intended to be published?	American Journal of Clinical Nutrition
Please list the paper's authors in the intended authorship order:	Lisa Miles, Elizabeth Allen, Kerry Mills, Robert Clarke, Ricardo Uauy, Alan Dangour
Stage of publication	Submitted

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	Lisa Miles designed the study, conducted the statistical analyses, interpreted findings, wrote the first draft of the manuscript and had primary responsibility for final content.
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Student Signature:

Date:

24/5/2016

Supervisor Signature: _____

Date: _____

24/5/2016

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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Sources of support

Funding for the OPEN study was provided by the Food Standards Agency (N05072) and the Department of Health. National Health Service Research and Development and King's College Hospital Trust Research and Development provided service

support costs. No further funding was received for the secondary analyses presented here.

Running head Vitamin B-12 status and neurologic function

Trial registration www.isrctn.com ISRCTN54195799

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

1 **Abstract**

2 **Background**

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

9 **Objective**

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

13 **Design**

14 We report cross-sectional analysis of baseline data from the Older People and
15 Enhanced Neurological Function study conducted in the South East of England. This
16 trial investigated the effectiveness of vitamin B-12 supplementation on
17 electrophysiological indices of neurological function in asymptomatic older people
18 (mean age 80y) with moderately low vitamin B-12 status (n=201). Vitamin B-12
19 status was assessed using total vitamin B-12, holotranscobalamin and a composite
20 marker, cB-12. Electrophysiological measures of sensory and motor components of
21 peripheral and central nerve function were assessed in all participants by a single
22 observer.

23 **Results**

24 In multivariate models, there was no evidence of an association between any
25 measure of vitamin B-12, holotranscobalamin or cB-12 and any of the nerve

conduction outcomes. There was no evidence of an association between vitamin B-12 status and clinical markers of neurologic function.

Conclusion

This secondary analysis of high quality trial data did not show any association between 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. Nevertheless, vitamin B-12-dependent impairment of neurological function in less healthy and more vitamin B-12 deplete populations cannot be excluded.

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). 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). As intakes of vitamin B-12 are mostly adequate (4), poor status in older people is largely attributable to age-related malabsorption of vitamin B-12 (5).

Vitamin B-12 is required for the initiation and maintenance of myelination of the nervous system (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, 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, 6, 7).

Although impaired neurologic function is a characteristic feature of overt vitamin B-12 deficiency, the neurologic and public health impact of moderately low vitamin B-12 status in older people is currently unclear. Neurological signs and symptoms associated with moderately low vitamin B-12 status can be non-specific and often go undetected because they are attributed to 'old age', yet can have an important impact on physical function. A recent systematic review (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 used composite measures of vitamin B-12 status involving both a biomarker of circulating vitamin B-12 and a functional biomarker (methyl malonic acid or homocysteine), as now recommended (9).

The Older People and Enhanced Neurological Function (OPEN) study afforded an opportunity to test whether there was an association between vitamin B-12 status and 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) (www.isrctn.com; ISRCTN54195799). The OPEN study was a randomised double-blind placebo-controlled trial that investigated the effectiveness of dietary supplementation with oral vitamin B-12 on electrophysiological indices of neurological function in older people with moderate B-12 deficiency (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 sample size for the trial was determined by a sample size calculation designed to achieve 90% power to detect a $\geq 28\%$ 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); holotranscobalamin (HoloTC;

117 Axis-Shield radioimmunoassay; Axis-Shield plc), total homocysteine (Hcy; Abbott IMx
118 analyzer; Abbott Laboratories), and folate (chloramphenicol-resistant microbiologic
119 assay) in a single laboratory in Trinity College Dublin. The Beckman Coulter method
120 (12) was used to assess vitamin B-12 status to screen participants for study
121 eligibility. A microbiological assay was used at study baseline to assess the vitamin
122 B-12 status of study participants. A full blood count was analysed for haematological
123 markers including haemoglobin, haematocrit and mean corpuscular volume.

124

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

132

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

conduction time from the distal stimulation site to the spinal cord) were also measured.

Transcranial magnetic stimulation, which painlessly and noninvasively excites the motor cortex (14), was used to measure central motor conduction in the corticospinal tract. A 13-cm diameter circular coil connected to a magnetic stimulator that provided a monophasic pulse was centred over the vertex to excite the hand area of the left motor cortex. A standard technique (15) determined the threshold for excitation. With the right ADM muscle partially activated voluntarily, 8 stimuli at 1.2 times the threshold were delivered to evoke motor evoked potentials (MEPs), the mean amplitude and minimal latency of which were measured. Similarly, 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, 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). 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) 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). cB-12 can be derived using equations that allow incomplete indicators, i.e. based on two or three of these markers (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). 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). 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 p-value of <0.01 used to test for statistical significance.

217

218 Age and sex are known confounders of the relationship between vitamin B-12 status
219 and neurological function and so have been adjusted for in analyses. In addition, the
220 following variables: alcohol frequency (daily, >once a week, approximately once a
221 fortnight, rarely/never); haemoglobin (g/l); haematocrit (%); mean corpuscular volume
222 (fl); and use of statins (yes/no) or proton-pump inhibitors (yes/no) were assessed as
223 potential confounders. If a potential confounder was found to be associated with both
224 an exposure and outcome, and its inclusion altered the effect size by $\geq 10\%$, then it
225 was included in the final model.

226

227 Skin temperature is a known confounder for nerve conduction outcomes, specifically
228 hand skin temperature for nerve conduction parameters in nerves of the upper limbs
229 (median and ulnar) and foot skin temperature for equivalent parameters in nerves of
230 the lower limbs (tibial, common peroneal, sural and superficial peroneal). The
231 analyses presented here combine outcomes in upper and lower limbs and so
232 inclusion of both hand and foot skin temperature in the models was considered.
233 However, hand and foot skin temperature were strongly positively correlated, so only
234 foot skin temperature was included in the final models due to concerns over
235 collinearity.

236

237 Sensitivity and subgroup analyses were conducted to test the robustness of the
238 findings. Sensitivity analyses were performed excluding participants with clinical
239 (previously decompressed nerves) or neurophysiological evidence [a median nerve
240 sensory conduction velocity < 40 m/s and (an ulnar sensory conduction velocity at
241 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, by testing for interaction between age 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 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. 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 between any measure of vitamin B-12, holoTC or cB-12 and 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 between 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 for any electrophysiological measure of nerve function (**Supplemental table 4**).

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.

Comparison with other studies

Few other studies have assessed neurologic function using nerve conduction tests.

In a longitudinal study (22), 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) (23), 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) (24). Heterogeneity in assays and cut-offs used to assess vitamin B-12 status (25) 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 (26, 27). 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). Furthermore, renal function, which is known to affect Hcy (28), was not measured in OPEN. In fact, cB-12 has recently been reported to be independently associated with renal function (29). 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 (25).

The OPEN study exclusion criteria means that the study participants had moderately low vitamin B-12 status. 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 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 is not 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 prevalent vitamin B-12 deficiency.

An important attribute 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.

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.

341

342 *Policy relevance and research needs*

343 In conclusion, this study did not identify an association between vitamin B-12 status
344 and peripheral or central neurologic function or clinical markers of neurologic function
345 in moderately vitamin B-12 deficient older people. The robustness of this finding is
346 supported by the use of a composite measure of vitamin B-12 status and a wide
347 range of nerve conduction tests to measure neurologic function. At a population level,
348 these findings cast doubt over concerns about moderately low vitamin B-12 status in
349 older people in relation to neurologic function.

350

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

355

356 **Acknowledgements**

357 The Older People and Enhanced Neurological Function (OPEN) Study was
358 supported by the Food Standards Agency (N05072) and the Department of Health.
359 National Health Service Research and Development and King's College Hospital
360 Trust Research and Development provided service support costs. No further funding
361 was received for the secondary analyses of OPEN study data presented here.

362

363 We thank all the participants and contributors involved in the OPEN study. We are
364 grateful to Dr. Anne Molloy for conducting biochemical analysis at Trinity College
365 Dublin, Ireland.

366

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

371

372 The funders had no role in the implementation, data collection, management,
373 analysis, or interpretation of the study or in the preparation, review, and approval of
374 the manuscript.

375

376 None of the authors declared a conflict of interest.

377

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Tables

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

	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	198	
None, n (%)		54 (27)
Basic or clerical, n (%)		34 (17)
Advanced or university, n (%)		52 (26)
Other, n (%)		58 (29)
Body mass index ²	201	26.8 (24.0, 29.3)
<18.5, n (%)		1 (0)
18.5 – 24.9, n (%)		69 (34)
25.0 – 29.9, n (%)		87 (43)
≥30, n (%)		44 (22)
Statins use, n (%)	162	67 (41)
Proton-pump inhibitor use, n (%)	162	53 (33)
Frequency of alcohol consumption	195	
Daily, n (%)		68 (35)
>once/week, n (%)		63 (32)
approx. once/fortnight, n (%)		19 (10)
rarely/never, n (%)		45 (23)
Frequency of meat consumption	191	
>once/week, n (%)		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-12 ¹	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	200	8.2 (5.6, 11.9)
Ulnar	200	6.6 (4.1, 9.1)
Sural	199	3.8 (1.6, 6.3)
Superficial peroneal	199	2.9 (1.1, 5.4)
Sensory nerve conduction velocities (m/s) ⁴		
Median	194	45.1 (5.5)
Ulnar	192	44.7 (4.6)
Sural	172	40.4 (5.3)
Superficial peroneal	159	41.1 (5.6)
CMAP amplitudes (mV) ²		
Median	200	3.8 (2.7, 5.0)
Ulnar	200	9.7 (8.5, 11.2)
Tibial	199	4.6 (2.1, 7.3)
Common peroneal	199	2.4 (1.1, 3.6)
Motor nerve conduction velocities (m/s) ⁴		
Median	200	51.3 (5.2)
Ulnar	200	54.5 (5.2)
Tibial	193	40.0 (5.1)
Common peroneal	189	42.8 (4.3)
Central motor conduction		
Left hemisphere ADM CMCT (ms) ⁴	198	5.5 (1.3)
Left hemisphere AH CMCT (ms) ⁴	182	13.6 (3.4)
Left hemisphere mean ADM MEP amplitude (mV) ²	200	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: Multivariate regression models to assess association between vitamin B-12 status and nerve conduction outcomes¹.

	Adjusted ² coefficients (95% CI)	B-12 (pmol/l)	HoloTC (pmol/l)	cB-12
<i>Sensory SAP amplitudes (μV)³</i>	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=0.87 ⁴	p=0.60 ⁴
<i>Sensory nerve conduction velocities (m/s)</i>	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
<i>Motor CMAP amplitudes (mV)</i>	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
<i>Motor nerve conduction velocities (m/s)</i>	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
<i>Central motor conduction (ms)</i>	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=0.99 ⁵	p=0.92 ⁵	p=0.86 ⁵

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.

²All 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)

Supplementary material

Unadjusted multivariate analyses (for online)

Clinical markers (for online)

Sensitivity and subgroup analyses (for online)

STROBE checklist (for reviewers only)

Published participant flowchart (for reviewers only)

ONLINE SUPPORTING MATERIAL

Supplemental table 1: Multivariate regression models to assess association between vitamin B-12 status and nerve conduction outcomes (unadjusted analyses)¹.

	Unadjusted coefficients (95% CI)	B-12 (pmol/l)	HoloTC (pmol/l)	cB-12
<i>Sensory SAP amplitudes (μV)²</i>	n	164	158	158
	Median	-0.01 (-0.03 – 0.00)	-0.01 (-0.04 – 0.03)	-0.84 (-3.10 – 1.26)
	Ulnar	-0.01 (-0.02 – 0.00)	-0.00 (-0.03 – 0.03)	-0.61 (-2.09 – 0.80)
	Sural	-0.00 (-0.01 – 0.01)	-0.01 (-0.04 – 0.03)	0.01 (-1.48 – 1.47)
	Superficial peroneal	-0.00 (-0.01 – 0.01)	-0.00 (-0.02 – 0.02)	0.33 (-0.92 – 1.44)
		p=0.17	p=0.99	p=0.75
<i>Sensory nerve conduction velocities (m/s)</i>	n	115	110	110
	Median	0.00 (-0.02 – 0.02)	0.02 (-0.02 – 0.06)	1.31 (-1.51 – 4.34)
	Ulnar	-0.01 (-0.03 – 0.00)	-0.03 (-0.07 – 0.00)	-1.80 (-3.64 – 0.10)
	Sural	-0.01 (-0.03 – 0.00)	-0.01 (-0.05 – 0.03)	-2.54 (-4.90 – -0.01)
	Superficial peroneal	-0.01 (-0.03 – 0.01)	0.00 (-0.05 – 0.05)	-1.05 (-3.67 – 1.79)
		p=0.10	p=0.10	p=0.00
<i>Motor CMAP amplitudes (mV)</i>	n	164	158	158
	Median	-0.01 (-0.01 – -0.00)	-0.00 (-0.02 – 0.01)	-0.18 (-0.76 – 0.54)
	Ulnar	0.01 (-0.00 – 0.01)	0.01 (-0.01 – 0.03)	0.85 (-0.11 – 1.75)
	Tibial	-0.01 (-0.01 – 0.00)	-0.00 (-0.03 – 0.02)	-0.11 (-1.79 – 1.38)
	Common peroneal	0.00 (-0.00 – 0.01)	0.00 (-0.01 – 0.02)	0.58 (-0.15 – 1.24)
		p=0.01	p=0.60	p=0.05
<i>Motor nerve conduction velocities(m/s)</i>	n	153	148	148
	Median	-0.00 (-0.02 – 0.01)	0.00 (-0.04 – 0.04)	-0.22 (-2.22 – 2.39)
	Ulnar	0.00 (-0.02 – 0.02)	0.01 (-0.04 – 0.05)	0.71 (-1.84 – 2.77)
	Tibial	0.00 (-0.01 – 0.02)	0.00 (-0.03 – 0.05)	0.28 (-1.88 – 2.44)
	Common peroneal	-0.01 (-0.02 – 0.01)	-0.02 (-0.05 – 0.02)	-0.38 (-2.17 – 1.84)
		p=0.79	p=0.76	p=0.93
<i>Central motor conduction (ms)</i>	n	147	142	142
	ADM CMCT	0.00 (-0.00 – 0.01)	-0.00 (-0.01 – 0.00)	0.17 (-0.34 – 0.62)
	AH CMCT	0.00 (-0.01 – 0.01)	-0.00 (-0.03 – 0.03)	0.02 (-1.78 – 1.84)
		p=0.40	p=0.66	p=0.74
	n	164	158	158
	Mean ADM MEP amplitude (mV)	-0.00 (-0.00 – 0.00)	-0.00 (-0.01 – 0.01)	-0.01 (-0.61 – 0.60)
		p=0.99	p=0.61	p=0.98

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.

