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Systematic Review

Vitamin B_{12} status, cognitive decline and dementia: a systematic review of prospective cohort studies

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Abstract

Poor vitamin B_{12} status may lead to the development of cognitive decline and dementia but there is a large variation in the quality, design of and results reported from these investigations. We have undertaken a systematic review of the evidence for the association between vitamin B_{12} status and cognitive decline in older adults. A database search of the literature to 2011 was undertaken, using keywords related to vitamin B_{12} and cognition. All prospective cohort studies assessing the association of serum vitamin B_{12} or biomarkers were included. Quality assessment and extraction of the data were undertaken by two researchers. The quality assessment tool assigns a positive, neutral or negative rating. Of 3772 published articles, thirty-five cohort studies (n 14 325 subjects) were identified and evaluated. No association between serum vitamin B_{12} concentrations and cognitive decline or dementia was found. However, four studies that used newer biomarkers of vitamin B_{12} status (methylmalonic acid and holotranscobalamin (holoTC)) showed associations between poor vitamin B_{12} status and the increased risk of cognitive decline or dementia diagnosis. In general, the studies were of reasonable quality (twenty-one positive, ten neutral and four negative quality) but of short duration and inadequate subject numbers to determine whether an effect exists. Future studies should be of adequate duration (at least 6 years), recruit subjects from the seventh decade, choose markers of vitamin B_{12} status with adequate specificity such as holoTC and/or methylmalonic acid and employ standardised neurocognitive assessment tools and not screening tests in order to ascertain any relationship between vitamin B_{12} status and cognitive decline.

Key words: Vitamin B_{12}: Cognition: Dementia: Systematic reviews

Cognitive decline and dementia have a significant impact on the independence and quality of life of sufferers and carers, and research into modifiable risk factors is paramount.

The most prevalent form of dementia is Alzheimer's disease (AD) which accounts for up to 70 % of cases[1], with other common forms including dementia with Lewy bodies, frontotemporal dementia and vascular dementia[2]. Risk factors for dementia include advanced age, genetics, low educational level as well as CVD, and its component vascular risk factors[1,2]. The most important known genetic risk factor for the development of dementia is possession of the apoE4 allele which substantially increases the risk of AD by two to three times[3].

Poor vitamin B_{12} status has been linked to cognitive decline for at least 50 years[4] but the role of vitamin B_{12} in this process is not clear. Vitamin B_{12} deficiency causes neurological degeneration with demyelination of the spinal cord and some initial studies have described a reversible dementia related to vitamin B_{12} deficiency[5–10].

A link between vitamin B_{12} status and cognitive decline is biologically plausible. Vitamin B_{12} is required for DNA and myelin synthesis, and it is a cofactor for the methylation of total homocysteine (tHcy) to methionine and for the conversion of methylmalonyl-CoA to succinyl-CoA[11]. Both tHcy and methylmalonic acid (MMA) accumulate while holotranscobalamin (holoTC), the active transport protein carrying vitamin B_{12}, decreases with inadequate vitamin B_{12} status.

The suggested aetiologies behind any association between cognitive decline related to low vitamin B_{12} status include inadequate methylation in the central nervous system[12], the accumulation of tHcy and/or MMA[13], the effects on the cerebral vasculature and brain atrophy and white matter damage[1].

Abbreviations: AD, Alzheimer's disease; holoTC, holotranscobalamin; MMA, methylmalonic acid; MMSE, Mini Mental State Examination; tHcy, total homocysteine.

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Recent reviews have linked high tHcy concentrations with an increased risk of cognitive decline and dementia[14–18]; however, it is not known whether tHcy is a marker of disease or a causative factor in the dementing process. A large number of studies have investigated the association between vitamin B_{12} status and cognition, but there is no consistency in outcomes and the independent role of vitamin B_{12} status in the development of neurocognitive decline is uncertain.

Cross-sectional studies showed positive associations between serum vitamin B_{12} and scores on cognitive tests, but cohort studies did not[19,20]. Balk *et al.*[19] assessed seven cohort studies, with only two showing associations of improved cognition with higher serum vitamin B_{12} concentrations. In three recent reviews, it was found that seven of fifteen, one of six and zero of three cohort studies showed associations between low serum vitamin B_{12} status and increased rates of cognitive decline[20–22]. These reviews, although finding no association between vitamin B_{12} and cognitive decline, have highlighted the methodological limitations of studies[15,19–21].

The most recent systematic reviews include studies published before 2007[19,21] and only include studies assessing vitamin B_{12} concentrations. Other studies that utilise sensitive biomarkers of vitamin B_{12} status, holoTC and MMA, have since been published. The aim of the present systematic literature review was to provide an up-to-date identification and critical appraisal of published studies of the longitudinal association between vitamin B_{12} status and the spectrum of cognitive decline and dementias in older adults.

Methods

A review protocol including library search strategy, inclusion and exclusion criteria, use of data extraction and quality tool templates was determined before the review.

Search strategy

A literature search was undertaken on 6 June 2010 and an update was performed on 12 August 2011. Database searches included Medline (1950–present), Pre-medline, Psyc INFO (1806–present), all EBM Reviews (ACP Journal Club, DARE and CCTR (1991–present), and Cochrane DSR (2005–present)), Cinahl (1982–present) and Embase (1980–present). Both medical subject headings and text words were used for dietary supplement terms, vitamin B_{12} and biomarkers (e.g. homocysteine, methylmalonic acid and holotranscobalamin) and common terms for cognition and dementia (e.g. cognition, dementia, memory and Alzheimer's disease) with appropriate truncation. The full search terms for the Medline database can be seen in the Appendix. Filters to limit publications to English language and to middle- and older-aged human subjects were applied at the end of each database search if available.

Study selection

Citations from each literature database search were downloaded into referencing software Endnote X1. Titles and abstracts were assessed for inclusion criteria. Inclusion criteria were prospective cohort studies assessing the association of serum vitamin B_{12}, MMA or holoTC and cognition or dementia in older adults. Studies that measured vitamin B_{12} status but only reported a lack of association in the text of the results were also included. All other studies were excluded. The reference lists of included studies and reviews identified in the literature search were checked for relevant articles. Studies with more than one publication from the same subject sample were reported as one study and studies reporting more than one outcome, e.g. dementia and AD, were reported separately.

