

## Systematic Review

# Is there an association of vitamin B<sub>12</sub> status with neurological function in older people? A systematic review

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### Abstract

Low vitamin B<sub>12</sub> 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 B<sub>12</sub> 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 B<sub>12</sub> status with some, but not all, neurological outcomes. Among groups with clinical and/or biochemical evidence of low vitamin B<sub>12</sub> status, one longitudinal study reported an association of vitamin B<sub>12</sub> 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 B<sub>12</sub> 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 B<sub>12</sub> status in older people.

**Key words:** Vitamin B<sub>12</sub>: Neurological function: Nerve conduction: Older people

Ageing is associated with a decline in vitamin B<sub>12</sub> status<sup>(1,2)</sup>, and there is widespread evidence of low vitamin B<sub>12</sub> status in older people<sup>(1,3)</sup>. In the UK, 5% of adults aged 65–74 years and 10% adults aged ≥75 years have low vitamin B<sub>12</sub> levels (defined as vitamin B<sub>12</sub> <150 pmol/l) or metabolically significant vitamin B<sub>12</sub> deficiency (defined as vitamin B<sub>12</sub> <200 pmol/l and homocysteine level >20 μmol/l)<sup>(1)</sup>. However, intakes of vitamin B<sub>12</sub> 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 B<sub>12</sub> intakes below the lower reference nutrient intakes<sup>(4)</sup>. Several factors relating to vitamin B<sub>12</sub> 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 B<sub>12</sub>-binding proteins, and lack of liver vitamin B<sub>12</sub> stores<sup>(5)</sup>.

Indeed, food-cobalamin malabsorption can account for up to 60–70% of confirmed cases of vitamin B<sub>12</sub> deficiency in older people<sup>(6)</sup>.

The clinical manifestations of vitamin B<sub>12</sub> deficiency can be haematological, neurological or both<sup>(7)</sup>; neurological symptoms can occur in the absence of anaemia in 20–30% of cases<sup>(8)</sup>. Pathological investigations in vitamin B<sub>12</sub> deficiency reveal demyelination in the spinal cord, peripheral nerves and/or the white matter of the brain<sup>(7)</sup> and an abnormal increase in astrocytes in the brain due to damage to nearby neurons<sup>(9)</sup>. It has been proposed that the mechanism by which vitamin B<sub>12</sub> deficiency affects neurological function involves impairment of vitamin B<sub>12</sub>-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<sup>(10,11)</sup>. 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<sup>(12,13)</sup>. Symptoms of peripheral neuropathy associated with vitamin B<sub>12</sub> deficiency commonly include symmetric paresthesias, numbness or gait problems. Other neurological defects include impaired vibration sense, impaired position and cutaneous sensation, ataxia, and weakness<sup>(2,7)</sup>. The relationship between vitamin B<sub>12</sub> status and cognitive function has been reviewed extensively elsewhere<sup>(14–17)</sup>.

The functional and public health significance of low vitamin B<sub>12</sub> status in older people is currently unclear. In the clinical setting, impaired neurological function is a known marker of frank vitamin B<sub>12</sub> deficiency. However, whether neurological impairment is associated with low vitamin B<sub>12</sub> status at a population level is unknown. To date, the epidemiological evidence of the association of vitamin B<sub>12</sub> status with neurological function has not been systematically reviewed. The present systematic review aims to evaluate the association of low vitamin B<sub>12</sub> 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 B<sub>12</sub> 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 B<sub>12</sub> 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  $\geq 50$  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 B<sub>12</sub> status (including bariatric surgery) or metabolites of vitamin B<sub>12</sub> (including renal insufficiency) were excluded. Included exposures were serum/plasma vitamin B<sub>12</sub>, transcobalamin (a binding protein for vitamin B<sub>12</sub>), holotranscobalamin (the form of vitamin B<sub>12</sub> able to cross into the cells), methyl malonic acid (a metabolite of vitamin B<sub>12</sub>) and dietary vitamin B<sub>12</sub> (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)<sup>(18)</sup>. 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 B<sub>12</sub> deficiency), category of neurological outcome, definition of low vitamin B<sub>12</sub> 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<sup>(19)</sup>. 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  $\leq 50$  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<sup>(20–27)</sup>, with the remaining studies conducted in the USA<sup>(28)</sup>, Asia<sup>(29,30)</sup> and Australia<sup>(31)</sup>. Of the European articles, three were from Denmark with overlapping samples of participants from the same study<sup>(20–22)</sup>. There was heterogeneity in the types of population from which subjects were drawn; eight articles<sup>(23,24,26–31)</sup> were based on general population groups of older people, though two of these recruited from hospitals (see online Supplementary Table S1), and four articles<sup>(20–22,25)</sup> (from two studies) were based on subjects with clinical and/or biochemical evidence of low vitamin B<sub>12</sub> 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 B<sub>12</sub> 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,