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

Supplemental table 2: Logistic regression models to assess association between vitamin B-12 status and clinical markers of nerve function

Vitamin B12 (pmol/L)

					Unadjusted	Adjusted for age and sex
Clinical marker	Absent	Mean (sd)	Present	Mean (sd)	OR (95% CI)	OR (95% CI)
Right knee jerk	15	246.71 (61.09)	150	231.83 (54.09)	1.01 (1.00 – 1.01)	1.01 (1.00 – 1.02)
Right ankle jerk	45	235.60 (51.75)	120	232.28 (55.99)	1.00 (1.00 – 1.01)	1.00 (0.99 – 1.01)
Joint position sense (right great toe)	12	257.27 (58.46)	153	231.30 (54.17)	1.01 (1.00 – 1.02)	1.01 (1.00 – 1.02)
Vibration sense (right great toe)	105	229.20 (52.73)	60	240.16 (57.85)	0.97 (0.99 – 1.00)	1.00 (0.99 – 1.00)

Holotranscobalamin (pmol/L)

					Unadjusted	Adjusted for age and sex
Clinical marker	Absent	Mean (sd)	Present	Mean (sd)	OR (95% CI)	OR (95% CI)
Right knee jerk	15	51.16 (18.81)	144	52.75 (19.93)	1.00 (0.97 – 1.02)	1.00 (0.97 – 1.03)
Right ankle jerk	43	51.57 (19.87)	116	52.98 (19.81)	1.00 (0.98 – 1.01)	1.00 (0.98 – 1.02)
Joint position sense (right great toe)	11	51.57 (19.82)	148	52.67 (19.84)	1.00 (0.97 – 1.03)	1.00 (0.97 – 1.03)
Vibration sense (right great toe)	101	51.55 (18.54)	58	54.43 (21.81)	0.99 (0.98 – 1.01)	0.99 (0.98 – 1.01)

cB-12

					Unadjusted	Adjusted for age and sex
Clinical marker	Absent	Mean (sd)	Present	Mean (sd)	OR (95% CI)	OR (95% CI)
Right knee jerk	15	-0.19 (0.35)	144	-0.23 (0.37)	1.32 (0.30 – 5.79)	1.32 (0.26 – 6.66)
Right ankle jerk	43	-0.24 (0.41)	116	-0.22 (0.35)	0.85 (0.33 – 2.18)	0.90 (0.33 – 2.43)
Joint position sense (right great toe)	11	-0.20 (0.52)	148	-0.22 (0.36)	1.17 (0.22 – 6.29)	1.34 (0.25 – 7.38)
Vibration sense (right great toe)	101	-0.25 (0.37)	58	-0.17 (0.36)	0.52 (0.21 – 1.30)	0.52 (0.21 – 1.30)

Supplemental table 3: Multivariate regression models to assess association between vitamin B12 status and nerve conduction outcomes (subjects with carpal tunnel syndrome excluded)^{1,2}.

	Adjusted coefficients ² (95% confidence interval)	B-12 (pmol/l)	HoloTC (pmol/l)	cB-12
<i>Sensory SAP amplitudes (μV)</i>		n=139	n=133	n=133
Median		-0.01 (-0.02 – 0.01)	-0.02 (-0.06 – 0.01) ³	-1.30 (-3.34 – 0.87) ³
Ulnar		-0.01 (-0.02 – -0.00)	-0.01 (-0.04 – 0.02) ³	-0.53 (-1.96 – 0.87) ³
Sural		-0.00 (-0.01 – 0.01)	-0.02 (-0.05 – 0.02) ³	-0.26 (-2.02 – 1.49) ³
Superficial peroneal		0.00 (-0.01 – 0.01)	-0.00 (-0.02 – 0.02) ³	0.55 (-1.03 – 1.76) ³
		p=0.23	p=0.73 ³	p=0.48 ³
<i>Sensory nerve conduction velocities (m/s)</i>		n=99	n=94	n=94
Median		0.00 (-0.02 – 0.01)	0.01 (-0.05 – 0.02)	0.17 (-2.45 – 2.31)
Ulnar		-0.01 (-0.03 – 0.00)	-0.03 (-0.07 – 0.01)	-1.33 (-3.44 – 0.70)
Sural		-0.01 (-0.03 – 0.00)	-0.01 (-0.05 – 0.03)	-1.62 (-4.12 – 0.53)
Superficial peroneal		-0.01 (-0.02 – 0.01)	-0.00 (-0.05 – 0.04)	-0.04 (-2.97 – 2.75)
		p=0.24	p=0.63	p=0.34
<i>Motor CMAP amplitudes (mV)</i>		n=139	n=133	n=133
Median		-0.01 (-0.01 – -0.00)	-0.01 (-0.02 – 0.01)	-0.38 (-0.95 – 0.26)
Ulnar		0.01 (-0.00 – 0.01)	0.02 (-0.00 – 0.03)	0.97 (-0.01 – 1.85)
Tibial		-0.00 (-0.02 – 0.01)	-0.01 (-0.03 – 0.02)	-0.16 (-1.82 – 1.33)
Common peroneal		0.00 (-0.00 – 0.01)	0.00 (-0.01 – 0.02)	0.58 (-0.24 – 1.33)
		p=0.01	p=0.14	p=0.01
<i>Motor nerve conduction velocities (m/s)</i>		n=131	n=126	n=126
Median		-0.00 (-0.02 – 0.01)	-0.00 (-0.04 – 0.04)	-0.73 (-2.57 – 1.19)
Ulnar		0.00 (-0.02 – 0.02)	0.02 (-0.03 – 0.06)	0.97 (-1.971 – 3.34)
Tibial		0.00 (-0.01 – 0.02)	0.01 (-0.03 – 0.06)	0.70 (-1.37 – 2.83)
Common peroneal		-0.00 (-0.02 – 0.01)	-0.02 (-0.05 – 0.01)	-0.28 (-2.09 – 1.96)
		p=0.85	p=0.52	p=0.72

¹SAP, sensory action potential; CMAP, compound muscle action potential.

²All analyses adjusted for age, sex and foot skin temperature, unless otherwise stated.

³Adjusted for age, sex, skin temperature (foot) and mean corpuscular volume.

Supplemental table 4: Multivariate regression models to assess interaction between vitamin B12 status and age for nerve conduction outcomes^{1, 2}.

	Coefficients (95% CI) for interaction parameter ²	B-12 (pmol/l)	HoloTC (pmol/l)	cB-12
<i>Sensory SAP amplitudes (μV)</i>	n=164	n=158	n=158	
Median	-0.00 (-0.01 -0.00)	-0.00 (-0.01 – 0.01) ³	0.03 (-0.73 – 0.63) ³	
Ulnar	-0.00 (-0.01 -0.00)	-0.00 (-0.01 – 0.00) ³	-0.28 (-0.60 – 0.05) ³	
Sural	-0.00 (-0.00 -0.00)	0.00 (-0.01 – 0.01) ³	0.07 (-0.39 – 0.52) ³	
Superficial peroneal	0.01 (-0.00 – 0.00)	-0.00 (-0.01 – 0.00) ³	0.05 (-0.42 – 0.46) ³	
	p=0.42	p=0.74 ³	p=0.35 ³	
<i>Sensory conduction velocities (m/s)</i>	n=115	n=110	n=110	
Median	0.00 (-0.01 – 0.01)	0.00 (-0.01 – 0.02)	0.40 (-0.33 – 1.34)	
Ulnar	0.00 (-0.00 – 0.01)	-0.00 (-0.01 – 0.00)	-0.11 (-0.62 – 0.31)	
Sural	-0.00 (-0.01 – 0.01)	-0.00 (-0.01 – 0.01)	-0.46 (-1.33 – 0.31)	
Superficial peroneal	0.00 (-0.00 – 0.01)	-0.01 (-0.02 – 0.00)	-0.70 (-1.45 - -0.05)	
	p=0.54	p=0.57	p=0.38	
<i>Motor CMAP amplitudes (mV)</i>	n=164	n=158	n=158	
Median	-0.00 (-0.00 – 0.00)	-0.00 (-0.01 – 0.00)	-0.13 (-0.29 – 0.07)	
Ulnar	-0.00 (-0.00 – 0.00)	0.00 (-0.00 – 0.00)	-0.02 (-0.30 – 0.25)	
Tibial	-0.00 (-0.00 – 0.00)	0.00 (-0.01 – 0.01)	0.03 (-0.36 – 0.44)	
Common peroneal	0.00 (-0.00 – 0.00)	-0.00 (-0.01 – 0.00)	-0.18 (-0.40 – 0.01)	
	p=0.95	p=0.06	p=0.23	
<i>Motor conduction velocities (m/s)</i>	n=153	n=148	n=148	
Median	0.00 (-0.00 – 0.01)	0.00 (-0.01 – 0.02)	0.45 (-0.17 – 1.26)	
Ulnar	-0.00 (-0.01 – 0.00)	-0.00 (-0.01 – 0.01)	-0.40 (-1.16 – 0.23)	
Tibial	0.00 (-0.00 – 0.01)	-0.00 (-0.01 – 0.01)	0.01 (-0.75 – 0.77)	
Common peroneal	0.00 (-0.00 – 0.00)	-0.01 (-0.02 - -0.00)	-0.40 (-0.99 – 0.44)	
	p=0.76	p=0.09	p=0.21	
<i>Central Motor Conduction Time (ms)</i>	n=147	n=142	n=142	
ADM	0.00 (-0.00 – 0.00)	-0.00 (-0.00 – 0.00)	-0.03 (-0.17 – 0.12)	
AH	-0.00 (-0.01 – 0.00)	-0.01 (-0.01 - -0.00)	-0.55 (-0.99 - -0.18)	
	p=0.70	p=0.06	p=0.03	
Mean ADM MEP amplitude (mV)	n=164	n=158	n=158	
	-0.00 (-0.00 – 0.00) ⁴	0.00 (-0.00 – 0.00) ⁴	-0.14 (-0.31 – 0.02) ⁴	
	p=0.16 ⁴	p=0.83 ⁴	p=0.11 ⁴	

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.

²All analyses adjusted for age, sex and foot skin temperature, unless otherwise stated.

³Adjusted for age, sex, skin temperature (foot) and mean corpuscular volume.

⁴Adjusted for age, sex, skin temperature (hand)

Chapter 4: Systematic review of evidence from interventions

4.1 Introduction

To date, the scientific evidence on effectiveness of vitamin B12 supplementation on neurological function in older people has not been systematically reviewed. A systematic review was conducted to assess the impact of vitamin B12 supplementation on neurological outcomes, with a research question as follows:

1b) Is there any evidence in the available literature that vitamin B12 supplementation improves neurological function in older people?

A common search strategy was used for both systematic reviews presented in Chapters 2 and 4. However, the systematic review presented in Chapter 2 sought epidemiological studies assessing the association between vitamin B12 and neurological function, whereas the systematic review presented here (Chapter 4) includes intervention studies that have measured the effectiveness of a vitamin B12 intervention on neurological outcomes. The common systematic search of nine bibliographic databases was conducted on 28 March 2013 as described in Chapter 2. This systematic search of the literature was updated with a further search of Medline covering the period from 1 January 2013 to 18 March 2016, using the same search terms. This second search of the literature was conducted to ensure the systematic review of interventions was up-to-date for thesis submission. Papers for the systematic review of interventions were assessed for inclusion based on the criteria in Box 1.

Box 1: Inclusion criteria for studies

Study design

All study types assessing the effects of a vitamin B12 intervention on neurological outcomes, except intervention studies without a placebo comparison group, case reports/series, narrative reviews, editorials, conference reports and observational studies comparing treatment status.

Subjects

Older people with a median or mean age ≥ 50 years resident in institutions or the community. Studies of subject groups with known existing medical conditions affecting neurological function (including alcoholism, HIV, diabetes-associated neuropathy or motor neurone disease), vitamin B12 status (including bariatric surgery) or metabolites of vitamin B12 (including renal insufficiency) were excluded.

Intervention

All forms of vitamin B12 (cobalamin, cyanocobalamin or hydroxocobalamin) supplementation were included, administered orally or intramuscularly. This included foods fortified with vitamin B12 but not multiple vitamin and mineral supplements.

Outcome

Included outcomes were peripheral sensory or motor nerve function/conduction, central motor conduction; peripheral neuropathy; clinical signs and symptoms of neurological (but not cognitive) function (somatosensory disorders, knee and ankle jerk/reflexes, joint, position and vibration sense, ataxia, and proprioception); and self-reported neurological (but not cognitive) symptoms (pain, altered sensation, unsteadiness, prickly feelings, weakness, numbness, and difficulty walking).

Methods for assessing eligibility of studies, assessing risk of bias, data extraction and synthesising results are as described in Chapter 2, and were the same for the original systematic review of interventions and its update. No study was excluded on the basis of quality. Forms for data extraction and assessment of risk of bias were developed based on controlled trial checklists developed by SIGN¹. Data were extracted into forms, defined according to the SIGN guidance, and risk of bias was assessed for each study based on the research question, randomisation procedures, blinding to treatment allocation, similarity of treatment and control groups at baseline, outcome assessment and percentage drop out.

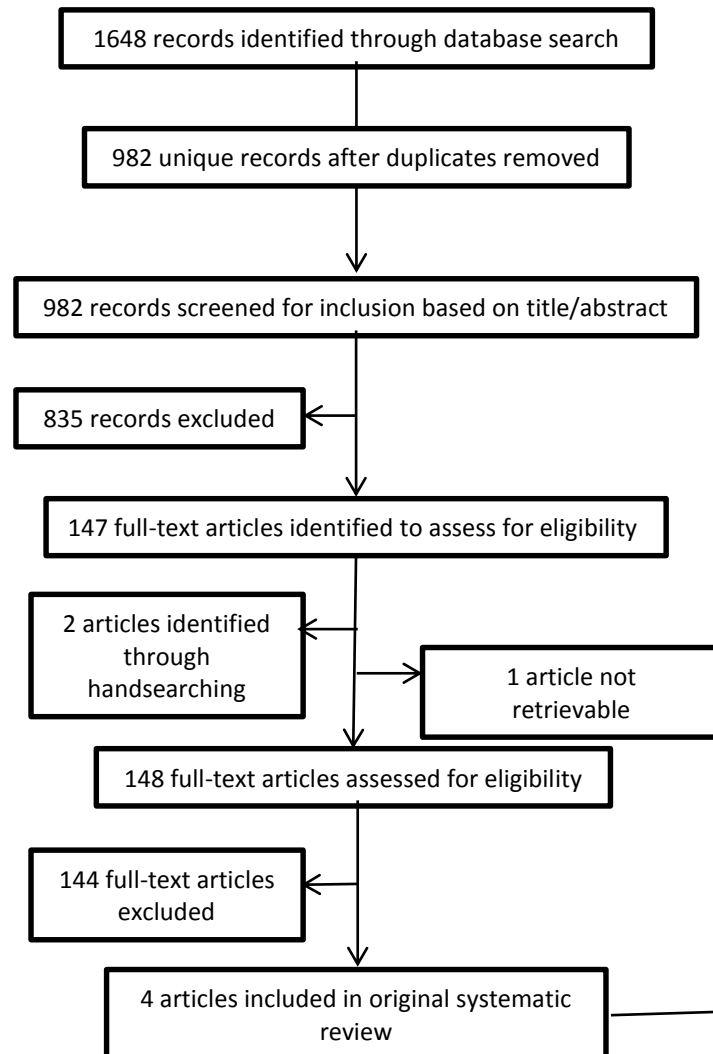
4.2 Study selection

The original search identified 982 records. Of these, 835 were excluded as not relevant following title and abstract review. Full-text articles for the remaining 147 records were

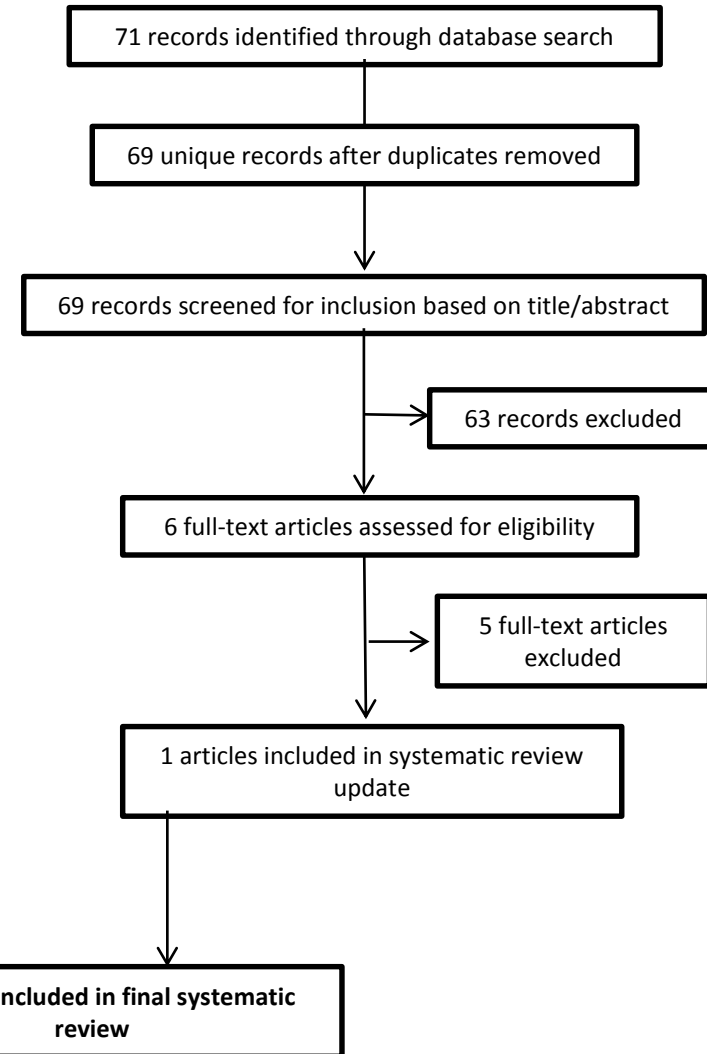
sought, though one was not retrievable². Two additional papers identified through handsearching did not meet the inclusion criteria. A total of 148 full-text articles were examined in detail. 144 of these articles did not meet the inclusion criteria leaving four articles³⁻⁶ included in the primary review. The systematic review update identified 69 unique records. Of these, 63 were excluded as not relevant following title and abstract review. Full-text articles for the remaining six articles were examined in detail. Five of these did not meet the inclusion criteria leaving one article⁷ to add to those identified in the primary review, giving a total of five articles (Figure 1).