Data extraction and synthesis

The Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) 2009 statement was used to guide the conduct and reporting of the present systematic review[23]. Data were extracted by two reviewers using the American Dietetic Association's Evidence Analysis data extraction template[24], with adjudication by a third reviewer if required. For each study, the following information was extracted: study description; participant selection and characteristics (including age, disease characteristics, baseline cognitive status and method of diagnosis); inclusion and exclusion criteria; subject numbers and withdrawals; statistical methods used; ascertainment and length of exposure; outcome measure characteristics; funding arrangements. No attempt was made to contact authors of included studies as only published data were included. A meta-analysis was deemed inappropriate due to the variability of baseline populations, cognitive and vitamin B_{12} status tests and reported outcome statistics.

Study quality

The quality of the studies was assessed by two reviewers using the American Dietetic Association Study Quality criteria guidelines[24], with a third reviewer resolving any disagreements. The quality assessment tool comprises ten questions related to the soundness and reporting of study design, methods and results and returns one of three scores of 'positive', 'neutral' or 'negative'. An overall positive score requires that the majority of the questions be answered 'yes' and that four essential validity questions be answered 'yes'. These questions assess bias in subject selection, comparability of subject groups, intensity and duration of exposure and the validity and reliability of the outcome measurements. For the present review, two of these questions were not able to be answered as the information is not known, so the questions were designated as 'not applicable' as outlined in the National Institute for Health and Clinical Excellence (NICE) guideline development manual[25]. These questions (numbers 6 and 7) relate to the 'intensity and duration of exposure' to low vitamin B_{12} status required to show an effect and the 'validity and reliability of outcome measures', i.e. of cognitive assessment tools. A neutral score is given if the answers to the four essential validity questions do not indicate that the study is exceptionally strong and a negative is awarded if most (six or more) of the answers to the validity questions are 'No'.

Results

A total of 3772 citations were downloaded for review with thirty-five cohort studies fulfilling the selection criteria. Fig. 1 illustrates the study selection process.

Summary of included studies

The studies were from ten countries encompassing the areas of North America (11), Europe (16), the UK (3), Asia (4) and Israel (1), and assessed a total of 14 325 subjects. Subject ages ranged from 47 to 101 years with a mean sample size of 409 subjects (median 271; range 24–1405), followed for a mean of 5·4 years (median 4·4 years; range 0·5–35 years).

Vitamin B_{12} status was determined predominantly by serum vitamin B_{12} concentrations alone (thirty-one studies). However, four studies used MMA[26,27] and/or holoTC[26,28,29] and one study used MMA in combination with serum vitamin B_{12}[27]. The majority of studies used multiple regression to assess the association of serum vitamin B_{12} and cognition with three studies applying cut-points of 110–251 pmol/l[30–32]. Moreover, seven studies reported only unadjusted data[30,33–38].

Cognitive decline was assessed in seventeen studies[26,27,35,37,39–51], the development of dementia or AD was determined in thirteen studies[28–34,40,52–56] and five studies assessed cognitive deterioration in subjects with diagnosed dementia or AD[36,38,57–59]. Futhermore, five studies reported two outcomes, one for dementia and one for AD[29–32,54].

Quality assessment and outcome

Quality assessment found that twenty-one studies were positive, ten were neutral and four were negative. Of the twenty-one positive studies with a low risk of bias, seven studies found positive associations between vitamin B_{12} status and cognitive decline[26,27,42,47], dementia[29,31] or AD[28,29,31] and fourteen studies did not[32,34,39,41,43–46,50,52–54,57,60]. In addition, nineteen[26,27,31,32,34,39,41–47,50,52–54,57,60] of the twenty-one studies of positive quality used serum vitamin B_{12} with three finding significant associations with cognitive decline[27,42,47]. All four studies using the markers holoTC and/or MMA were of positive quality and all showed significant associations with cognitive decline, dementia or AD[26–29].

Neuropsychological assessment was performed using standardised tools. Dementia and AD diagnosis were defined by the Diagnostic and Statistical Manual of Mental Disorders (third and fourth edition), the Mattis Dementia Rating Scale

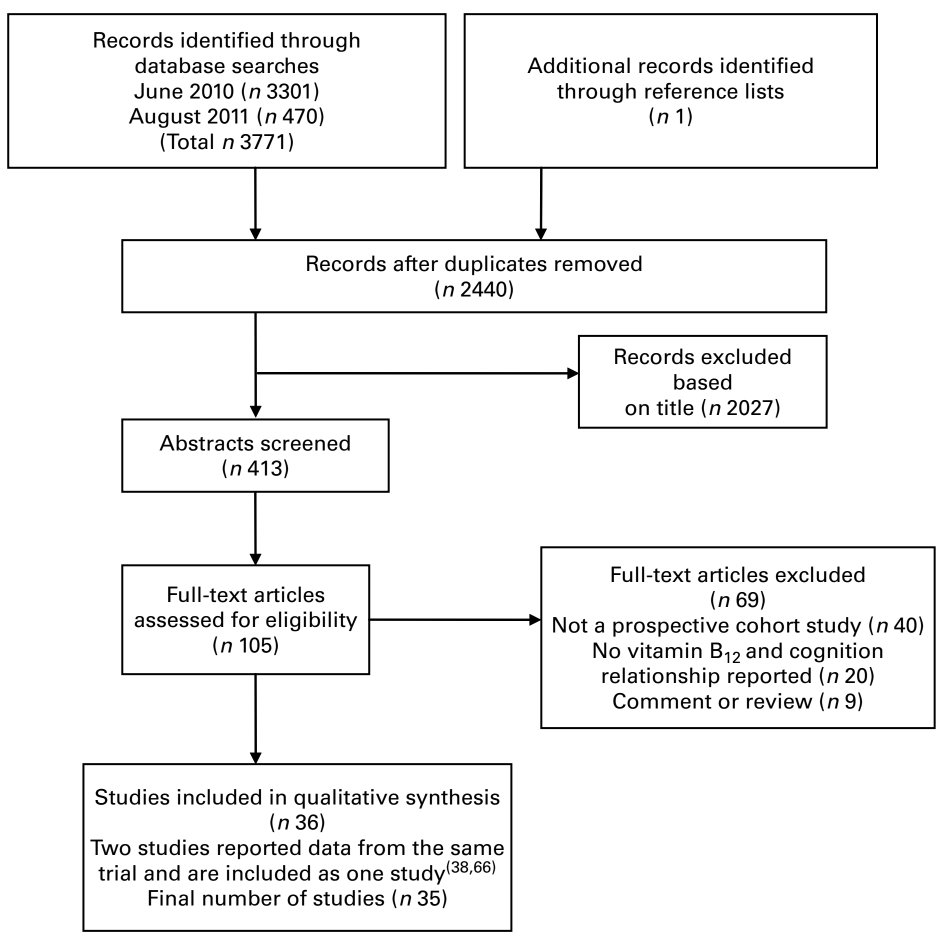

Fig. 1. Flow chart of literature search and study selection.