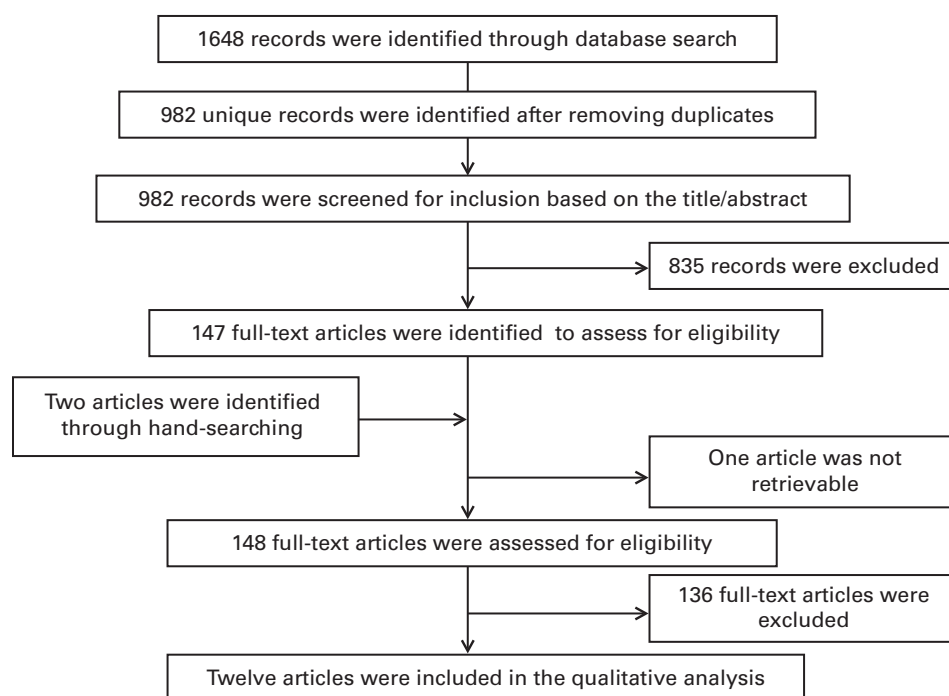


Fig. 1. Study selection process for the systematic review of the association of vitamin B<sub>12</sub> status with neurological function in older people.

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<sup>(23)</sup> and seven cross-sectional<sup>(24,26–31)</sup> analyses were based on general population groups of older people (see online Supplementary Table S1). One longitudinal study<sup>(23)</sup> with a follow-up of 3 and 6 years and low risk of bias provided no evidence of an association of vitamin B<sub>12</sub> status or change in vitamin B<sub>12</sub> status with electrophysiological measures of nerve function or peripheral neuropathy. One cross-sectional analysis<sup>(28)</sup> with a low risk of bias suggested an association of low vitamin B<sub>12</sub> status with some, but not all, electrophysiological measures of nerve function. One small cross-sectional analysis<sup>(29)</sup> with an identified risk of bias found no association of vitamin B<sub>12</sub> status and electrophysiological measures of nerve function. Five further cross-sectional studies with low<sup>(24,26)</sup> or identified<sup>(27,30,31)</sup> risk of bias reported clinically measured signs and symptoms of neuropathy and self-reported neurological symptoms, and provided mixed results. Three analyses<sup>(24,27,30)</sup> identified a statistically significant association of vitamin B<sub>12</sub> status with some, but not all, clinical signs of neuropathy or self-reported symptoms, the remaining two cross-sectional studies<sup>(26,31)</sup> 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 B<sub>12</sub> status*

One longitudinal analysis<sup>(22)</sup> (mean follow-up 1.0–3.9 years) and three cross-sectional analyses<sup>(20,21,25)</sup> were based on subjects with clinical and/or biochemical evidence of low vitamin B<sub>12</sub> status (see online Supplementary Table S2). One small cross-sectional study<sup>(25)</sup> with an identified risk of bias found no association of vitamin B<sub>12</sub> 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<sup>(20,21)</sup> from the Danish study did not identify any association of vitamin B<sub>12</sub> 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 B<sub>12</sub> 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 B<sub>12</sub> status and neurological function, three used composite definitions of low vitamin B<sub>12</sub> status involving plasma/serum vitamin B<sub>12</sub> plus elevated methyl malonic acid or homocysteine levels.

The exercise to identify selective reporting bias showed that two cross-sectional studies<sup>(27,30)</sup> did not report results for all neurological outcomes measured. In addition, one longitudinal analysis of participants with low vitamin B<sub>12</sub> status<sup>(22)</sup>

measured dietary vitamin B<sub>12</sub> 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 B<sub>12</sub> 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 B<sub>12</sub> status and nerve conduction<sup>(23)</sup>. The limited evidence for an association comes from four of seven cross-sectional analyses<sup>(24,27,28,30)</sup> that identified positive associations of vitamin B<sub>12</sub> 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 B<sub>12</sub> status. None of the three cross-sectional analyses (from two studies)<sup>(20,21,25)</sup> reported any association of vitamin B<sub>12</sub> status with neurological function, and in the single longitudinal analysis<sup>(22)</sup>, results were mixed.

This is the first systematic review investigating the association of vitamin B<sub>12</sub> 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 B<sub>12</sub> 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 B<sub>12</sub> deficient) and in how low vitamin B<sub>12</sub> status was defined. Debate continues about the most appropriate method for assessing vitamin B<sub>12</sub> 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 B<sub>12</sub> status using both a biomarker of circulating vitamin B<sub>12</sub> and a functional biomarker such as methyl malonic acid or homocysteine<sup>(32)</sup>. 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 B<sub>12</sub> 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 B<sub>12</sub> 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 B<sub>12</sub> status in older people. Well-designed observational studies that comply with the latest recommendations on measuring vitamin B<sub>12</sub> status<sup>(32)</sup> 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 B<sub>12</sub> 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>

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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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