Figure 1: Study selection process for the systematic review of the effects of vitamin B12 supplementation on neurological function in older people.

PRIMARY SYSTEMATIC REVIEW TO 28/03/2013



SYSTEMATIC REVIEW UPDATE (01/01/2013-18/03/2016)



4.3 Study characteristics

The five included articles reported three randomised controlled trials (RCTs)^{3-5, 7} and one non-randomised controlled trial (nRCT)⁶. Two articles describing RCTs are from the same study. One article reports the original RCT findings⁴ and the second article⁵ describes subsequent sub-group analyses from the same RCT based on baseline holoTC status.

One RCT reported by Kwok *et al.*³ was based on subjects in Hong Kong, with subjects for the remaining three studies based in Europe. RCTs by Kwok *et al.*³ and Hvas *et al.*^{4, 5} were conducted in subjects with biological evidence of low vitamin B12 status; the RCT by Dangour *et al.*⁷ was conducted in asymptomatic adults with moderately low vitamin B12 status; and the nRCT by Bjorkegren and Svardsudd⁶ was conducted in a general population group. However, the nRCT intervention varied according to the presence of neurological symptoms.

Interventions used in studies by Kwok *et al.*³ and Hvas *et al.*^{4, 5} were similar and based on cyanocobalamin administered intramuscularly and follow-up was at 3-6 months. The study by Dangour *et al.*⁷ used a 1 mg vitamin B12 oral supplement intervention with 12 months follow-up. Kwok *et al.*³ and Hvas *et al.*^{4, 5} assessed neurological function through clinical examination; Hvas *et al.*^{4, 5} also measured self-reported neurological symptoms. Bjorkegren and Svardsudd⁶ assessed neurological function through clinical examination and self-reported neurological symptoms. The study by Dangour *et al.*⁷ was the only one to measure nerve function through electrophysiological measures; a wide range of nerve conduction outcomes were reported covering peripheral motor and sensory nerve function and central motor conduction. Clinical markers of nerve function were reported in addition. All study characteristics and findings are summarised in Table 1. Table 2 displays study characteristics used to assess risk of bias (based on the adapted SIGN checklist).

Table 1: Study characteristics and findings of intervention studies

Author (year)	Subject characteristics	Intervention(s)	Blinding and follow-up	Outcomes	Effects (sizes) of intervention (s)
Kwok <i>et al.</i> (1998) ³	Subjects with serum B12<120 pmol/l, based in Hong Kong (n=50), mean age=76.6y (intervention), 77.4y (control).	1) 3X 1mg cyanocobalamin administered i/m for first week, one dose weekly for further 3 weeks, followed by one monthly dose thereafter until follow-up (n=23) 2) No placebo given to control group (n=27)	3-6 Mo follow-up. Not blinded to subjects or investigators.	NP: Motor function scale of the adult Luria-Nebraska neuropsychological battery: measure of kinesthesia, drawing, fine motor, spatial movement and oral motor skills	NP: Baseline versus follow-up in intervention group: - Kinesthesia deteriorated at follow-up [4.2 (2.8) vs 2.6 (2.4), p<0.05] - Fine motor skills deteriorated at follow-up [9.8 (3.0) vs 8.6 (3.4), p<0.05] - Drawing deteriorated at follow-up [10.1 (2.5) vs 7.2 (2.8), p<0.005] - No stat sig change in spatial movement or oral motor scores. Baseline versus follow-up in control group: - Drawing deteriorated at follow-up [9.1 (3.9) vs 7.3 (3.5), p<0.005] - Oral motor deteriorated at follow-up [0.2 (0.6) vs 1.1 (1.5), p<0.005] - No stat sig change in spatial movement, kinesthesia or fine motor scores. Differences in changes in score between intervention and control groups: - Stat sig deterioration in kinesthesia, fine motor and oral motor scores in intervention group compared to controls (p<0.05)
Hvas <i>et al.</i> (2001) ⁴ Hvas and Nexø (2005) ⁵	Patients with elevated MMA (0.40-2.00 µmol/l), based in Denmark (n=140), median age=75y (intervention group), 74y (placebo group).	1) Intervention: i/m injections of 1mg cyanocobalamin once weekly for 4 wks (n=70) 2) Placebo: i/m injections of 1ml isotonic NaCl once weekly for 4 wks(n=70)	3 Mo follow-up Double-blinded to investigators and subjects	NP: Vibration sensation, neurological disability score based on finger-nose test, heel-knee-shin test, dysdiadochokinesis, Romberg test and gait. NS: Neurological symptom score based on presence of muscle weakness, sensory disturbances or autonomic symptoms.	NP: - Vibration sensation: small improvements in treatment (upper extremities p=0.04, lower extremities p=0.89) and placebo (upper extremities p=0.01, lower extremities p=0.08) groups; no stat sig improvement in treatment group compared to placebo group. - No stat sig difference between treatment/placebo in change in neurologic disability score. NS: - No stat sig difference between treatment/placebo in change in neurologic symptom score or neurologic disability score. <i>Subgroup analyses based on baseline P-MMA, P-B12, P-Hcy or holoTC:</i> NP: - Vibration sensation: no stat sig improvement in treatment group with P-MMA≥0.6 µmol/l compared to the placebo group. - No stat sig difference between treatment/placebo in change in neurologic disability score.

					<p>- No stat sig differences between treatment group and placebo group in neurological disability score, vibration sensation (upper or lower extremities) if stratified by baseline holoTC (< or $\geq 40\text{pmol/l}$).</p> <p>NS:</p> <p>- Stat sig association between improvement in neurologic symptoms in treatment group compared to placebo if P-MMA$\geq 0.6\text{ }\mu\text{mol/l}$ (p=0.014) (n=24 treatment/21 placebo) or if P-tHcy $\geq 15\text{ }\mu\text{mol/l}$ (p=0.034) (n=25 treatment/26 placebo).</p> <p>- Borderline stat sig association if P-B12<250pmol/l (p=0.06) (n=29 treatment/34 placebo).</p> <p>- No stat significant differences between treatment group and placebo group in neurological symptoms if stratified by baseline holoTC (< or $\geq 40\text{pmol/l}$).</p>
Bjorkegren and Svardsudd (2004) ⁶	General population in Sweden (n=118). Treated group had serum B12 <300 pmol/l and (serum MMA $\geq 0.37\text{ }\mu\text{mol/l}$ or serum Hcy $\geq 15\text{ }\mu\text{mol/l}$). B12 status of untreated group unknown. Mean age=78.5y (treated group), 74.6y (untreated group).	<p>1) Treatment for 6 Mo:</p> <p>(a) If neurological or psychiatric symptoms were present 1mg of hydroxocobalamin given i/m 5d/wk for 2 wk, then once weekly for 2 Mo and then once monthly.</p> <p>(b) If such symptoms not present given 2mg cyanocobalamin tablets twice a day for 1 Mo then 1 mg daily. Those subjects with elevated serum MMA or Hcy at 6 Mo, then received 5mg folic acid daily (n=61).</p> <p>2) Untreated group did not receive a placebo (n=57)</p> <p>Treatments continued until follow-up.</p>	36 Mo follow-up Not blinded to subjects or investigators	<p>NP: Vibration perception threshold, mean reflex intensity</p> <p>NS: Neurological symptom score assessed by questionnaire</p>	<p>NP:</p> <p>- No stat significant differences between treated/untreated groups in any of the outcomes at 36 Mo**.</p> <p>- No stat significant change across the study period within groups for any outcome (except a stat sig decline in mean reflex intensity in the treated group)**.</p> <p>NS:</p> <p>- No stat significant differences between treated/untreated groups in any of the outcomes at 36 Mo**.</p> <p>- No stat significant change across the study period within groups for any outcome**.</p>

Dangour <i>et al.</i> (2015) ⁷	Asymptomatic people based in the UK, with moderately low vitamin B12 status (≥ 107 and < 210 pmol/l) who did not have anaemia. Mean age=79.9y (intervention group), 80.1y (placebo group).	1) Daily 1mg cyanocobalamin as oral supplement (n=99). 2) Daily oral placebo supplement (n=102)	12 Mo follow-up Double-blinded to investigators and subjects	NF: Motor nerve conduction (tibial and common peroneal CMAP amplitude and conduction velocity); sensory nerve conduction (sural and superficial peroneal SAP amplitude and conduction velocity); central motor conduction (abductor digiti motor evoked potential amplitude, abductor digiti minimi CMCT and abductor hallucis CMCT) NP: Knee and ankle jerk reflexes, great toe position and vibration sense.	NF: - No stat sig difference between intervention and placebo groups in motor nerve conduction outcomes* - No stat difference between intervention and placebo groups in sensory nerve conduction outcomes* - No stat difference between intervention and placebo groups in central motor conduction outcomes* NP: - No stat sig difference between intervention and placebo groups in knee or ankle jerk reflexes* - No stat sig difference between intervention and placebo groups in great toe position or vibration sense*
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*Adjustments made for age and sex.

** Adjustments made for age, sex and serum creatinine.

Abbreviations

B12, Vitamin B12; CMAP, Compound muscle action potential; CMCT, Central motor conduction time; d, days; Hcy, homocysteine; HoloTC, holotranscobalamin; i/m, intramuscular; MMA, methyl malonic acid; Mo, months; NaCl, sodium chloride; NF, electrophysiological measure of nerve function; NP, clinically assessed signs of neuropathy; NS, self-reported neurological symptoms; P-B12, Plasma vitamin B12; P-Hcy, Plasma homocysteine; P-MMA, plasma methyl malonic acid; SAP, sensory action potential; stat sig, statistically significant; vs, versus; wk, weeks; y, years.

Table 2: Study characteristics used to assess risk of bias (based on adapted SIGN checklist)

Study characteristic	Kwok <i>et al.</i> (1998) ³	Hvas <i>et al.</i> (2001) ⁴ Hvas and Nexø (2005) ⁵	Bjorkegren and Svardsudd (2004) ⁶	Dangour <i>et al.</i> (2015) ⁷
Focussed research question	Poorly addressed	Well covered	Poorly addressed	Well covered
The assignment of subjects to treatment groups is randomised	Adequately addressed	Well covered	Not addressed	Well covered
Subjects and investigators are kept blind about treatment allocation	Not addressed	Adequately addressed	Not addressed	Well covered
The treatment and control groups are similar at the start of the trial	Poorly addressed	Adequately addressed	Not addressed	Well covered
All relevant outcomes are measured in a standard, valid and reliable way	Poorly addressed	Adequately addressed (neurological markers based on clinical examination Poorly addressed (self-reported neurological symptoms))	Adequately addressed (neurological markers based on clinical examination Poorly addressed (self-reported neurological symptoms))	Well covered
Percentage of subjects recruited into each treatment arm that dropped out before the study completed	0% both arms	7% treatment 1% placebo	36% treatment 0% control referents	5% both arms

4.4 Findings

Included studies varied in their risk of bias (see Table 2). The study by Bjorkegren and Svardsudd ⁶ has a risk of bias for a number of reasons: the research question was not clearly defined; the vitamin B12 intervention varied accordingly to the neurological symptom status of the subjects; over a third of the subjects receiving the intervention were lost to follow-up, there was no blinding and the controls did not receive a placebo. Therefore, limited conclusions can be drawn from this study.

The remaining RCTs varied in risk of bias. Dangour *et al.*⁷ reported a well designed, double-blinded RCT with low-drop out and a wide range of nerve conduction outcomes. Accordingly, it was assessed to have a low risk of bias. The study did not detect any benefits of daily 1mg vitamin B12 supplementation over one year on a wide range of neurologic outcomes.

The RCT by Hvas *et al.*^{4, 5} showed relatively low risk of bias. All study characteristics used to assess bias were at least adequately addressed but follow-up was short and nerve function was assessed by clinical examination and self-reported symptoms only. This study reports on the effectiveness of 1mg cyanocobalamin administered intramuscularly weekly for four weeks on neurological outcomes at three months. Primary analyses show no statistically significant difference in vibration sensation, neurological disability score (based on clinical markers) or neurological symptom score between intervention and control groups. There is, however, some suggestion of effectiveness of the intervention in further subgroup analyses to isolate subjects with the lowest vitamin B12 status. A statistically significant association between improvement in neurologic symptom score (but not vibration sensation or neurological disability score) in the treatment group compared to placebo if P-MMA \geq 0.6 μ mol/l ($p=0.014$) or if P-tHcy \geq 15 μ mol/l ($p=0.034$) was reported⁴. The association between improvement in neurologic symptom score in treatment versus placebo reached borderline statistical significance ($p=0.06$) if P-B12 $<$ 250pmol/l. These subgroup analyses were based on subject numbers ranging from 21-34 in each placebo/treatment group. However, in analyses stratified according to patients' holoTC status at baseline ($<$ or \geq 40pmol/l), there was no statistically significant difference between placebo and treatment group⁵.

The RCT by Kwok *et al.*³ suffered from lack of blinding (to investigators or subjects), a poorly defined outcome based on an aggregate measure of clinical measures of neurological function and a poorly defined research question. This study reported a statistically significant deterioration in kinesthesia, fine motor and oral motor scores in

the intervention compared to the control group, but the poor quality of the study means that this finding needs to be interpreted with caution.

4.5 Discussion

Very few studies are available to help understand whether vitamin B12 supplementation improves neurological function or clinically relevant neurological outcomes in older people.

Only four studies were available to answer the research question, and two of these were subject to risk of bias. The remaining two studies assessed the effectiveness of different types of intervention (oral supplementation versus intramuscular injection) and only one used electrophysiological measures of nerve conduction, which are the most sensitive and objective measures of neurologic function. It difficult to reach firm conclusions from heterogeneous evidence so limited in size and quality.