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Table 1. Relationship between vitamin B_{12} and cognitive decline in non-demented subjects

(Mean values, ranges and medians)

Study	Age* and sex	Follow-up years, *n*	Exclusion criteria	Adjustments	Vitamin B_{12} biomarkers and cognitive outcome	Outcome summary	Quality score
Kim *et al.*[39], South Korea	71·9 years 43 % M	2·4 years *n* 607	Dementia	Age, sex, education	No association of the change in MMSE-K with ascending baseline serum vitamin B_{12} quintiles	No effect	P
Haan *et al.*[40], USA	60–101 years 42 % M	4·5 years *n* 1405	Dementia, CIND, subjects without blood results	Age, education, tHcy, excluding baseline stroke	U-shaped association between vitamin B_{12} and dementia/CIND (HR 1·07, 95 % CI 1·02, 1·11). Vitamin B_{12} modified the positive association between tHcy and outcome. Rates of dementia or CIND associated with tHcy for those in the lowest and highest tertiles of vitamin B_{12} were higher (HR 1·61, *P* for interaction = 0·04) and lower (HR 0·94, *P* = 0·02) *v.* those in the middle tertile, respectively	Increased risk. Interaction effect of tHcy and serum vitamin B_{12}	O
Kado *et al.*[41] and Brown *et al.*[74], USA	74 years 60 % M	7 years *n* 370	Based on physical activity, activities of daily living and cognitive functions	Age, sex, education, baseline cognitive and physical function, smoking, vitamin B_6, folate, tHcy	No association of quartiles of serum vitamin B_{12} and composite score of five tests (confrontation naming test, delayed recognition span test, test to assess geometric figure copying, similarities subtest of WAIS-R and a test to assess abstract concept formation). Reanalysis of data in 2011: no interaction of low vitamin B_{12} (< 294·1 pg/ml) with apoE4 status in predicting cognitive decline (cognitive tests as above)	No effect	P
Elias *et al.*[42], USA	60–82 years 49 % M	7·6 years *n* 705	Stroke, dementia, tHcy > 90 μmol/l	Age, sex, education, plasma B_6 and folate, stroke, renal and CVD risk factors, apoE4, smoking, alcohol, coffee	Serum vitamin B_{12} significantly associated with improved global composite score, visual reproductions – immediate and delayed recall, visual reproductions – delayed recognition, logical memory – immediate and delayed recall (β = 0·021, 0·0356, 0·041, 0·0413, 0·033 and 0·043, respectively, all at least *P* < 0·05)	Yes, reduced risk	P
Teunissen *et al.*[43], The Netherlands	57 years 59 % M	6 years *n* 92	CVD, stroke, PD, dementia, epilepsy, mental disability, psychotropic drug use	Age, sex, education	No association of baseline serum vitamin B_{12} with Stroop test, Letter-Digit Coding Test, Word Learning Test or Delayed Recall	No effect	P
McCaddon *et al.*[44], UK	74 years† 31 % M	5 years *n* 32	Dementia, MMSE ≤ 25, pernicious anaemia, gastric surgery, hepato–renal disease, cancer, malabsorption, vegetarian, taking medications affecting tHcy	Age, HT, smoking, education, folate, tHcy, creatinine	No independent association between serum vitamin B_{12} and MMSE	No effect	P

M, male; MMSE-K, Mini Mental State Examination – Korean; P, positive; CIND, cognitive impairment no dementia; tHcy, total homocysteine; HR, hazard ratio; O, neutral; WAIS-R, Wechsler Adult Intelligence Scale-Revised; PD, Parkinson's disease; MMSE, Mini Mental State Examination Score; HT, hypertension.

* Mean or range (years).

† Median.

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and the Mental Deterioration Battery of the Neuroepidemiology Branch of the National Institute of Neurological Disorders and Stroke criteria. Studies assessing cognitive decline used a variety of neuropsychological tool sets or individual tests, singly or in combination, and included the Mini Mental State Examination (MMSE) Score, the Wechsler Adult Intelligence Scale subsets, the Boston Naming Test, the Stroop Colour Word Test and the Wechsler Memory Scale.

Cognitive decline and vitamin B_{12} status in non-demented subjects

The association of cognitive decline with vitamin B_{12} status in non-demented subjects was assessed in six studies (Table 1). Haan *et al.*[40] found that vitamin B_{12} was associated with an increased hazard ratio for the development of dementia or cognitive impairment (hazard ratio 1·07, 95 % CI 1·02, 1·11). Interactions between vitamin B_{12} and tHcy were also found, with vitamin B_{12} modifying the association between tHcy and the development of dementia or cognitive impairment. For those in the first tertile of serum vitamin B_{12} (<340 pg/ml), the rates of dementia or cognitive impairment associated with tHcy were higher, and for those in the third tertile of vitamin B_{12} (≥498 pg/ml), the rates of dementia or cognitive impairment were lower compared with the second tertile[40]. Elias *et al.*[42] found a small significant association of serum vitamin B_{12} with the Wechsler Memory Scale composite score and its subsets. However, four studies showed no associations of cognitive decline and serum vitamin B_{12}[39,41,43,44] after a mean follow-up period of 5·1 years.

Cognitive decline and vitamin B_{12} status in subjects with unspecified cognition

The association of cognitive decline in subjects with normal or unspecified cognition was assessed in eleven studies using multiple tests of cognition. Of these eleven studies, four found an association of vitamin B_{12} status and at least one test of cognition (Table 2). Tucker *et al.*[47] studied male subjects for 3 years and found a positive association of serum vitamin B_{12} and construction praxis (a neuropsychological assessment tool), but no other cognitive measures. Further, two studies followed subjects for 6 years, with Nurk *et al.*[49] finding a trend for increasing risk of memory deficit with decreasing quintiles of baseline vitamin B_{12} and the Kendrick Object Learning Test, and Tangney *et al.*[27] found associations between higher serum vitamin B_{12} and a slower decline in memory, and a faster decline in memory with higher MMA concentrations. Clarke *et al.*[26] followed 472 subjects for 10 years and reported no association of cognitive decline (MMSE score) with serum vitamin B_{12} concentrations, but found that a doubling of holoTC or MMA was associated with a slower and faster cognitive decline, respectively. Moreover, seven studies[35,37,45,46,48,50,51] found no associations between serum vitamin B_{12} and individual or composite cognition scores or the MMSE after a follow-up ranging between 2·3 and 6·0 years.

Development of dementia in subjects with mild cognitive impairment

The development of dementia or AD in subjects with cognitive impairment was assessed in three studies. Of these, one study from Sweden[33] analysed unadjusted data and found that females who developed AD had lower baseline vitamin B_{12} concentrations compared with those without AD, but no effect was found in the other two studies assessing subjects with cognitive impairment[34,60] (Table 3).

Development of dementia in subjects with no dementia at baseline

A further eight studies assessed non-demented subjects and the development of dementia (Table 4). Of these, three studies found associations between serum vitamin B_{12} or holoTC and the development of dementia[29,31,40], and five found no associations[30,32,52–54]. The development of AD was assessed in eight studies, with three finding associations with holoTC or serum vitamin B_{12} alone or in combination with low serum folate concentrations[28,29,31] (Table 5). Wang *et al.*[31] detected no association between serum vitamin B_{12} or folate and AD alone but found a doubling of risk of AD with serum vitamin B_{12} ≤150 pmol/l or serum folate ≤10 nmol/l compared with normal concentrations. A 7-fold additional risk was found for subjects with a MMSE >26 and serum vitamin B_{12} ≤250 pmol/l or folate ≤12 nmol/l *v.* normal[31]. Kivipelto *et al.*[29] found that subjects with baseline holoTC concentrations in the third, compared with the first, quartile had a reduced relative risk for the development of AD after nearly 7 years (relative risk 0·38, 95 % CI 0·15, 0·94). However, no difference between the fourth and first quartiles was found[29]. Hooshmand *et al.*[28] also found that subjects with lower holoTC had a reduced OR of 0·977 for each 1 pmol/l increase in holoTC.

Cognitive decline in subjects with existing dementia

The association of baseline vitamin B_{12} and cognitive deterioration was assessed in five studies of subjects with dementia (Table 6). No associations between vitamin B_{12} and cognition were observed[36,38,57–59].

Discussion

The present review finds that there is insufficient evidence to determine whether vitamin B_{12} status is associated with cognitive decline or dementia. The assessment of the thirty-five cohort studies or, more particularly, of the twenty-one studies of positive quality, does not support a role for the association of serum vitamin B_{12} concentrations in the aetiology of cognitive impairment or dementia. Of the twenty-one studies of positive quality, seven found significant associations between vitamin B_{12} status and cognitive decline[26,27,42,47], dementia[29,31] or AD[28,29,31]. An interesting finding of the review is that the markers of vitamin B_{12} status with greater specificity, i.e. holoTC and MMA, showed consistent results with all four

Table 2. Relationship between vitamin B_{12} and cognitive decline in subjects with unspecified cognition
(Mean values and ranges)

Study	Age* and sex	Follow-up years, *n*	Exclusion criteria	Adjustments	Vitamin B_{12} biomarkers and cognitive outcome	Outcome summary	Quality score
van den Kommer *et al.*[45], The Netherlands	75·4 years 48·5 % M	6 years *n* 895	Subjects with high serum vitamin B_{12} or Cr, blood results	Age, sex, education, time, Cr, HDL, HT, TAG, ACT	Association of tHcy and cognition confounded by vitamin B_{12} for immediate recall, information processing speed, fluid intelligence, but not for MMSE or retention. No data on the independent effect of serum vitamin B_{12} concentrations	No effect	P
Tangney *et al.*[27], USA	80 years 61 % M	6 years *n* 498	Not reported	Age, sex, education, race, frequent cognitive activities, serum Cr, smoking, alcohol, SFA (g/d), diet, vitamin E, niacin, total vitamin C, serves fish/week	Higher serum vitamin B_{12} associated with a slower decline and higher MMA associated with a faster decline in cognition based on a composite score of the East Boston Test of immediate and delayed recall, MMSE and Symbol Digit Modalities Test ($\beta = 0{\cdot}00\,013$ and $-0{\cdot}00\,016$, $P<0{\cdot}005$, respectively)	Yes, reduced risk	P
Clarke *et al.*[26], UK	71·9 years 38 % M	10 years *n* 472	Serum vitamin B_{12} >1000 pmol/l or holoTC >400 pmol/l or vitamin B_{12} injections or supplement use	Sex, education, smoking, history of vascular disease, MMSE, systolic BP, apoE, tHcy, holoTC, MMA	Using the MMSE score, a doubling of holoTC from 50 to 100 pmol/l was associated with a 30 % slower cognitive decline; an increase of MMA from 0·25 to 0·5 μmol/l was associated with a >50 % more rapid cognitive decline. No association with serum vitamin B_{12}	Yes, reduced risk	P
Kang *et al.*[46], USA	>70 years 100 % F	4 years *n* 389†	History of stroke, CHD, breast or colon cancer, subjects without blood results	Assay batch, time between blood and cognitive test, age, education, DM, BP, TC, HRT use, menopause age, BMI, smoking, mental health, antidepressant use, aspirin use, alcohol, PA, vitamin E supplement use	No association between serum B_{12} and mean difference in the rate of cognitive decline assessed by the global score, telephone interview for cognitive status or verbal score	No effect	P
Tucker *et al.*[47], USA	67 years 100 % M	3 years *n* 280–284‡	Subjects with less than two cycles of cognitive testing	Time from cognitive measure, baseline score, age, education, BMI, smoking, alcohol, Cr, systolic BP, DM, FA fortification introduction, tHcy, serum folate concentrations	Positive association of serum vitamin B_{12} and construction praxis (spatial copying, sum of drawings, $\beta^2 = 0{\cdot}59$, $P<0{\cdot}05$) but no association of serum vitamin B_{12} with language (verbal fluency), working memory (backward digit span, longest span recalled), recall memory (word lists) or MMSE	Yes, reduced risk	P
Mooijaart *et al.*[48], The Netherlands	>85 years 34 % M	4 years *n* 341	<85 years	Sex, education, depression, B_{12} and FA supplements, living arrangements, tHcy, folate concentrations	No association between serum vitamin B_{12} and decrease in MMSE, global score, Stroop test, Letter-Digit Coding Test or 2-Word-Learning Test	No effect	O
Nurk *et al.*[49], Norway	65–67 years 45 % M	6 years *n* 235	Baseline tHcy >40 μmol/l	Sex, apoE genotype, education, CVD, HT, depression score	Trend for increasing risk of memory deficit (using the Kendrick Object Learning Test) with lower vitamin B_{12} quintiles (*P* for trend$=0{\cdot}042$, Q1 *v.* Q5: OR 1·63, 95 % CI 1·0, 2·7)	Yes, reduced risk	O
Garcia *et al.*[50], Canada	72·9 years 28 % M	2·3 years *n* 104	Vitamin B_{12} supplement use, gastric/ileal surgery, Cr >130 μmol/l, neurological disease, MMSE <24, depression, institutional living	Age, sex, education, time between visits, erythrocyte folate, DM, HT	In subjects with more than 40 % increase in tHcy from baseline, tHcy associated with declining Stroop scores ($P=0{\cdot}001$) but no association with serum vitamin B_{12}. Rate of change of the Stroop score not related to serum vitamin B_{12}	No effect	P