One RCT by Dangour *et al.*⁷ assessed the effects of daily 1mg vitamin B12 supplementation, and reported no difference between intervention and placebo in a wide range of outcomes covering all aspects of neurologic function. Theoretically, if the benefits of vitamin B12 supplementation are limited to a particular aspect of nerve function, this study would have been able to detect such differences; for example if benefits were limited to the PNS or CNS, sensory or motor peripheral nerve function, or measures reflective of axonal degeneration (CMAP and SAP amplitudes) or impaired myelination (motor or sensory conduction velocities). As there was no evidence of an effect of vitamin B12 supplementation, this suggests no such aspects of neurologic function were affected. However, there remains the possibility that the follow-up period was not long enough to detect an effect of vitamin B12 supplementation or that dose of vitamin B12 was too low. Furthermore, as subjects were asymptomatic and had only a moderately low vitamin B12 status, it is possible that subjects were too replete in vitamin B12 for supplementation to have a benefit.

A further RCT by Hvas *et al.*^{4, 5} provided information about the effectiveness of a 1mg weekly cyanocobalamin intervention administered intramuscularly. Subjects already

had elevated MMA and the study⁴ provided no evidence of effectiveness. However, a statistically significant association between improvement in neurologic symptom score (but not vibration sensation or neurological disability score) was reported in the treatment group compared to placebo, in subjects with the lowest baseline vitamin B12 status. Such subgroup analyses were not reported in any other study included in the review but further investigation in future studies may be warranted.

Systematic reviews specifically aim to minimise bias resulting from partial identification, evaluation and reporting of the available evidence base. The search strategy used in the present systematic review was comprehensive. However, the literature search was limited to English language and published literature, and may have missed relevant studies published in other languages or in the grey literature such as conference proceedings. The search strategy for the systematic review update was limited to Medline and so relevant studies indexed in other bibliographic databases could have been missed. However, 75% of the studies identified in the original search were indexed in Medline. Both assessment of papers for inclusion and data extraction have been conducted by one reviewer. Ideally these processes would have been conducted in duplicate independently by two reviewers. Therefore, it is possible that some studies could have been included or excluded from the review inappropriately. The heterogeneity of the available evidence precluded the conduct of meta-analysis.

4.5.1 Comparison with other studies

To the best of my knowledge, this is the first systematic review investigating the impact of vitamin B12 supplementation on neurological function in older people. Existing evidence has been shown to be limited. A clustered RCT designed to compare two modes of vitamin B12 supplementation (daily 1mg vitamin B12 oral supplementation alongside consumption of standard fortified foods; consumption of fortified foods to supply 1mg vitamin B12 daily) on nerve conduction among healthy older people living in Chile has also been conducted⁸ but the results are not yet published.

4.6 Conclusion

This systematic review shows that the currently available evidence indicates that vitamin B12 supplementation does not improve neurological function or clinically relevant neurological outcomes in older people. However, the available evidence was limited in size and quality so definitive conclusions cannot be drawn and some questions remain unanswered. One RCT provides evidence to suggest those with the lowest vitamin B12 status benefit from an intramuscular vitamin B12 intervention in terms of improvement in neurological symptoms. It is not known whether this extends to clinical markers of neurologic function or nerve conduction or whether the same effect would be seen with an oral vitamin B12 intervention.

Impaired neurological function is a recognised consequence of vitamin B12 deficiency but it is not known what needs to be done to identify and treat those at risk. It has been suggested that routine vitamin B12 supplementation is needed in older people in light of the high prevalence of mild, preclinical deficiency⁹ but it is important to be clear whether there is sufficient evidence to infer those at risk will benefit. Further high quality research is warranted to investigate whether those older people with low vitamin B12 status, but not clinically identified as vitamin B12 deficient, would benefit from vitamin B12 supplementation in terms of neurologic function.

4.7 References

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Chapter 5: Impact of baseline or change in vitamin B12 status on the effects of vitamin B12 supplementation on neurologic function in older people in the OPEN study

Preface

As described in Chapter 4, the systematic review of intervention studies identified four studies that overall show that the currently available evidence indicates that supplementation with vitamin B12 does not improve neurologic function in older people (research question 1b). However, subgroup analyses from a single study suggested that it is possible that improvement in neurologic function from vitamin B12 supplementation is only apparent in people with the lowest vitamin B12 status. This hypothesis could be tested further in order to advance knowledge about the effects of vitamin B12 supplementation on neurologic function in older people.

Data from the OPEN study afforded an opportunity to test whether the effectiveness of oral vitamin B12 supplementation on electrophysiological indices of neurologic function alters according to baseline B12 status or change in B12 status. Conducting such secondary analyses in OPEN had the advantage of using a high quality dataset derived from asymptomatic older people with moderately low vitamin B12 status.

Chapter 5 presents a secondary analysis of data from the OPEN study to address the following research question:

- 2b) Does baseline vitamin B12 status or change in vitamin B12 status alter the effectiveness of dietary supplementation with vitamin B12 on electrophysiological indices of neurologic function in asymptomatic older people with moderately low vitamin B12 status?

My role in this research was to design the study, conduct the statistical analyses, interpret findings and write the manuscript. This report has been submitted for publication in a peer-reviewed journal. The manuscript is presented here.



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Student	Lisa Miles
Principal Supervisor	Alan Dangour
Thesis Title	Vitamin B12 and neurological function in older people

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SECTION B – Paper already published

Where was the work published?			
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Please list the paper's authors in the intended authorship order:	Lisa Miles, Elizabeth Allen, Robert Clarke, Kerry Mills, Ricardo Uauy, Alan Dangour
Stage of publication	Submitted

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	Lisa Miles designed the study, conducted the statistical analyses, interpreted findings, wrote the first draft of the manuscript and had primary responsibility for final content.
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Student Signature: _____

Lisa Miles

Date: _____

24/5/2016

Supervisor Signature:



Date: 24/5/2016

Impact of baseline or change in vitamin B12 status on the effects of vitamin B12 supplementation on neurologic function in older people in the OPEN study.

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Sources of support

Funding for the OPEN study was provided by the Food Standards Agency (N05072) and the

Department of Health. National Health Service Research and Development and King's

College Hospital Trust Research and Development provided service support costs. No

further funding was received for the secondary analyses presented here.

Trial registration www.isrctn.com ISRCTN54195799

24 **Abstract**

25 **Background**

26 Studies to date suggest that vitamin B12 supplementation does not improve neurologic
27 function in older people but it is possible that improvement is only apparent in people with
28 the lowest vitamin B12 status. This study assessed whether baseline or change in vitamin
29 B12 status (from baseline over 12 months) altered the effectiveness of dietary vitamin B12
30 supplementation on electrophysiological indices of neurologic function in asymptomatic
31 older people with moderately low vitamin B12 status.

32 **Methods**

33 Vitamin B12 status was assessed using vitamin B12, holotranscobalamin, homocysteine and
34 combination of all three such measures (cB12). Eleven electrophysiological measures of
35 sensory and motor components of peripheral and central nerve function were also
36 assessed. Analyses were restricted to the intervention arm (n=91) of the trial.

37 **Results**

38 Linear regression analyses revealed some moderate associations between several measures
39 of baseline vitamin B12 status and neurologic responses to supplementation for some
40 outcomes. However, the directions of effect varied and heterogeneity in findings across
41 outcomes cannot be explained according to type of neurologic outcome. There were no
42 differences in the neurologic response to vitamin B12 supplementation according to any
43 indicator of *change* in B12 status.

44 **Conclusions**

45 This study provides no evidence of a difference in the effect of vitamin B12 supplementation
46 on peripheral or central nerve conduction dependent on baseline or change in vitamin B12
47 status. There is insufficient evidence of efficacy on neurological function to support

- 48 population-wide recommendations for vitamin B12 supplementation in healthy
- 49 asymptomatic older people, even among those with the lowest vitamin B12 status.

Introduction

Ageing is associated with a decline in vitamin B12 status, and prevalence of deficiency increases with age¹. As dietary intakes are usually adequate in healthy populations², the age-related decline in vitamin B12 status is usually attributed to atrophic gastritis which reduces absorption of vitamin B12³. 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^{4, 5}.

It has been suggested that routine vitamin B12 supplementation is needed in older people in light of the high prevalence of mild, preclinical deficiency^{6 7}. Indeed, US adults aged >50 years are advised to meet their vitamin B12 recommended daily allowance of 2.4 µg/day mainly by consuming food fortified with vitamin B12 or a vitamin B12-containing supplement⁷. Yet, few studies⁸⁻¹¹ are available to help understand whether vitamin B12 supplementation improves neurological function in older people. Overall, these studies suggest that vitamin B12 supplementation does not improve neurologic function in older people but the available evidence is limited in size and quality.

It is possible that improvement in neurologic function from vitamin B12 supplementation is only apparent in people with the lowest vitamin B12 status. This may explain the lack of treatment effect reported in studies available to date. Although primary analyses from the study by Hvas *et al.*,⁹ showed no statistically significant differences in neurologic outcomes between intervention (1mg cyanocobalamin administered intramuscularly weekly for four weeks) and control groups, there was evidence of effectiveness of treatment in individuals

with the lowest vitamin B12 status. A statistically significant association between improvement in neurologic symptom score was reported in the treatment group compared to placebo if plasma methyl malonic acid $\geq 0.6 \mu\text{mol/l}$ or plasma total homocysteine (Hcy) $\geq 15 \mu\text{mol/l}$ at baseline. There is further evidence to support greater benefits of vitamin B12 supplementation in people with the lowest vitamin B12 status from studies considering the effects of B vitamin supplementation on cognitive outcomes. The VITACOG trial 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 Hcy levels ^{12, 13}.

The Older People and Enhanced Neurological function (OPEN) study afforded an opportunity to further explore in a high quality dataset whether improvement in neurologic function from vitamin B12 supplementation is limited to people with the lowest vitamin B12 status. The OPEN study was a double-blind placebo-controlled randomised controlled trial (RCT) investigating the effects of 1 mg vitamin B12 oral supplementation on electrophysiological indices of neurologic function in older people with moderately low vitamin B12 status. The results demonstrated no effect of supplementation with vitamin B12 on any measure of nerve conduction compared with placebo¹¹, but it is possible that overall the participants were too replete in vitamin B12 to be able to detect any benefit of vitamin B12 supplementation. The present study involves a secondary analysis of data from the OPEN study designed to explore whether differences in baseline vitamin B12 status or change in vitamin B12 status alters the effectiveness of vitamin B12 supplementation on electrophysiological indices of neurologic function in asymptomatic older people with moderately low vitamin B12 status.

98

99 **Methods**

100 This study is a secondary analysis of data from the OPEN study, the protocol of which has
101 been published¹⁴ (www.isrctn.com; ISRCTN54195799). Screening for participation in the
102 OPEN study took place between November 2008 and February 2010. Participants were aged
103 ≥ 75 years and recruited from 7 general practices in South East England. Individuals with
104 dementia, epilepsy, alcohol addiction, diabetes, pacemakers or other implanted metallic
105 devices, residents of nursing homes, or a previous diagnosis of pernicious anaemia were
106 excluded. Potential participants with significant cognitive impairment or who reported
107 current consumption of vitamin B12 supplements or who had received a vitamin B12
108 injection in the previous 6 months were excluded. Individuals with moderate vitamin B12
109 deficiency [serum vitamin B12 concentrations ≥ 107 and < 210 pmol/L (Beckman Coulter
110 assay)] who did not have anaemia (haemoglobin concentrations ≥ 110 g/L for women and
111 ≥ 120 g/L for men) were eligible to join the study.

112

113 Participants were enrolled in the trial and randomised to treatment (n=99) or placebo arms
114 (n=102). Allocation to treatment was balanced by age and sex and all participants and
115 investigators were blinded to treatment allocation. Allocated treatment consisted of a
116 single tablet containing 1 mg vitamin B12 (cyanocobalamin) administered daily. 91
117 participants in each arm of the trial provided follow-up data on the primary trial outcome,
118 posterior tibial compound muscle action potential (CMAP) amplitude. The size of the study
119 was determined by a sample size calculation designed to achieve 90% power to detect a
120 $\geq 28\%$ difference in the primary outcome (with 5% significance) between arms of the trial.

121

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 (microbiologic assay); holotranscobalamin (HoloTC; Axis-Shield radioimmunoassay; Axis-Shield plc), total Hcy (Abbott IMx analyzer; Abbott Laboratories), and folate (chloramphenicol-resistant microbiologic assay) in a single laboratory in Trinity College Dublin. 88% of 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 participants had moderately low vitamin B12 status.

A single neurophysiologist 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 limbs¹⁵. 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^{16, 17}; 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 myelination¹⁸. All nerve conduction outcomes were measured in the right side of the body.

Central motor conduction tests were measured using transcranial magnetic stimulation, which painlessly and noninvasively excites the motor cortex¹⁹. Detailed methods are reported by Dangour *et al.*¹¹. 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, the mean amplitude of which were measured. Similarly, the leg area of motor cortex was excited to measure MEPs evoked in the AH muscle. ADM and AH CMCT was 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 Hcy²⁰. Although Hcy 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

Analyses were performed to explore whether baseline or change in vitamin B12 status alters the impact of dietary vitamin B12 supplementation on neurologic function in the OPEN study. Analyses have been 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 negligible¹¹; minor changes in vitamin B12 status or nerve conduction in the placebo arm are assumed to be due to variability in repeated measures. All analyses were exploratory in nature with the aim to look for consistent patterns in findings rather than applying stringent p values to test for statistical significance.

Firstly, the effects of vitamin B12 supplementation on vitamin B12 status according to baseline status were explored graphically, using vitamin B12, HoloTC, Hcy and cB12 as indicators of vitamin B12 status. Secondly, the effects of vitamin B12 supplementation on neurologic outcomes according to baseline or change in vitamin B12 status were assessed visually using scatter plots and lowess smoother curves to explore the nature and functional form of any potential associations.

Linear regression models were used to test for associations between baseline and change in vitamin B12 status (measured by vitamin B12, HoloTC, Hcy 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 study¹¹. 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 bootstrapped to allow for non-normal distributions of exposures and outcomes. Results were 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 Hcy (mean change -2.8 µmol/l) decreased at 12 months follow-up. Levels of vitamin B12 and cB12 at follow-up are relatively constant across quartiles of baseline status, suggesting a plateau effect (**Figure 1**). There is no significant differences in follow-up vitamin B12 (F test p=0.44) or cB12 (F test p=0.21) across quartiles of baseline vitamin B12. The effects of vitamin B12 supplementation on Hcy and HoloTC

differs across baseline quartiles (**Figure 1**). There is a statistically significant difference in follow-up Hcy across baseline quartiles (F test $p < 0.001$) and a borderline statistically significant difference in follow-up HoloTC across baseline quartiles (F test $p = 0.07$). Figure 1 shows that follow-up HoloTC increases across quartiles of baseline HoloTC ($p = 0.01$ for linear trend), and follow-up Hcy increases across quartiles of baseline Hcy ($p < 0.001$ for linear trend).

Linear regression models found no evidence of a difference in impact of vitamin B12 supplementation on the primary trial outcome (posterior tibial CMAP amplitude) across quartiles of baseline vitamin B12, HoloTC, Hcy or cB12 status (**Table 1**). However, there is evidence of a very small improvement in common peroneal CMAP amplitude in response to supplementation, in participants with lower baseline vitamin B12 status [$\beta = -0.01$ (-0.01 - -0.00), $p = 0.02$] for vitamin B12; $p > 0.05$ for all other measures of vitamin B12 status.

There is evidence of a small inverse association between baseline vitamin B12 and cB12 and change in posterior tibial motor conduction velocity; participants with the lowest baseline status tended to have a greater positive change in outcome in response to supplementation over 12 months. However, small associations in the opposite direction are detected in analyses between HoloTC and cB12 and change in common peroneal conduction velocity in response to supplementation. Further, there is evidence of a very small improvement in sensory sural conduction velocity in response to supplementation, in participants with higher baseline vitamin B12 status [$\beta = 0.02$ (0.00 - 0.03), $p = 0.05$] for vitamin B12; $p > 0.05$ for all other measures of vitamin B12 status.