Table 2. *Continued*

Study	Age* and sex	Follow-up years, *n*	Exclusion criteria	Adjustments	Vitamin B_{12} biomarkers and cognitive outcome	Outcome summary	Quality score
Dufouil *et al.*[51], France	67 years 41·4 % M	4 years *n* 1241	Not reported	Age, sex, education, BMI, alcohol intake, smoking, HT, increased cholesterol, glycaemic status, vascular disease, serum folate	No association of serum vitamin B_{12} with tHcy or cognitive decline as determined by the MMSE or Digit Symbol Test, Finger-Tapping Test or Trail-Making Test scores	No effect	O
Eussen *et al.*[35], Europe	75–80 years 45 % M	5 years *n* 262	Subjects living in psycho-geriatric institution, no language fluency, unable to answer questions independently	No adjustments	No association between of serum vitamin B_{12} and MMSE	No effect	N
La Rue *et al.*[37], USA	77 years No data	6 years *n* 137	Diabetes, CHD, uncontrolled HT	No adjustments	No association of baseline serum vitamin B_{12} with Shipley-Hartford Intelligence Test, Abstraction Scale, Shipley-Hartford Intelligence Test Abstraction scale	No effect	N

M, male; Cr, creatinine; HT, hypertension; ACT, α-1-antichymotrysin; tHcy, total homocysteine; MMSE, Mini Mental State Examination; P, positive; MMA, methylmalonic acid; holoTC, holotranscobalamin; BP, blood pressure; F, female; DM, diabetes; TC, total cholesterol; HRT, hormone replacement therapy; PA, physical activity; FA, folic acid; O, neutral; Q, quintile; N, negative.

* Mean or range (years).

† For global score, *n* 389; for telephone interview for cognitive status, *n* 391; for verbal score, *n* 391.

‡ For construction praxis, *n* 280–284; for language, *n* 239–243; for working memory, *n* 236–240; for recall memory, *n* 235–239; for MMSE *n* 271–275.

studies finding associations with cognitive decline[26,27], dementia[29] and AD[28,29]. However, the study by Kivipelto *et al.*[29] found associations of holo TC and dementia development only for the third quartile.

The subject's age at recruitment and the length of time of exposure to a low vitamin B_{12} status for a change (if any) in cognition to be noted are not known; however, cognitive impairment and dementia generally develop over many years and studies of inadequate duration may not show any effect. The median duration of studies was 4 years, with only seven of the thirty-five studies assessing cognition for more than 6 years. The majority of studies recruited subjects aged greater than 75 years at baseline, with only two studies recruiting subjects from a midlife stage, with a follow-up of 35 and 6 years, respectively; however, neither showed any association with serum vitamin B_{12} and cognition[43,52]. Of the nine studies recruiting subjects from the seventh decade or earlier, five[33,40,42,47,49] found associations between cognition and vitamin B_{12} status, while only five[26–29,31] of the twenty-five studies commencing in later life found associations. The effect of vitamin B_{12} status is likely to commence in midlife with a long period for disease development, hence the baseline age of subjects needs consideration[1].

In order to ascertain whether any effect exists, the correct diagnosis of vitamin B_{12} status must be made; however, there is no 'gold standard' for the determination of vitamin B_{12} status and each diagnostic assay has limitations. High vitamin B_{12} concentrations generally indicate sufficiency, but the interpretation of the lower concentrations of vitamin B_{12} concentrations is unclear[61,62]. Vitamin B_{12} is carried by two proteins, with the active form of holoTC making up only 20–30 % of the total serum vitamin B_{12} measured. Studies have shown that the determination of the active form of vitamin B_{12}, holoTC and/or MMA, an indicator of tissue stores, improves the prediction of low vitamin B_{12} status[62], and a recent review has found the use of serum vitamin B_{12} concentrations alone unreliable in diagnosing a vitamin B_{12} deficiency[63]. Of the thirty-two studies that assessed serum vitamin B_{12}, ten showed associations with cognition, while four of four studies assessing the newer and more specific markers of vitamin B_{12} status showed an effect. Of the two studies that assessed holoTC or MMA in addition to serum vitamin B_{12}[26,27], only the study by Tangney *et al.*[27] showed an association with serum vitamin B_{12}, and this was in a folate-fortified population using neuropsychological assessment tools and after a follow-up of 6 years. Futhermore, one cohort study published after the literature evaluation for the present review was completed has been located. This study assessed non-demented subjects from the Cardiovascular Risk Factors, Aging and Dementia study[64] and supports the association of low holoTC concentrations with a decline in cognition.