In terms of central nerve conduction, **Table 1** shows that AH CMCT following vitamin B12 supplementation improves (decreases) in participants with lower baseline cB12 status [$\beta=2.19$ (0.10 – 4.04), $p=0.03$]. Results for change in ADM CMCT are consistent in direction of effect but smaller in magnitude and not statistically significant [$\beta=0.53$ (-0.75 – 1.43), $p=0.31$]. However, results for mean ADM MEP amplitude suggest that neurologic function improves in response to supplementation in participants with higher baseline vitamin B12 status (as measured by Hcy and cB12).

Linear regression analyses show that neurologic response to vitamin B12 supplementation does not differ by change in vitamin B12 status (**Table 2**). Null results are consistent across each measure of vitamin B12 status and all nerve conduction outcomes.

Discussion

Key findings

This study demonstrated no differences in the effect of vitamin B12 supplementation on the primary trial outcome across quartiles of baseline vitamin B12 status. However, when considering secondary 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: as many analyses showed suggestive evidence of greater neurologic improvement in response to supplementation in participants with *higher* baseline vitamin B12 status as 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 cannot 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, there is no evidence of differences in the effect of vitamin B12 supplementation on measures of nerve conduction based on baseline vitamin B12 status. This study also finds no evidence of difference in neurologic response to vitamin B12 supplementation according to any indicator of *change* in B12 status (vitamin B12, holoTC, Hcy or cB12) and any measure of peripheral or central nerve conduction. In addition, this study shows that different indicators of vitamin B12 status reveal different patterns in vitamin B12 status achieved after 12 months oral vitamin B12 supplementation: follow-up Hcy and HoloTC increase across baseline quartiles, whereas a relatively constant follow-up vitamin B12 and cB12 is achieved across baseline quartiles.

Comparison with other studies

Whilst previous reports⁹ 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. The findings of the present study differ from the results from the VITACOG trial which reported greater benefits of supplementation on cognitive outcomes in people with the lowest baseline status^{12, 13}. This study also extends the primary findings of the OPEN RCT¹¹ 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 intervention would have been effective if participants had had slightly poorer vitamin B12 status 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^{21, 22}. The use of cB12 has the advantage of combining biomarkers of circulating vitamin B12 and a functional biomarker of vitamin B12 status²⁰, but it has been reported to be associated with renal function²³ which 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 which eliminated inter-observer variability.

It is recognised that this study is limited in statistical power and so analyses have been exploratory. It is possible that trends would have been more easily detected in a larger study. It is also possible that the 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.

314 *Policy relevance and research needs*

315 This study provides no evidence to suggest that oral supplementation with vitamin B12 has
316 any beneficial effect on neurological function in individuals with the lowest vitamin B12
317 status. These results suggest that concerns over the neurologic impact of moderately low
318 vitamin B12 status in older people are not justified by the available evidence. In terms of
319 peripheral and central neurologic function, the authors suggest there is insufficient evidence
320 to support population screening for moderate vitamin B12 deficiency in the absence of
321 anaemia or neurological symptoms, nor support population-wide recommendations for
322 vitamin B12 supplementation in healthy asymptomatic older people, even among those
323 with the lowest vitamin B12 status. Treatment for neurological impairment attributed to
324 vitamin B12 deficiency should be managed as appropriate by clinicians, rather than handled
325 at the population level.

326

327 **Acknowledgements**

328 The Older People and Enhanced Neurological Function (OPEN) Study was supported by the
329 Food Standards Agency (N05072) and the Department of Health. National Health Service
330 Research and Development and King's College Hospital Trust Research and Development
331 provided service support costs. No further funding was received for the secondary analyses
332 of OPEN study data presented here.

333

334 We thank all the participants and contributors involved in the OPEN study. We are grateful
335 to Dr. Anne Molloy for conducting biochemical analysis at Trinity College Dublin, Ireland.

336

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

341

342 The funders had no role in the implementation, data collection, management, analysis, or
343 interpretation of the study or in the preparation, review, and approval of the manuscript.

344

345 None of the authors declared a conflict of interest.

346

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Table 1: Linear regression analyses to assess relationship between baseline vitamin B12 status and change in nerve conduction in response to supplementation¹

Change in outcome		Baseline B12 (pmol/L)	Baseline HoloTC (pmol/L)	Baseline Hcy (μmol/L)	Baseline cB12
Motor tibial CMAP amplitude (mV)	n	73 ²	70 ²	72 ²	70 ²
	β	-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
Motor common peroneal CMAP amplitude (mV)	n	71 ³	69 ⁴	70 ³	69 ⁴
	β	-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
Motor tibial conduction velocity (m/s)	n	72	69	71	69
	β	-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
Motor common peroneal conduction velocity (m/s)	n	71	69	70	69
	β	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
Sensory sural SAP amplitude (μV)	n	59 ⁵	58 ⁶	59 ⁷	58 ⁶
	β	-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
Sensory superficial peroneal SAP amplitude (μV)	n	49 ⁸	48 ⁹	49 ¹⁰	48 ⁹
	β	-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
Sensory sural conduction velocity (m/s)	n	59	58	59	58
	β	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
Sensory superficial peroneal conduction velocity (m/s)	n	49	48	49	48
	β	-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
ADM CMCT (ms)	n	72	69	71	69
	β	0.00 (-0.00 - 0.01) ¹¹	0.01 (-0.01 - 0.03) ¹¹	-0.00 (-0.08 - 0.09) ¹¹	0.53 (-0.75 - 1.43) ¹¹
	p	0.77 ¹¹	0.21 ¹¹	0.98 ¹¹	0.31 ¹¹
AH CMCT (ms)	n	66	63	65	63
	β	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
Mean abductor digiti motor (ADM) MEP amplitude (mV)	n	74	71	73	71
	β	0.00 (-0.00 - 0.01) ¹¹	0.00 (-0.01 - 0.01) ¹¹	-0.07 (-0.13 - -0.03) ¹¹	0.72 (0.04 - 1.29) ¹¹
	p	0.17 ¹¹	0.33 ¹¹	0.00 ¹¹	0.03 ¹¹

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

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

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

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

⁵Seventeen 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.

⁶Fifteen 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.

⁷Sixteen 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.

⁸Twenty-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.

⁹Twenty-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.

¹⁰Twenty-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.

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

Table 2: Linear regression analyses to assess relationship between change in vitamin B12 status and change in nerve conduction in response to supplementation¹

Change in outcome		Change in B12	Change in HoloTC	Change in Hcy	Change in cB12
Motor tibial CMAP amplitude (mV)	n	71 ²	68 ²	70 ²	68 ²
	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
Motor common peroneal CMAP amplitude (mV)	n	69 ³	67 ⁴	68 ³	67 ⁴
	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
Motor tibial conduction velocity (m/s)	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
Motor common peroneal conduction velocity (m/s)	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
Sensory sural SAP amplitude (μV)	n	58 ⁵	57 ⁶	58 ⁷	57 ⁶
	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
Sensory superficial peroneal SAP amplitude (μV)	n	49 ⁸	48 ⁹	49 ¹⁰	48 ⁹
	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
Sensory sural conduction velocity (m/s)	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
Sensory superficial peroneal conduction velocity (m/s)	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
ADM CMCT (ms)	n	70	67	69	67
	β	-0.00 (-0.00 – 0.00) ¹¹	-0.00 (-0.00 – 0.00) ¹¹	0.02 (-0.19 – 0.17) ¹¹	-0.19 (-1.01 – 0.90) ¹¹
	p	0.15 ¹¹	0.35 ¹¹	0.81 ¹¹	0.68 ¹¹
AH CMCT (ms)	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
Mean abductor digiti motor (ADM) MEP amplitude (mV)	n	72	69	71	69
	β	0.00 (-0.00 – 0.00) ¹¹	0.00 (-0.00 – 0.00) ¹¹	-0.03 (-0.13 – 0.07) ¹¹	0.50 (-0.27 – 1.23) ¹¹
	p	0.36 ¹¹	0.72 ¹¹	0.52 ¹¹	0.19 ¹¹

¹ Adjusted for baseline measure of neurologic outcome, baseline B12/HoloTC/Hcy/cB12 status, baseline age, baseline sex and change in skin temperature (foot) unless otherwise stated.

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

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

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

⁵ 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.

⁶ 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.

⁷ 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.

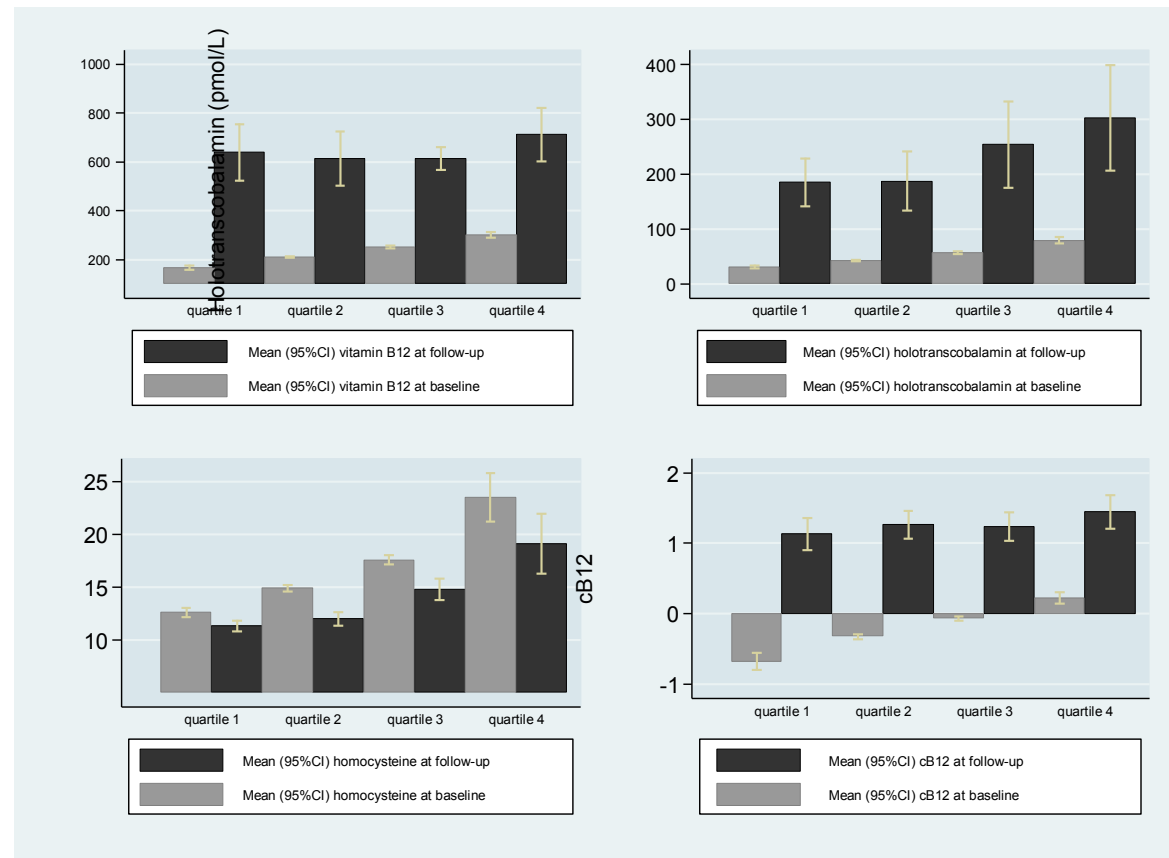
⁸ Twenty-five subjects with 0 values for superficial peroneal SAP at baseline or follow-up excluded; 10 of these had detectable superficial peroneal SAP amplitude at baseline and no detectable (0) superficial peroneal SAP amplitude at follow-up; and 9 of these had undetectable (0) superficial peroneal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

⁹ 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.

¹⁰ 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.

¹¹ Adjusted for baseline measure of neurologic outcome, baseline B12/HoloTC/Hcy/cB12 status, baseline age, baseline sex and change in skin temperature (hand).

Figure 1: Baseline and follow-up vitamin B12 status according to quartiles of serum vitamin B12, HoloTC, Hcy and cB12 at baseline.



Chapter 6: Discussion and conclusions

6.1 Introduction

Older people contribute to society in many ways, for example through informal care of family members and as an experienced workforce that can benefit local communities and the economy. Yet, making the most of older people in society is enhanced if their extra years are spent in good health, and this presents a public health challenge. Maintaining functional ability in older age reduces the need for dependence on carers and health services, and the costs associated with them, and can improve quality of life¹⁻³. Healthy ageing is affected by many factors, including physiological changes that affect nutrition. Vitamin B12 is a nutrient whose status is affected by physiologic changes seen with ageing. A decline in vitamin B12 status is associated with ageing and this is largely due to impaired absorption rather than reduced intakes⁴⁻⁶. As neurologic impairment is a known consequence of frank vitamin B12 deficiency⁷⁻⁹, it is plausible that inadequate vitamin B12 has implications for functional ability in older age through its relationship with neurologic function.

Neurological impairments associated with low vitamin B12 status are often assumed to be part of the natural ageing process, and so can be easily missed. This causes potential opportunities for prevention or treatment to be overlooked. In such cases, the neurologic signs and symptoms of low vitamin B12 status can have an important impact on physical function, social independence, mobility and ability to conduct activities of daily life. Accordingly, the neurologic impact of low vitamin B12 status in older people could contribute significantly to the burden of loss of functional ability in older age. However, very little is known about the public health consequences of neurologic implications of low vitamin B12 status at a population level. This study has aimed to improve our understanding of the contribution of vitamin B12-neurological impairment to the public health challenge of ageing.

This study aims to assess the relationship between low vitamin B12 status and neurologic function in older people by firstly systematically reviewing existing evidence, and secondly, by conducting secondary analyses using data from the OPEN Study.

Here, I briefly synthesise the findings of work presented in Chapters 2-5 and relate them to the research questions that are the basis of this thesis; highlight some overall strengths and weaknesses of the study; provide some further reflections on vitamin B12 status and neurological function in older people; and discuss the study implications, including for policy, and suggest directions for future research.

The present study aimed to address the following two research questions in systematic reviews of the literature:

- 1a) Is there any evidence in the available literature that vitamin B12 status is associated with neurological function in older people?
- 1b) Is there any evidence in the available literature that vitamin B12 supplementation improves neurological function in older people?

The findings of the systematic reviews were then used to identify gaps in the evidence-base. Secondary analyses (using data from the OPEN study) were then conducted to address these evidence gaps. Based on the findings of the systematic reviews, the following research questions for the secondary analyses were developed:

- 2a) Is vitamin B12 status associated with electrophysiological indices of peripheral or central neurologic function in asymptomatic older people with moderately low vitamin B12 status?
- 2b) Does baseline vitamin B12 status or change in vitamin B12 status alter the effectiveness of dietary supplementation with vitamin B12 on electrophysiological indices of neurologic function in asymptomatic older people with moderately low vitamin B12 status?

6.2 Synthesis of study findings by research questions

6.2.1 Research questions 1a and 2a

Chapter 2 presents findings from a systematic review designed to assess whether vitamin B12 status is associated with neurological function in older people (research question 1a). Twelve relevant reports from ten studies were identified. Among eight studies of general population groups of older people, one longitudinal study reported no association¹⁰, and four¹¹⁻¹⁴ of seven cross-sectional studies reported limited evidence of an association of vitamin B12 status with some, but not all, neurological outcomes. Among four articles reporting on groups of older people with clinical and/or biochemical evidence of low vitamin B12 status, one longitudinal study¹⁵ reported an association of vitamin B12 status with some, but not all, neurological outcomes and three cross-sectional analyses reported no association¹⁶⁻¹⁸. Overall, there is limited evidence from observational studies of older people to suggest an association of vitamin B12 status with neurological function. However, few studies are available, and the majority are cross-sectional in design so this limits the ability to infer causation or lack of it.