The present review of papers indicates that the direction of any likely effect is consistent, with low vitamin B_{12} status being associated with increased rates of dementia or cognitive impairment. There was one study showing that both low and high serum vitamin B_{12} concentrations were associated with an increased risk of dementia or cognitive impairment. However, this result was confounded by an interaction effect

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Table 3. Relationship between vitamin B_{12} and dementia or Alzheimer's disease (AD) in subjects with mild cognitive impairment

(Mean values and ranges)

Study	Age* and sex	Follow-up years, *n*	Exclusion criteria	Adjustments	Vitamin B_{12} biomarkers and cognitive outcome	Outcome summary	Quality score
Annerbo *et al.*[34], Sweden	65·1 years 48 % M	6 years *n* 93	Non-MCI subjects	No adjustments	No difference in serum vitamin B_{12} concentrations between subjects who converted to AD and those who did not	No effect	P
Ravaglia *et al.*[60], Italy	76·0 years 49 % M	2·8 years *n* 77	Non-MCI subjects, MMSE <24, poor ADL function, cancer, neurological, metabolic, haematological, psychiatric disorders, depression, normal routine blood tests	Age, sex, education	Vitamin B_{12} <217 pmol/l not related to conversion to dementia (HR 0·6, 95% CI 0·26, 1·39, P=0·234)	No effect	P
Annerbo *et al.*[33], Sweden	64·5 years 42 % M	3 years *n* 96	Subjects taking B_{12} supplements, diagnosis of commotio cerebri (concussion)	No adjustments	Females who developed AD had lower baseline B_{12} (234 (SD 102) *v.* 304 (SD 100) pmol/l, P<0·01). No difference in males	Yes, reduced risk in females	O

M, male; MCI, mild cognitive impairment; P, positive; MMSE, Mini Mental State Examination; ADL, activities of daily living; HR, hazard ratio, O, neutral.
* Mean or range (years).

between vitamin B_{12} and tHcy that was not included in the reported statistical model[40].

The cognitive assessment tools that are chosen also need to have the sensitivity to detect changes in cognition affected by low vitamin B_{12} status. The majority of studies in the present review used neuropsychological tests for the detection of cognitive decline or dementia. However, nine studies used the MMSE alone or in combination with other cognitive tests. The MMSE is a validated screening, rather than a diagnostic tool, and, as such, may be less sensitive to cognitive change[65]. Vitamin B_{12} status was found to be associated with the MMSE in only one study, and this study used vitamin B_{12} markers with greater specificity, i.e. holoTC and MMA[26]. A combination of the MMSE and other cognitive tests was used in three studies[47,48,51], with one finding an association with vitamin B_{12}[47] but none with the MMSE. The sensitivity of cognitive tests to changes in tHcy has been investigated[66] but there is little information on the sensitivity of cognitive tests to vitamin B_{12} status, and the development of this knowledge is an important area of research. Studies using brain scans to assess brain atrophy and white matter changes have shown associations with vitamin B_{12} status and may be a more sensitive outcome measure than tests of cognition[67–69].

The study populations assessed in the present review varied with populations drawn from ten countries, with differing levels of cognitive decline, chronic disease profiles and folate fortification strategies. Some studies excluded subjects with dementia or cognitive impairment while others included all subjects without assessment of cognition. Baseline vitamin B_{12} concentrations were not always described as a number of studies were primarily assessing the effect of tHcy on cognition. All measures of vitamin B_{12} status are confounded by diseases such as renal and liver disease[62], and the adjustment for confounders based on the chronic disease profile, e.g. stroke and renal diseases, is required to ensure that the true effect of vitamin B_{12} status can be seen.

Numerous genes have been implicated in the development of cognitive decline and dementia[70], with the most common genetic polymorphism being the apoE4 genotype which doubles the risk of AD development[71]. Low vitamin B_{12} concentrations have been found to increase the risk of cognitive decline in apoE4 carriers in some[72,73] but not all studies[51,74]. Of the studies reviewed, eight[26,28,29,32,42,49,54,55] controlled for apoE4 in statistical analysis, with five[26,28,29,42,49] showing associations of vitamin B_{12} status and cognitive decline after adjustment for confounders. Due to the potential impact of genotype on the risk of disease, known genetic traits must be controlled for in any analysis assessing the role of vitamin B_{12} status.

Higher folate status has been associated with improved cognition[1], but in subjects with low vitamin B_{12} status, high serum folate concentrations have been associated with increased concentrations of MMA and tHcy[75] and increased cognitive decline[76]. In the present review, half (four out of seven) of the studies performed in folate-fortified subjects found negative associations between vitamin B_{12} status and cognitive decline[27,40,42,47] compared with approximately one-third of studies overall[26–29,31,33,40,42,47,49]. This is consistent with the postulated detrimental effect of high

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Table 4. Relationship between vitamin B_{12} and development of dementia in subjects without dementia at baseline
(Mean values, medians and ranges)

Study	Age* and sex	Follow up years, *n*	Exclusion criteria	Adjustments	Vitamin B_{12} biomarkers and dementia outcome	Outcome summary	Quality score
Zylberstein *et al.*[52], Sweden	47 years 100 % F	35 years *n* 1368	Not reported	Age, creatinine, education, BMI, TC, TAG, BP, smoking	Vitamin B_{12} not associated with dementia diagnosed using the DSM III-R (either alone or in the full model)	No effect	P
Kivipelto *et al.*[29], Sweden	81·0 years 25 % M	6·7 years *n* 83	Dementia, aged < 75 years, without tHcy, holoTC, folate or vitamin B_{12} supplement use	Age, sex, education, baseline BMI, Alb, Hb, Cr, apoE4, MMSE, holoTC, vitamin B_{12}, serum folate	Subjects with holoTC in the third *v.* first quartile had a reduced risk of dementia using the DSM III criteria (RR 0·47, 95 % CI 0·23, 0·96). No risk reduction for the fourth quartile and no association of holoTC and dementia as a continuous variable	Yes, reduced risk with Q3 holoTC	P
Kim *et al.*[53], South Korea	71·8 years 43 % M	2·4 years *n* 518	Dementia	Age, sex, education, disability, depression, alcohol use, PA, vascular risk factors, creatinine, vitamin intake, weight change, serum folate and tHcy change	No association between baseline serum vitamin B_{12} and MMSE-K. Subjects who developed dementia had smaller increases in serum vitamin B_{12} over the 2·4-year follow-up (0·3 *v.* 56 pmol/l, $P=0·01$)	No effect	P
Haan *et al.*[40], USA	60–101 years 42 % M	4·5 years *n* 1405	Dementia, CIND, no blood tests	Age, education, tHcy, excluding baseline stroke	U-shaped association between vitamin B_{12} and dementia/CIND (HR 1·07, 95 % CI 1·02, 1·11). Vitamin B_{12} modified the positive association between tHcy and outcome. Rates of dementia or CIND associated with tHcy for those in the lowest and highest tertiles of vitamin B_{12} were higher (HR 1·61, *P* for interaction $=0·04$) and lower (HR 0·94, $P=0·02$) *v.* those in the middle tertile, respectively	Increased risk. Interaction effect of tHcy and serum vitamin B_{12}	O
Ravalgia *et al.*[32], Italy	73·6 years 47 % M	3·8 years *n* 816	Dementia, no blood tests	Age, sex, education, apoE genotype, vascular risk factors, tHcy, serum folate	No difference in dementia rates for subjects with serum vitamin $B_{12} \leq 251$ *v.* >251 pmol/l using the NINCDS–ADRDA criteria	No effect	P
Seshadri *et al.*[54], USA	76 years 38·9 % M	8 years†, range 1–13 years *n* 932	Dementia	Age, sex, apoE genotype	Serum vitamin B_{12} not independently related to the risk of dementia	No effect	P
Wang *et al.*[31], Sweden	75–101 years 19 % M	3 years *n* 370	Dementia, subjects who refused blood tests, vitamin B_{12} or FA supplements	Age, sex, education	No difference in the risk of dementia (using the DSM III criteria) for vitamin $B_{12} \leq 150$ *v.* >150 pmol/l. Subjects with vitamin $B_{12} \leq 150$ pmol/l or folate ≤ 10 nmol/l *v.* normal had an increased risk of dementia (RR 1·8, 95 % CI 1·1, 2·8)	Yes, reduced risk	P
Crystal *et al.*[30], USA	75–85 years No data	5 years *n* 410	Not reported	No adjustments	No difference in dementia incidence using the DSM III criteria in subjects with serum vitamin $B_{12} < 110$ pmol/l *v.* >110 pmol/l	No effect	N

M, male; MMSE, Mini Mental State Examination; TC, total cholesterol; BP, blood pressure; DSM III-R, Diagnostic and Statistical Manual of Mental Disorders (third edition revised); P, positive; tHcy, total homocysteine; holoTC, holotranscobalamin; Alb, albumin; Cr, creatinine; RR, relative risk; Q, quartile; PA, physical activity; MMSE-K, Mini Mental State Examination – Korean; CIND, cognitive impairment no dementia; HR, hazard ratio; O, neutral; NINCDS–ADRDA, National Institute of Neurological and Communicative Disease and Stroke–Alzheimer's Disease and Related Disorders Association; FA, folic acid; N, negative.
* Mean or range (years).
† Median.

Table 5. Relationship between vitamin B_{12} and the development of Alzheimer's disease (AD) in subjects with no dementia
(Mean values, medians and ranges)

Study	Age* and sex	Follow-up years, *n*	Exclusion criteria	Adjustments	Vitamin B_{12} biomarkers and AD outcome	Outcome summary	Quality score
Hooshmand *et al.*[28], Sweden	70·7 years 38 % M	7·4 years *n* 271	Dementia	Age, sex, BMI, education, apoE4 allele, BP, tHcy, folate, MMSE, smoking, stroke, renal disease, follow-up time	Subjects with lower holoTC had an increased risk of AD using the NINCDS–ADRDA criteria. OR for the risk of AD for a 1 pmol/l increase in holoTC was 0·977 (95 % CI 0·958, 0·997). Adjusting for holoTC attenuated the tHcy–AD link, with an OR decrease from 1·16 to 1·10 (95 % CI 0·96, 1·25)	Yes, reduced risk	P
Crystal *et al.*[30], USA	75–85 years no data	5 years *n* 410	Not reported	No adjustments	No difference in AD incidence using the DSM III criteria in subjects with serum vitamin B_{12} <110 *v.* >110 pmol/l	No effect	N
Wang *et al.*[31], Sweden	75–101 years 19 % M	3 years *n* 370	Not reported	Age, sex, education	No difference in AD risk using the DSM III-R criteria for vitamin B_{12} cut-point of 150 pmol/l. Doubling of the risk of AD for vitamin B_{12} ≤ 150 pmol/l or serum folate ≤ 10 nmol/l (RR 2·1, 95 % CI 1·2, 3·5); seven times increased risk of AD for MMSE >26 and vitamin B_{12} ≤ 250 pmol/l or serum folate ≤ 12 nmol/l *v.* normal (RR 7·0, 95 % CI 1·2, 31·6); no increased risk for MMSE ≤ 26; three times increased risk of AD for MMSE >26 and vitamin B_{12} ≤ 150 pmol/l or folate ≤ 10 nmol/l *v.* normal (RR 3·1, 95 % CI 1·1, 8·4) but no association for those with MMSE <26	Yes, no effect of vitamin B_{12} alone, but increased risk with low vitamin B_{12} or folate	P
Luchsinger *et al.*[55], USA	76·2 years 28·3 % M	4·7 years *n* 679	Aged <65 years, dementia	Ethnicity; apoE genotype, smoking, DM, HT, CHD, Cr	No association between serum vitamin B_{12} and the risk of AD using the NINCDS–ADRDA criteria	No effect	O
Ravalgia *et al.*[32], Italy	73·6 (SD 6·3) years 48·6 % M	3·8 years *n* 816	Dementia	Age, sex, education, apoE genotype, vascular risk factors, tHcy, serum folate	No difference in AD incidence using the NINCDS–ADRDA criteria for serum vitamin B_{12} ≤ 251 *v.* >251 pmol/l	No effect	P
Kivipelto *et al.*[29], Sweden	81·0 years 25 % M	6·7 years *n* 61	Dementia, aged <75 years, no blood results, vitamin B_{12} or folate supplement use	Age, sex, education, baseline BMI, Alb, Hb, Cr, MMSE, holoTC, tHcy, serum folate	Subjects with holoTC in the third *v.* first quartile had a reduced risk of AD using the DSM III criteria (RR 0·38, 95 % CI 0·15, 0·94). No risk reduction for the fourth quartile and no association of holoTC and AD as a continuous variable	Yes, reduced risk with Q3 holoTC	P
Seshadri *et al.*[54], USA	76 years 38·9 % M	8 years†, *n* 932	Dementia	Age, sex, apoE genotype	Serum vitamin B_{12} not independently related to the risk of AD using the DSM IV and NINCDS–ADRDA criteria	No effect	P
Bowirrat *et al.*[56], Israeli Arabs	No data	20 months, *n* 158	Not reported	Birth year, sex	Subjects in the lowest vitamin B_{12} tertile did not have a greater risk of developing AD	No effect	N

M, male; MMSE, Mini Mental State Examination; BP, blood pressure; tHcy, total homocysteine; holoTC, holotranscobalamin; NINCDS–ADRDA, National Institute of Neurological and Communicative Disease and Stroke–Alzheimer's Disease and Related Disorders Association; P, positive; DSM III and DSM IV, Diagnostic and Statistical Manual of Mental Disorders (third and fourth edition); N, negative; DSM III-R, Diagnostic and Statistical Manual of Mental Disorders (third edition Revised); RR, relative risk; DM, diabetes; HT, hypertension; Cr, creatinine; O, neutral; Alb, albumin.
* Mean or range (years).
† Median.