Importantly, the systematic review identified several sources of heterogeneity and risks of bias in the available evidence, which have been useful to inform further research. There was considerable heterogeneity between studies in the definition of vitamin B12 status and in the neurological outcomes reported. As discussed in Chapter 1, the use of composite measures of vitamin B12 status involving plasma/serum vitamin B12 plus MMA or hcy levels are now recommended¹⁹. This approach was used in three of the included studies, and interestingly all of these studies reported some positive associations between vitamin B12 status and neurologic function. It is possible that composite measures of vitamin B12 status are able to detect associations with neurologic function more effectively. Further, the systematic review found that only five of the included studies used electrophysiological measures of nerve conduction to assess neurologic function; these are the most sensitive and objective measures of neurological function. Measuring of self-reported neurologic symptoms and/or clinical markers of neurologic function, both of which are subjective, is much more widespread

in the available literature. Many existing studies also failed to identify and adjust appropriately for confounders.

By identifying these limitations in the currently available evidence, the subsequent component of this study (presented in Chapter 3) could be designed to address these evidence gaps. The OPEN study afforded an opportunity to test whether there was a cross-sectional association between vitamin B12 status and neurologic function in a high quality dataset with data on electrophysiological indices of peripheral and central neurologic function. A composite indicator of vitamin B12 status (cB12) was included in the analyses and an extensive exercise was conducted to identify potential confounders. A secondary analysis of baseline data from the OPEN study was conducted to determine whether vitamin B12 status is associated with electrophysiological indices of peripheral or central neurologic function in asymptomatic older people with moderately low vitamin B12 status (research question 2a). These analyses showed no evidence of an association of measures of vitamin B12 status with nerve conduction outcomes. There was also no evidence of an association of vitamin B12 status with clinical markers of neurologic function. Null results were consistent in all measures of vitamin B12 status and across a wide range of neurologic outcomes measuring sensory and motor components of peripheral nerve function in upper and lower limbs, as well as central motor conduction.

Taken together, the currently available evidence-base (presented in Chapters 2 and 3) suggests that concerns about the neurologic implications of moderately low vitamin B12 status in older people at the population level may be unwarranted. Nevertheless, the majority of the studies reviewed systematically, as well as the results from the OPEN study, are based on general population groups of healthy older people without clinical or biochemical evidence of vitamin B12 deficiency. As high quality studies have not been conducted to assess associations in less healthy and more vitamin B12 deplete populations, vitamin B12-dependent impairment of neurological function in such groups cannot be excluded. It is likely that older people identified as clearly vitamin B12 deplete would be selected for treatment and so simple observational studies on these people may be difficult to justify ethically.

6.2.1.1 Comparison with other studies

Since the systematic review of observational studies (Chapter 2) was published, a further relevant small (n=53) cross-sectional study in older people (median age 74 years) has been identified²⁰. Matamala *et al.*²⁰ reported a statistically significant lower peripheral motor conduction time in subjects with low vitamin B12 status (pre-defined as plasma vitamin B12 <221 pmol/l; mean 155 pmol/l and measured using a radioassay) versus normal vitamin B12 status (pre-defined as plasma vitamin B12 ≥221 pmol/l; mean 316 pmol/l) but no difference in CNS outcomes. However, analyses did not adjust for potential confounders and a composite measure of vitamin B12 status was not used, which are limitations. Although healthy participants were recruited, comparability of their vitamin B12 status to OPEN participants is difficult due to use of different vitamin B12 assays. When considered alongside all existing evidence, this study does not alter a conclusion of limited evidence from observational studies of older people to suggest an association of vitamin B12 status with neurological function.

6.2.2 Research questions 1b and 2b

Chapter 4 presents findings from a systematic review designed to assess whether vitamin B12 supplementation improves neurologic function in older people (research question 1b). The review identified three relevant placebo-controlled RCTs^{16, 17, 21, 22} and one nRCT²³; two of these were subject to risk of bias^{22, 23} which limits the conclusions that can be drawn from these studies; and the remaining two studies used different types of vitamin B12 intervention (oral²¹ versus intramuscular injection^{16, 17}). The RCT by Dangour *et al.*²¹ was the only study to use electrophysiological indices of nerve function as outcomes and recruited older people without biochemical or clinical evidence of vitamin B12 deficiency. Primary analyses from all these studies indicate that supplementation with vitamin B12 does not improve neurologic function in older people. However, it is difficult to reach firm conclusions from heterogeneous evidence so limited in size and quality.

Nevertheless, by performing a detailed assessment of the currently available evidence, the subsequent component of this study (presented in Chapter 5) could be designed to further advance knowledge about the effects of vitamin B12 supplementation on

neurologic function in older people. In subgroup analyses, Hvas *et al.*¹⁷ showed a statistically significant association between improvement in neurologic symptom score (but not vibration sensation or neurological disability score) in the treatment group compared to placebo, in subjects with the lowest baseline vitamin B12 status. Consequently, it is possible that improvement in neurologic function from vitamin B12 supplementation is only apparent in people with the lowest vitamin B12 status. It is already known from case series that people with frank vitamin B12 deficiency show neurologic improvement after vitamin B12 treatment^{9, 24}. Accordingly, this may explain the lack of treatment effect reported in the studies available to date. Interestingly, there is evidence to support greater benefits of vitamin B12 supplementation in people with the lowest vitamin B12 status from studies considering the effects of B vitamin supplementation on cognitive outcomes^{25, 26}, lending further support to this hypothesis.

Questions therefore remained about whether greater benefits of vitamin B12 supplementation in people with the lowest vitamin B12 status extend to improvement in nerve conduction or whether the same effect would be seen with an oral vitamin B12 intervention. Data from the OPEN study afforded an opportunity to test whether the effectiveness of oral vitamin B12 supplementation on electrophysiological indices of neurologic function alters according to baseline vitamin B12 status or change in vitamin B12 status (research question 2b). Conducting such secondary analyses in OPEN had the advantage of using a high quality dataset derived from asymptomatic older people with moderately low vitamin B12 status.

The secondary analyses presented in Chapter 5 showed that some indicators of vitamin B12 status (but not others) revealed evidence of associations between baseline vitamin B12 status and some peripheral and central neurologic responses to supplementation. However, directions of effect were inconsistent and such differences could not be explained by the aspect of neurologic function each outcome measured. Overall, no evidence of a difference in the impact of oral vitamin B12 supplementation on nerve conduction based on baseline vitamin B12 status or change in vitamin B12 status was observed. These findings do not support the hypothesis that improvement in neurologic function from oral vitamin B12 supplementation is only apparent in people with the

lowest vitamin B12 status. It is unlikely that the OPEN intervention would have been effective if participants had had slightly poorer vitamin B12 status alongside absence of neurologic or haematological symptoms of deficiency.

Taken together, the currently available evidence-base (presented in Chapters 4 and 5) indicates that vitamin B12 supplementation does not improve neurologic function in asymptomatic older people. Although a single study suggests that those with the lowest vitamin B12 status (elevated vitamin B12 metabolites) benefit from an intramuscular injection of vitamin B12 in terms of neurologic symptoms, this finding has not been replicated in a high quality study assessing neurologic function by nerve conduction and using an oral vitamin B12 intervention. For healthy older people with no clinical or biochemical evidence of vitamin B12 deficiency, this thesis suggests that supplementation with vitamin B12 will not improve neurologic function, even if vitamin B12 status is at the lower end of the sub-clinical spectrum.

6.2.2.1 Comparison with other studies

Although it did not meet the inclusion criteria for the systematic review of intervention studies (Chapter 4) (because there was no control group), it is useful to reflect on a recent study by Brito *et al.*²⁷ A small (n=51) pre- and post-treatment study of asymptomatic older subjects excluded from taking part in a larger cluster RCT²⁸ was conducted in Chile; vitamin B12 levels were below <120 pmol/l in accordance with the exclusion criteria of the RCT²⁷. The response to a single dose of intramuscular treatment of 10 mg cyanocobalamin (B12), 100mg pyridoxine and 100 mg thiamin was assessed after 4 months, in terms of sensory and motor nerve conduction. Vitamin B12 status was defined by combining vitamin B12, holoTC, hcy and MMA into cB12. Sensory latency for left and right sural nerves and of the right median nerve statistically significantly decreased (improved) after treatment but no statistically significant differences in SAP amplitudes for these nerves were seen. The response to treatment in motor nerves was mixed but did include a faster nerve conduction velocity in the right peroneal nerve. There was no significant differences in central nerve conduction at 4 months. This study is limited by its small size and lack of a placebo group. Its comparability to results from the OPEN study is constrained by its different mode of intervention, short follow-up

period and the vitamin B12 status of its participants. Although asymptomatic, this group of subjects had clear biochemical evidence of vitamin B12 deficiency.

A further pre- and post-treatment study in patients diagnosed with vitamin B12 neurological syndrome was conducted by Kalita *et al.*²⁴ Sixty-six patients were treated with 1000 µg intramuscular cyanocobalamin daily for 10 days followed by weekly for a month and monthly thereafter. Clinical examination, sensory testing and nerve conduction studies were done at 3 and 6 months follow-up. The majority of patients improved both in clinical and nerve conduction parameters following treatment; improvement in nerve conduction was more marked at 6 months. Again this study was in patients with clinical or biochemical evidence of vitamin B12 deficiency (vitamin B12 <141 pmol/l) and so is not directly comparable to findings from the OPEN study.

In line with the findings from Brito *et al.*²⁷ and Kalita *et al.*²⁴, the conclusions drawn from this thesis do not exclude the possibility that people with clinical or biochemical evidence of vitamin B12 deficiency will benefit from vitamin B12 supplementation. This thesis, including the systematic review of interventions (Chapter 4), was not specifically focussed on this question and therefore inappropriate in scope to be able to answer this question adequately (see Section 6.5.1 for reflections on clinical implications).

6.3 Study strengths and limitations

6.3.1 Study strengths

Overall, this thesis adds to a relatively small body of existing literature, identifies gaps in knowledge in the current literature, and provides a cohesive synthesis of existing literature with new findings; and thereby offers new insights into the relationship between vitamin B12 and neurological function.

A strength of the research presented in this thesis is the use of systematic methods to review existing scientific literature. Search strategies were comprehensive and standard systematic procedures were followed for study selection, data extraction and assessment of risk of bias. The systematic reviews presented in Chapters 2 and 4 are, to

the best of my knowledge, the first to address the relationship between vitamin B12 and neurologic function in older people.

The secondary analyses presented in Chapters 3 and 5 are conducted on an existing high quality dataset. The OPEN study was a well-designed double-blinded placebo controlled trial with robust procedures followed for the selection of participants and data collection. Drop-out was very low and compliance to interventions was very high²¹. A particular strength of the OPEN study is the use of electrophysiological indices of nerve function, which were all collected by a single expert observer using state-of-the-art methods and are objective measures of nerve function.

Many previous studies have been limited by the use of a single biomarker of circulating vitamin B12 as the sole marker of vitamin B12 status. The use of composite measures of vitamin B12 status involving plasma/serum vitamin B12 plus MMA or hcy levels are now recommended¹⁹, and therefore the novel use of the composite marker cB12 in Chapters 3 and 5 is a strength of the study. Indeed, findings presented in Chapter 3 are, to my knowledge, the first to report on associations between cB12 and neurologic function.

6.3.2 Study limitations

Addressing research questions 1a) and 1b) has been limited by a heavy reliance on cross-sectional studies. The systematic review of observational studies identified only two longitudinal analyses (of 12 analyses in total) and the secondary analysis of baseline data from the OPEN study is cross-sectional in design. As cross-sectional studies measure associations at one point in time they cannot be used to determine whether the exposure proceeded or followed the outcome.

Of consideration for all aspects of the study, is the lack of a 'gold standard' test for assessing vitamin B12 status. Debate still remains around the use of various vitamin B12 assays and cut-offs to define vitamin B12 deficiency and/or marginal status. In particular, there is a lack of age or sex-specific local reference ranges against which to evaluate individual study results and this hinders comparability across studies.

This study has taken an inclusive approach by reporting vitamin B12 status by several measures: vitamin B12, holoTC and cB12. Yet, each measure has its limitations (see Chapter 1). Vitamin B12 measures both active and inactive forms of the vitamin. Although it measures the active form only, factors that influence holoTC have not been widely studied²⁹. Whilst advantageous as a composite marker of vitamin B12 status, the use cB12 has not been fully explored yet or specifically validated against neurologic outcomes. cB12 has been used in only four^{27, 30-32} studies to date, one of which reported that cB12 was independently associated with renal function but not age³⁰. Findings reported in Chapter 3 showed that use of cB12 did not alter study results. Similarly, Kobe *et al.*³¹ found that associations between serum vitamin B12 and memory performance were largely unchanged when cB12 was used as a measure of vitamin B12 status (see further reflections on composite measures and cB12 in Section 6.4).

Investigators of the OPEN study did not measure renal function or MMA, which is a limitation. Ideally analyses using hcy and cB12 would have adjusted for a measure of renal function if this had been available. Further, the NHANES roundtable work on measurement procedures for assessing vitamin B12 status suggested that MMA has advantages over hcy as the functional marker of vitamin B12 status because MMA increases with vitamin B12 inadequacy but hcy increases with both folate and vitamin B12 inadequacies¹⁹. The availability of MMA in the OPEN study would have allowed cB12 to be based on four markers rather than three; when using three markers, having MMA missing is less reliable than having any of the other three markers missing³³. Recent studies have investigated the use of urinary MMA as a biomarker of vitamin B12 status which may be an option for consideration in future studies. It has been shown that urinary MMA ratio responds to vitamin B12 supplementation in a comparable manner to plasma MMA³⁴ and may have advantages in studies where blood sampling proves difficult.

Analyses using data from the OPEN study may also be limited by the relatively healthy characteristics of the study population. 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, thereby limiting the generalisability of findings from

the study. Further, it is also possible that the duration of the vitamin B12 intervention was too short to have an effect on neurologic function. It is also recognised that there is some missing data in the OPEN study which may present a limitation. In particular, the number of observations for follow-up sensory nerve SAP responses (used in analyses presented in Chapter 5) was lower than for other neurologic outcomes. It is therefore possible that associations between vitamin B12 status and sensory nerve SAP responses were more difficult to detect.

Whilst the OPEN study measured a wide range of peripheral and central neurologic outcomes, it remains possible that vitamin B12 status and/or supplementation is more closely related to a neurologic outcome not measured in OPEN. It has been suggested that somatosensory- and visual- evoked potentials (electrical activity from the somatosensory or visual systems) are likely to be more revealing in assessing neurologic improvement in asymptomatic vitamin B12-deficient people³⁵, based on clinical and electrophysiological observations in case series⁷⁻⁹. Nevertheless, findings from a recent study in patients with vitamin B12 deficiency neurological syndrome suggest that it has been prudent to measure neurologic function by a wide range of nerve conduction outcomes that assess axonal function and myelination as used in OPEN. Kalita *et al.*²⁴ reported that 54.5% of 66 patients with vitamin B12 neurological syndrome had abnormal nerve conduction; 22.2% of these had axonal, 11.1% had demyelinating, and 66.7% had mixed features (see further reflections on neurologic outcomes in Section 6.4).

6.4 Further reflections related to vitamin B12 status and neurological function in older people

Preparing for, conducting and synthesising results presented in this thesis has enabled me to reflect further on research on vitamin B12 and neurological function, beyond the conclusions drawn to address the research questions (Section 6.2).

As mentioned in Section 6.3.2, the lack of consensus on how to measure vitamin B12 status in terms of cut-offs and assays used is a major consideration for research on vitamin B12 status and neurological function. Completely objective comparison across

studies using different measures and assays of vitamin B12 is simply not possible. Although the debate continues to some extent, it is preferable for studies on vitamin B12 status to use a functional measure of vitamin B12 status (hcy or MMA) in addition to serum/plasma vitamin B12 or holoTC. It is possible that composite measures of vitamin B12 status are able to detect associations with neurologic function more effectively if they exist (suggestive evidence in Chapter 2). Similarly, a systematic review on vitamin B12 status and cognitive decline and dementia concluded that studies that used composite measures of vitamin B12 status including a measure of hcy or MMA showed associations between poor vitamin B12 status and increased risk of cognitive decline or a dementia diagnosis³⁶. Yet, uncertainty remains around the optimal composite marker for vitamin B12 status in terms of measuring its relationship with neurological function. The use of cB12 has some advantages (previously discussed) but its cut-off values are validated using haemoglobin and cognitive score rather than a measure of neurologic function. Neurologic symptoms of vitamin B12 deficiency can occur without concurrent anaemia³⁷, so these cut-offs require further scrutiny if they are to be used to assess risk of vitamin B12-dependent neurological impairment. For this reason, analyses using cB12 presented in Chapters 3 and 5 are based on cB12 as a continuous variable rather than categorised according to the published cut-offs. Furthermore, generation of cB12 values could be regarded as a 'black box' process where the inputs are well-defined but the mechanisms by which cB12 values are generated remain unclear. MMA and hcy can become elevated in cases of poor renal function. There is a need to consider further the relationship between cB12 and renal function to allow investigators to be well-informed about whether possible confounding should be considered in future studies of older people.

Screening for eligibility to join the OPEN study was based on serum vitamin B12 concentrations of ≥ 107 and < 210 pmol/L using the Beckman Coulter chemiluminescent immunoassay, with the aim to select individuals with moderate vitamin B12 deficiency who did not have anaemia. At baseline, vitamin B12 was then assessed using a microbiologic assay that estimated serum concentrations approximately 25% higher than did the Beckman Coulter method. The different values estimated by the two assays highlights the issue of comparability discussed earlier. Although at baseline, 88% of

recruited participants had vitamin B12 status below the median value for the microbiologic assay reference standard, it could be argued that vitamin B12 status should have been assessed at screening by measuring MMA or hcy in combination with vitamin B12, or by cB12, to be sure that participants had marginal vitamin B12 status. It can be said that selecting study participants with marginal nutrient status at baseline for trials of effectiveness of nutrient supplementation is critical. It has been proposed that many randomised trials of nutritional supplements have not targeted individuals with low or marginal status and so a likely explanation of null findings is that nutrient status of participants is already at a protective level and so further supplementation offers no further benefit³⁸.

Selecting the nutrient status of participants entering nutrient supplement randomised trials requires careful consideration depending on how the study results are to be used. For example, participants in the OPEN study were selected on the basis of moderately low vitamin B12 status so that findings were relevant to population health in older people and could be used to inform public health recommendations. However, as assessment of vitamin B12 status at screening was based on vitamin B12 alone, it is possible that the participants were too replete in vitamin B12 to be able to detect any effect of vitamin B12 supplementation on neurological function. If participants had been selected with biochemical or clinical evidence of frank vitamin B12 deficiency, then it might be expected that neurologic markers of deficiency would improve after supplementation (because a deficiency is being corrected), but this type of study could not be reliably generalised for application to populations or be the basis for public health recommendations. It is also difficult to design a controlled-trial using vitamin B12 deficient subjects because it is unethical to prevent clearly deficient subjects from receiving treatment. In such cases a study design without a control group would be most appropriate. Still, it is important to identify whether findings of studies intend to be applied to older people at risk of vitamin B12 deficiency in the population (and so used to inform public health recommendations) or older people already living with signs and symptoms of frank deficiency (and so used to inform clinical practice). Balancing the likelihood of effectiveness of nutrient supplementation versus desired application of findings is a difficult challenge.

Suggestive findings that composite measures of vitamin B12 status are able to detect associations with neurologic function more effectively imply that hcy and/or MMA may have an important role (or be markers for biochemical changes) affecting nerve function. Yet, mechanistic evidence on the role of hcy and/or MMA in vitamin B12-dependent neurological impairment remains inconclusive. As discussed in Section 1.6.1 plausible mechanisms exist whereby impaired function of vitamin B12-dependent enzymes MCM (causing elevated MMA) and/or MeS (causing elevated hcy) elucidate biochemical changes affecting neurological function but direct mechanistic evidence in humans appears lacking. A thorough review of the scientific literature on potential mechanisms by which vitamin B12 status can affect neurological function (outside the scope of this thesis) would certainly be valuable to shed light on the importance of classical biochemical enzyme mechanisms versus other proposed mechanisms. The World Cancer Research Fund is currently testing a new methodology for conducting systematic reviews of mechanistic studies which may provide a helpful steer for such a review.

A thorough review of mechanistic evidence could also help direct further observational or intervention research on vitamin B12 and neurological function by identifying which components of nerve function are most susceptible to low vitamin B12 status (for example sensory or motor outcomes, PNS or CNS). To date, there appears to be debate in the literature around whether axonal or demyelinating neurological impairment is likely to predominate with vitamin B12 deficiency^{24, 39-41} or whether the PNS or CNS is predominantly affected^{42, 43}. If well-informed by mechanistic evidence, it may be possible to focus further research on vitamin B12 on the most relevant neurologic outcomes.

6.5 Policy implications and directions for future research

6.5.1 From a clinical perspective

It is possible to reflect on the clinical implications of the findings of this study with reference to recent evidence-based guidance on how to diagnose and manage vitamin

B12 deficiency provided by Hunt⁴⁴. This guidance recognises the limitations of vitamin B12, holoTC and hcy as measures of vitamin B12 status but still suggests vitamin B12 as the preferred choice for biochemical assessment of vitamin B12 status. I would suggest that the preferred first choice is both vitamin B12 and MMA if available. This guidance also importantly recognises that neurological symptoms can occur in vitamin B12 deficiency in isolation so it is important to consider a diagnosis of vitamin B12 deficiency in the presence of neurological symptoms of unknown cause, as neurological features may progress and become irreversible. This is helpful to address the risk of missing vitamin B12-dependent neurological impairments because they are often assumed to be part of the natural ageing process or a result of 'wear and tear'.

The expert consensus for standard treatment of vitamin B12 deficiency in the UK is to begin parenteral treatment with intramuscular hydroxocobalamin, to avoid the possibility of treatment being absorbed and metabolised ineffectively. Standard initial treatment for patients without neurological involvement is 1000 µg intramuscularly three times a week for two weeks (or alternate days for up to three weeks in patients with neurological involvement). It is suggested that oral treatment be considered in mild or subclinical deficiency when problems with absorption and compliance can be ruled out ⁴⁴.

Clinical guidance indicates that expectations about neurological recovery after treatment are less clear than haematological responses; this is consistent with the findings of this study. Although not a primary focus, findings presented in this study highlight that research on the impact of vitamin B12 supplementation on neurologic function in people with clinically or biochemically defined vitamin B12 deficiency is limited in size and quality (Chapter 4). Clinical guidance highlighted a separate systematic review investigating the effectiveness of oral versus intramuscular administration of vitamin B12 to correct deficiency that included two RCTs^{45, 46}; these trials were not blinded or placebo-controlled and detail on assessment of neurologic response was scant but appears to be restricted to self-reported symptoms and clinical markers⁴⁷.

The systematic review of interventions performed as part of this thesis (Chapter 4) was not specifically focussed on neurologic effects in people with frank vitamin B12 deficiency and was therefore inappropriate in scope to be able to fully assess the impact of vitamin B12 supplementation on neurologic function in people with clinically or biochemically defined vitamin B12 deficiency. Further research may be warranted in this group of subjects to assess the most effective mode and duration of vitamin B12 treatment and which aspects of neurologic function are most responsive. The WHO has also identified a research need to establish objective criteria to assess early neurological impairment caused by vitamin B12 deficiency, ideally using neurophysiologic measurements⁴⁸. A systematic review of evidence on effectiveness of all modes and duration of vitamin B12 treatment on neurologic function in older people with frank vitamin B12 deficiency would be valuable to inform clinical practice. Such a review would benefit from broader inclusion criteria than used in the systematic review of interventions that has formed part of this study (Chapter 4); for example inclusion of case series, pre- and post-treatment and other study designs not utilising a control group.

6.5.2 From a public health perspective

The results of this thesis suggest that concerns over the neurologic impact of moderately low vitamin B12 status in otherwise healthy older people may not be warranted. In accordance with limited and inconsistent evidence of association of vitamin B12 status and neurologic function, it is proposed that there is insufficient **evidence of a benefit of population screening for moderate vitamin B12 deficiency in the absence of anaemia or neurological symptoms.**

However, the majority of the studies reviewed systematically, as well as the results from the OPEN study, are based on general population groups of healthy older people without clinical or biochemical evidence of vitamin B12 deficiency. As high quality datasets have not been used to assess associations in less healthy and more vitamin B12 deplete populations, vitamin B12-dependent impairment of neurological function in such groups cannot be excluded.

This study concludes that evidence does not support supplementation with vitamin B12 to improve neurologic function in healthy older people, even if vitamin B12 status is at the lower end of the sub-clinical spectrum. Accordingly, there is **insufficient evidence to support population-wide recommendations for vitamin B12 supplementation in healthy asymptomatic older people**. The conclusions drawn in this thesis do not exclude the possibility that people with clinical or biochemical evidence of vitamin B12 deficiency will benefit from vitamin B12 supplementation, but treatment for neurological impairment attributed to vitamin B12 deficiency should be managed as appropriate by clinicians, rather than handled at the population level.

The nutritional status of the UK population is monitored in the NDNS. At the moment vitamin B12 status is assessed by serum vitamin B12 using a threshold of 150 pmol/l (chemiluminescence immunoassay), which is based on a recommendation by WHO. This threshold was developed using data from the NHANES and based on concentrations below which plasma MMA becomes elevated⁴⁸. This threshold is appropriate for use with serum vitamin B12 alone, but measurement of population-wide vitamin B12 status would benefit from the additional measurement of a functional biomarker, preferably MMA as it has a greater specificity for vitamin B12 than hcy. Nevertheless, continued use of the same chemiluminescence assay and threshold across subsequent years of the NDNS rolling programme will allow trends in vitamin B12 status in older people to be monitored. Further, there may be an opportunity to develop an age- and sex-specific UK reference standard for assessing vitamin B12 status by the chemiluminescence assay for serum vitamin B12, by using a representative sample of the NDNS, in a similar fashion to what has been done in Ireland (Dr. A. Molloy personal communication).

Treatment with folic acid can mask the diagnosis of vitamin B12 deficiency and therefore exacerbate neurologic impairment⁴⁹; therefore the findings of this study may be of interest to countries that fortify grains with folic acid. Mandatory folic acid fortification has been introduced in over 70 countries since 1991 and on the whole has been cited a success in terms of reducing incidence of neural tube defects in pregnancy⁵⁰. This thesis' finding of limited and inconsistent evidence of association of vitamin B12 status and

neurologic function in healthy older people supports no need for concern around unrecognised vitamin B12-associated neurological impairment amongst populations. However, the OPEN study was conducted in the UK where flour is not currently fortified with folic acid, and so its results may not be directly generalisable to countries that fortify grains or other foods with folic acid. The UK's Scientific Advisory Committee on Nutrition has issued recommendations for folic acid fortification to UK governments in 2006, and again further refined in 2009^{49, 51}, so this is possible if taken forward by current or successive governments. In such an event, it may be important for future studies to revisit the research questions addressed in this thesis with further consideration of interaction with folate status. For example in Chile, where flour is fortified with folic acid, Brito *et al.*²⁷ reported that subjects with higher baseline serum folate had statistically significantly less improvement in vitamin B12 status in response to vitamin B12 supplementation than those with lower baseline folate. The influence of the interaction between folate and neurologic response to vitamin B12 supplementation is being further explored in this study⁵².

The present study has focussed on a single nutrient, vitamin B12, and a single component of physical function, neurologic function, as a potentially important contributor to loss of functional ability in older age. Considered alone, this study concludes that neurological impairment attributed to low vitamin B12 does not make a recognisable contribution to the public health challenge of ageing. But in terms of improving functional ability in older age and the opportunities afforded by improved nutrition, there is a need to take a broader view than simply supplementing with individual nutrients that might be linked to specific diseases seen in old age. Amongst the research literature, trials that have evaluated the effectiveness of single nutrient interventions on age-related health outcomes are prevalent, and many do not show evidence of effectiveness. For example, on vitamin B12 specifically, several reviews have concluded that there is no or limited evidence of effectiveness of vitamin B12 supplementation on cognitive function⁵³⁻⁵⁵. Even when a wide range of lifestyle, behavioural and therapeutic interventions were reviewed for effectiveness to reduce cognitive decline and risk of Alzheimer's disease, overall single-domain interventions were found to yield mainly negative results⁵⁶. It may be that a holistic approach to

dietary interventions involving wholesale changes to dietary patterns (and improvements in physical activity) would be expected to change the levels and balance of a range nutrients and therefore be more effective in improving outcomes related to functional ability in older age. Such multi-domain interventions can target several risk factors and disease mechanisms simultaneously, and are important for chronic disease prevention as well as optimising functional ability in later years⁵⁷⁻⁶⁰. It can be claimed that multi-domain interventions in combination are likely to be more effective on age-related health outcomes than those that use single-component interventions. A successful example is the FINGER trial⁵⁸, a large RCT that combined diet, exercise, cognitive training and vascular risk monitoring in a multi-domain intervention to improve or maintain cognitive function in at-risk older people. After 2 years, significant improvements in cognition were detected; further findings from a 7 year extended follow-up will be even more informative about incidence of dementia and AD.

6.6 Concluding remarks

This thesis concludes that there is limited evidence from observational studies of older people to suggest an association of vitamin B12 status with neurological function. Overall, the available evidence suggests that concerns about the neurologic implications of moderately low vitamin B12 status in older people at the population level may be unwarranted. Further, the currently available evidence-base indicates that vitamin B12 supplementation does not improve neurologic function in asymptomatic older people, even if vitamin B12 status is at the lower end of the sub-clinical spectrum.

Research on vitamin B12 and neurological function is constrained by a lack of consensus on how to measure vitamin B12 status in terms of cut-offs and assays. The use of composite measures of vitamin B12 status show promise but need to be specifically validated using neurologic outcomes. Future research on vitamin B12 and neurologic function would benefit from careful selection of study participants with marginal vitamin B12 status and a greater understanding of the mechanisms by which vitamin B12 status can affect neurological function.

6.7 References

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Appendices

Coeliac UK Research Strategy

Research Strategy

2013 - 2016

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1 Executive summary

Coeliac disease is an autoimmune condition elicited by gluten and related prolamines from barley and rye, in genetically susceptible individuals. It is characterised by the presence of a variable combination of gluten-dependent clinical manifestations, coeliac disease specific antibodies, HLA DQ2 and DQ8 haplotypes and enteropathy. Currently, the only treatment is strict adherence to a lifelong gluten-free diet.

During the last 10 years, we have spent over £1.3M on research into coeliac disease and dermatitis herpetiformis (DH), the skin manifestation of coeliac disease that appears as a rash of raised red patches, often with blisters.

Coeliac UK supported an Organisational Policy Analysis (OPA) to overview the Charity's research activities including funding research. The findings of the OPA have been used to develop this Research Strategy in collaboration with our Members, Health Advisory Council, Food Standards Committee and in conjunction with our Board of Governors. This strategy provides a framework for the Charity's research activities for 2013-2016.

Working together with our Members, healthcare professionals, our Health Advisory Council and Health Associates Network, Food Standards Committee, researchers, research organisations and policy makers, we will improve the lives of people living with coeliac disease and DH.

We will fund research that underpins and integrates with our business plan activities whilst also considering and where possible, funding, the wider long term research agenda in understanding coeliac disease and DH.

We will ensure our funds are committed to research which provides the maximum benefit to people with coeliac disease and/or DH. We will diversify our income streams, proactively seeking new and alternative sources of funding and develop partnerships both nationally and internationally in order to achieve our objectives.

However funding research is only one way the Charity supports research. The Charity's Membership is a valuable resource for the research community providing the largest register of patients with the condition in the UK. In addition, the employees within the Charity are an important source of expertise and as such they are increasingly becoming involved as active members of research teams and can facilitate an outreach to the wider research community.

2 Introduction

Coeliac UK was founded in 1968. It is the largest charity for people with coeliac disease in the world representing more than 65,000 Members with coeliac disease and/or dermatitis herpetiformis (DH).

Coeliac disease is an autoimmune condition elicited by gluten and related prolamines from barley and rye in genetically susceptible individuals. It is characterised by the presence of a variable combination of gluten-dependent clinical manifestations, coeliac disease specific antibodies, HLA DQ2 and DQ8 haplotypes and enteropathy. Currently, the only treatment is strict adherence to a lifelong gluten-free diet.

Screening studies in the UK show that the prevalence of coeliac disease is 1 in 100 (*West J et al. 2003*) people but only 24% of them are diagnosed (*West J et al. 2014*).

Our vision is to see a world full of choice for people with coeliac disease and DH and eventually, a world free of the condition. Our mission is to improve the lives of people with coeliac disease and DH.

The Charity has committed significant funds into medical research in the last 10 years. Research of this kind is increasingly expensive, requiring innovative approaches to provide solutions to funding, such that a move to develop partnerships and collaborative working, both nationally and internationally is required.

Funding research is only one way that the Charity supports research. The Charity's Membership is a valuable resource for the research community providing the largest register of patients with the condition in the UK. In addition, the employees within the Charity are an important source of expertise and as such they are increasingly becoming involved as active members of research teams and can facilitate an outreach to the wider research community.

3 Research Strategy

3.1 The Charity's strategic aims

Our Research Strategy for 2013-2016 is in alignment with the Charity's 10 year Strategy approved by the Board of Governors.

For people with coeliac disease and DH:

- We will maintain our position as the authoritative source of support and advice.
- We want to see an accelerated rate of diagnosis and uniformly high quality care and management.
- We will improve cost, availability and access to a wider range of good quality products.
- We want more gluten-free choices on menus so people with coeliac disease and DH can eat out with confidence and pleasure.
- We want an effective use of funds for research to build a strong evidence base supporting the work of the Charity.
- We shall ensure the Charity's resources are used in line with the strategic aims of the Charity and provide value for money.

3.2 How the research strategy was developed

The Research Strategy has been developed in the context of the Charity's 10 year Strategy and from the findings of an Organisational Policy Analysis (OPA) conducted in 2012. The OPA provided an overview of the Charity's research activities, including funding research, between 2007-2011. The process involved a review of documentation, interviews with key stakeholders and shadowing of day to day operations within the Charity.

The Research Strategy was also developed taking into account existing guidance and bench marking against comparable organisations also involved in funding research.

3.3 Key principles

- We will fund research that underpins and integrates with our business plan activities whilst also considering and where possible, funding, the wider long term research agenda in understanding coeliac disease and DH.
- Research proposals will be reviewed for both relevance to the Charity's research priorities and scientific excellence; only the best will be considered for funding.

- All research funded by Coeliac UK must benefit people with coeliac disease and DH and if this is not realised in the short or medium term, the potential to make a difference with time must be demonstrated.
- Only appropriately qualified individuals, as determined during the peer review process, may apply for research funding.
- Applicants must be contracted to work in an appropriate institution which has the necessary networks to support education, training and professional development.
- Applications from within the UK and overseas will be considered.
- All research proposals must have a statement of originality; replication of work carried out elsewhere will not be accepted. However there may be exceptions eg where the same research question may have a different outcome depending on the cultural environment.
- The Charity will consider exceptional proposals of novel research, not included within the scope of this strategy. Such proposals must demonstrate how the research will further the understanding of coeliac disease and DH and the potential to improve the lives of people living with the condition.

3.4 Research areas

The Charity supports research covering a wide range of topics (with past examples):

3.4.1 Underpinning Research

Research that underpins investigations into the cause, development, detection, treatment and management of coeliac disease and DH. (Comprehensive gluten T-cell epitope mapping in coeliac disease – Professor Bob Anderson)

3.4.2 Aetiology

Identification of determinants that are involved in the cause, risk or development of coeliac disease and DH. (Identifying rare large effect size genetic variants predisposing to coeliac disease – Professor David van Heel)

3.4.3 Prevention of disease and promotion of wellbeing

Research aimed at the primary prevention of disease, conditions or ill health or promotion of well-being. (Psychological and social factors associated with coeliac disease: a UK based study – Dr Ruth Howard)

3.4.4 Detection, screening and diagnosis

Discovery and development of diagnostic, prognostic and predictive markers and technologies. (Pre-endoscopy serological testing for coeliac disease – a novel approach using rapid antibody testing - Prof Dave Sanders and Prospective coeliac disease diagnosis evaluation - Dr Adrian Thomas)

3.4.5 Development of treatments

Discovery and development of treatments, in addition to the gluten-free diet, in preclinical settings. (We have supported researchers working in this area by providing advice and facilitating a survey of Members for their views on the acceptability of enzyme technology)

3.4.6 Evaluation of treatments

Testing and evaluation of treatments in clinical, community or applied settings. (Investigations of the morbidity, mortality and effect of treatment in coeliac disease and DH – Dr Nina Lewis)

3.4.7 Management of coeliac disease and DH

Research into the individual care needs and management of the disease and associated conditions. (Osteoporosis in coeliac disease associated with novel auto-antibodies – Dr Philip Riches)

3.4.8 Health and social care services research

Research into the provision and delivery of health and social care services and health policy. (We have provided local healthcare teams with available evidence to support the role of the dietitian in delivery of post diagnosis care and education in coeliac disease.

3.4.9 The gluten-free diet

Including but not limited to the investigation of; ingredients, preparation, nutritional composition, access, cost and availability of foods to support a gluten-free diet. (Preparation of gluten-free food in a kitchen where wheat flour is being used simultaneously - Coeliac UK)

3.5 Research Strategy objectives

- We will review allocation of research funding by the Charity to ensure that research supports the key evidence gaps in promoting improved healthcare and food options whilst achieving value for money.
- We will extend our research portfolio and develop international research networks using links with European and global research partners eg the Association of European Coeliac Societies and Coeliac UK grant holders, to secure partnerships.
- We will take a leading role in the creation of an international research fund which will fund research into greater understanding of the diverse nature of the disease and effective management solutions.
- We will continue to commit at least 5% of the Charity's income to supporting research.

3.6 How we will achieve our research objectives

3.6.1 Research call

A call for research, the focus and priority, is ultimately approved by the Board of Governors, usually in the summer. The research call and application process will be clearly advertised on the Coeliac UK website and networks, with relevant professional bodies and in a peer reviewed journal.

Types of research call:

- Specific, where only research proposals supporting a given research question will be accepted. ie research commissioned to directly underpin the Charity's business activities.
- Open, where research proposals on any topic related to improving long term understanding of the fundamentals of coeliac disease and DH will be accepted ie researcher led, response mode

Grant applications are screened for completeness by the Research Manager and qualifying applications are internally reviewed by Members of the Charity's Health Advisory Council (HAC) and/or the Food Standards Committee (FSC) before external independent peer review. The advice of the HAC and/or FSC and the independent peer review is considered by the Board of Governors before making an ultimate decision on applications to be approved for funding.

All grant holders are required to:

- provide progress updates every 6 months as a condition of funding; annual reports will be reviewed by the HAC or the FSC before the release of ongoing funds (refer to associated documentation)
- provide lay summaries of progress for our Members, to be uploaded on our website.
- attend Charity events to present findings and also contribute to publications and other conferences.
- complete an end of grant report (refer to associated documentation)

This is to ensure the achievements and the implications of the research Coeliac UK has funded are recorded and communicated. This information allows us to determine if the research has been carried out in accordance with the grant conditions and objectives of the Charity. It also helps with the reporting to our Members and for our future planning and strategy development.

3.6.2 Commissioning research outside of a research call

There may be occasions throughout a year when the Charity needs to commission research, outside a traditional research call, to underpin the Charity's business activities and inform a food or health policy on a shorter term basis. In this situation,

depending on the research question to be answered, the research need will either be advertised for open tender or via a more targeted approach.

Where the required funding is less than £30K, the research proposals will not require peer review by the HAC or the FSC. The proposals will be reviewed within the Coeliac UK Policy, Research and Campaigns (PRC) team and where considered necessary, the expertise and advice of one or more Members of the HAC and/or the FSC or other external experts may be sought. In this case the final decision for funding is made by the Charity's Chief Executive Officer.

Where the required funding is more than £30K, external expertise and advice (from the HAC and/or the FSC with possible additional advice from other experts) will be consulted and funding approval will be sought from the Board of Governors.

The reporting structure will be specified in the terms and conditions of contract.

3.6.3 Researcher-led proposals submitted to the Charity on speculation

There are times when researcher-led proposals are submitted on speculation to the Charity outside a research call. All submissions will be recorded on a Research Register. They will, in the first instance, be screened by the Coeliac UK Research Manager.

The Charity is receptive to such research ideas so as not to overlook opportunities which it may otherwise not have considered but which are innovative and may be important to underpinning the work of the Charity or for the greater understanding of coeliac disease and DH.

Where the requested funding is more than £30K, the research proposal will either be filed for consideration during the next research call or if considered appropriate by the PRC team and the Charity's Chief Executive officer, the proposal will be forwarded to the HAC and/or the FSC or other external experts for formal peer review and where applicable, funding approval sought from the Board of Governors.

Where the requested funding is less than £30K the proposal will be reviewed within the Coeliac UK PRC team and, where necessary, the expertise and advice of one or more Members of the HAC and/or the FSC or other external experts may be sought. In this case the final decision for funding is made by the Charity's Chief Executive Officer.

3.6.4 Research Fellowship

The objective of the fellowship (clinical or non-clinical) is to build capacity in research into coeliac disease and DH, where there is currently a deficit, by encouraging the best postgraduates to embark on an early career in coeliac disease and DH.

Research fellowships will be advertised and applications processed in the same way as for a research call.

3.6.5 Sponsored dissertation

The Charity will provide a small grant (max. £1000 per student) to encourage undergraduates or postgraduates (including but not limited to dietitians,

immunologists, food technologists, clinicians, epidemiologists, social scientists, psychologists, medical students) studying at UK institutions, to focus on coeliac disease or DH in their project work.

The primary objective is to place coeliac disease, a culturally specific condition, on the research agenda of UK academic institutions, whilst commissioning research that underpins the Charity's business plan activities.

Applications will be reviewed within the PRC team and the decision for funding will be made by the Charity's Chief Executive Officer based on the advice of the PRC team.

The grants will be advertised on the Coeliac UK website and networks.

3.6.6 Our Members

In a survey of our Members, 79% indicated research to be a priority (*Coeliac UK 2012*).

Our Members and their carers/families play a vital role in research, their participation in the past has advanced the understanding of coeliac disease and DH.

We will use our available communication channels to connect researchers with our Members; via our website, electronic newsletter, Crossed Grain magazine and social media networks.

3.6.7 Our Staff

Our staff have a wealth of experience, expertise and knowledge in coeliac disease and DH. Where appropriate we will support researchers in the preparation of their research proposals and/or applications for funding. Where resource allows and the expertise is available we contribute to steering committees and facilitate an outreach to the wider research network.

3.6.8 Professional and public engagement

We will aim to hold an annual research conference for healthcare professional Members of the Charity: dietitians, clinicians and researchers. This will provide the opportunity for researchers, including those funded by Coeliac UK, to share their research findings with others and also contribute to their continuing professional development.

We will develop and promote training and education opportunities into coeliac disease and DH.

The results of our research can inform the Charity's work on policy and campaigning, as well as influence the research agenda of others.

3.6.9 Collaborations

Where research into coeliac disease and DH is a common interest to other national charities in Europe and beyond, we will lead the way for cofunding.

We will develop successful relationships with other charities and research networks where there are synergies in research, to strengthen our voice and raise the profile of coeliac disease and DH.

We will also work to influence the multi-national research agenda into coeliac disease and DH.

3.6.10 Research priorities

Our research priorities are those identified to fill the evidence gaps of relevance to our strategic aims. They will be determined through an interpretative 'listening model' approach, based on the engagement and exchange between research funders, researchers and the potential users and beneficiaries of research. They are established as part of our annual business planning and in conjunction with:

- Healthcare professionals including the HAC, the FSC and the Health Associates Network
- Research experts in the field of coeliac disease and DH
- Relevant professional bodies and funders of research
- Our Members and patient representatives
- The Senior Management team

The Board of Governors has the ultimate decision on the research priorities of the Charity.

We will also endeavour to achieve a wider identification and agreement on the uncertainties in coeliac disease and DH.

3.7 Funding

Investment in research is a priority for Coeliac UK and the Charity will commit to invest a minimum 5% of annual income. Currently our income streams originate from a combination of Membership fees, donations and legacies.

The Charity is also committed to diversifying its current income streams to extend its research portfolio; we will proactively survey external grant opportunities such as those from the NHS, research councils, other charities and industry and submit applications to obtain additional funding to close the gap.

3.8 Research Register

The Charity has access to a network of researchers working in the field of coeliac disease and maintains a register of known research projects. If you are researching in the field of coeliac disease and DH and would like to be added to the Research Register or placed in contact with other researchers, please contact our Research Manager at heidi.urwin@coeliac.org.uk

3.9 Associated documentation

Application for a Coeliac UK research grant award
Application for a Coeliac UK research fellowship
Application for a sponsored dissertation
Information for grant applicants
Grant conditions
Grant progress report form
Grant final report form
Research priorities
Terms and conditions of the HAC and the FSC

3.10 Acknowledgements

The Charity wishes to acknowledge the work of Lisa Miles, former Research Manager at Coeliac UK, for the findings of the Organisational Policy Analysis.

3.11 Your views

If you have any comments or questions about our Research Strategy 2013-2016 please contact our Research Manager at heidi.urwin@coeliac.org.uk

3.12 References

Coeliac UK (2012) Member Survey

West J, Logan RFA, Hill PG et al. (2003) Seroprevalence, correlates and characteristics of undetected coeliac disease in England. *Gut* 52; 960-65

West J, Fleming KM, Tata LJ et al. (2014) Incidence and prevalence of coeliac disease and dermatitis herpetiformis in the UK over two decades; population based study

Appendices

Ethical approval for secondary analyses on OPEN study



Observational / Interventions Research Ethics Committee

Ms. Lisa Miles
DPH / EPH
LSHTM

16 April 2014

Dear Ms. Miles,

Submission Title: Secondary analyses on the OPEN (Older People and Enhanced Neurological Function) study

LSHTM Ethics Ref: 7176

Thank you for your application of 3 April 2014 for the above research, which has now been considered by the Observational Committee via Chair's Action.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved is as follows:

Document Type	File Name	Date	Version
Investigator CV	CV ea 2 pages_v3.docx	19/12/2013	1
Investigator CV	CV Dangour June 2013.docx	19/12/2013	1
Local Approval	OPEN study LSHTM ethics application.doc	19/12/2013	1
Local Approval	FORMB - Dangour 23.04.08.pdf	19/12/2013	1
Investigator CV	cv201213LMiles.docx	20/12/2013	1
Protocol / Proposal	Secondary Analyses of OPEN Protocol v2.docx	07/02/2014	2

After ethical review

Any subsequent changes to the application must be submitted to the Committee via an Amendment form on the ethics online applications website. All studies are also required to notify the ethics committee of any serious adverse events which occur during the project via an Adverse Event form on the ethics online applications website. At the end of the study, please notify the committee via an End of Study form on the ethics online applications website. Ethics online applications website link: <http://leo.lshtm.ac.uk>

Yours sincerely,

Professor John DH Porter
Chair

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<http://www.lshtm.ac.uk/ethics/>