Table 6. Relationship of vitamin B_{12} and cognitive decline in subjects with dementia or Alzheimer's disease (AD)
(Mean values and ranges)

Study	Age (years)*, sex	Follow-up years, *n*	Exclusion criteria	Adjustments	Vitamin B_{12} biomarkers and AD outcome	Outcome summary	Quality score
Oulhaj *et al.*[57], UK	71·9 years 44 % M	4 years *n* 97	Subjects with no CAMCOG scores or baseline score ≤35	Age at baseline, stroke, education, treatment with centrally acting drugs, tHcy and interaction terms	No effect of serum vitamin B_{12} on the decline in CAMCOG score from baseline	No effect	P
Small *et al.*[58], Sweden	83·5 years 23 % M	2·5 years *n* 24	Not reported	Age, sex, education, AD or vascular dementia	No effect of serum vitamin B_{12} on the decline in memory, visuospatial or verbal decline scores	No effect	O
Small *et al.*[59], Sweden	85·9 years 22 % M	2·6 years *n* 27	Not reported	Baseline MMSE, dementia diagnosis, years between testing, age, sex, education	Serum vitamin B_{12} did not predict the MMSE decline	No effect	O
Tu *et al.*[38], Taiwan	73·8 years 33 % M	0·5 years *n* 92	Cr $>$15 mg/l, stroke, modified Hachinski ischaemia score $>$4, abnormal LFT, FA or vitamin B_{12} supplements	No adjustments	No correlation between Cognitive Ability Screening score or between the Cognitive Ability Screening ratio (time 1:time 2) and serum vitamin B_{12}	No effect	O
Huang *et al.*[36], Taiwan	72·8 years 41 % M	2 years *n* 133	Cr $>$15 mg/l, stroke, modified Hachinski ischaemia score $>$4, abnormal LFT, FA or vitamin B_{12} supplements	No adjustments	No difference in serum vitamin B_{12} concentrations between AD subjects who had a decline in MMSE by <3 *v.* ≥ 3	No effect	O

M, male; CAMCOG, Cambridge Cognitive Assessment; P, positive; O, neutral; MMSE, Mini Mental State Examination; Cr, creatinine; LFT, liver function tests; FA, folic acid.
* Mean or range (years).

folate concentrations on the progression of cognitive impairment[77].

The studies may have been underpowered to show an effect of vitamin B_{12} with only ten studies having sample sizes of more than 500. Power calculations were generally not performed or reported; however, many studies cited inadequate power as a possible explanation for the lack of effect seen. The population standard deviation for serum vitamin B_{12} and biomarkers can be large and any effect is only likely to be seen in subjects with a low vitamin B_{12} status which is found in 10–20 % of older people[78], indicating that the number of study subjects may need to be larger.

The present systematic review has limitations, as it assessed only cohort studies and did not attempt to locate unpublished results or include publications not in English. A meta-analysis was deemed not appropriate due to the variability of subjects, vitamin B_{12} status and outcome measures used. The strengths of the study include the inclusion of multiple database and hand searches. The present review included all located studies, including those primarily interested in tHcy, with any reported associations between vitamin B_{12} and cognition, and included studies with only in-text associations of no effect between vitamin B_{12} and cognition. This increased the number of included studies and thus reduced reporting bias. The use of a quality tool strengthened the study, but the lack of knowledge of the time frame of cognitive decline or dementia development and the inability to determine vitamin B_{12} adequacy limited its application.

Future studies should aim to use MMA and/or holoTC as well as serum vitamin B_{12} and describe the analysis fully, including both significant and non-significant outcome results for any tested cut-points and continuous measures of vitamin B_{12} status. Studies should be of adequate duration (more than 6 years) and choose sensitive cognitive assessment rather than screening tools. Further research into the sensitivity and specificity of these tests is needed. Where possible, studies should aim to include the assessment and adjustment for confounders of age, sex, smoking, physical activity, socio-economic status, vitamin and genetic factors, and others known to be associated with the increased risk of cognitive decline, e.g. CVD, diabetes and chronic kidney disease.

In summary, current studies examined in the present review do not show a clear link between serum vitamin B_{12} concentrations and cognitive decline, but these studies were limited by recruitment age, inadequate subject numbers, lack of adjustment for confounders, study duration and, in some studies, the choice of cognitive outcome measure. The biomarkers of vitamin B_{12} status showed consistent significant associations between lower vitamin B_{12} status and increased rates of cognitive decline or dementia diagnosis, and these biomarkers deserve more study.

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References

1. Smith AD (2008) The worldwide challenge of the dementias: a role for B vitamins and homocysteine? *Food Nutr Bull* **29**, S143–S172.
2. Camicioli R & Rockwood K (2010) Dementia diagnosis. In *Brocklehurst's Textbook of Geriatric Medicine and Gerontology*, 7th ed., pp. 385–391 [HM Fillit, K Rockwood and K Woodhouse, editors]. Amsterdam: Elsevier.
3. Corder EH & Beaumont H (2007) Susceptibility groups for Alzheimer's disease (OPTIMA cohort): integration of gene variants and biochemical factors. *Mech Ageing Dev* **128**, 76–82.
4. Droller H & Dossett JA (1959) Vitamin B_{12} levels in senile dementia and confusional states. *Geriatrics* **14**, 367–373.
5. Martin DC, Francis J, Protetch J, *et al.* (1992) Time dependency of cognitive recovery with cobalamin replacement: report of a pilot study. *J Am Geriatr Soc* **40**, 168–172.
6. Cunha UG, Rocha FL, Peixoto JM, *et al.* (1995) Vitamin B_{12} deficiency and dementia. *Int Psychogeriatr* **7**, 85–88.
7. Healton EB, Savage DG, Brust JC, *et al.* (1991) Neurologic aspects of cobalamin deficiency. *Medicine (Baltimore)* **70**, 229–245.
8. Abyad A (2002) Prevalence of vitamin B_{12} deficiency among demented patients and cognitive recovery with cobalamin replacement. *J Nutr Health Aging* **6**, 254–260.
9. Nilsson K, Gustafson L & Hultberg B (2001) Improvement of cognitive functions after cobalamin/folate supplementation in elderly patients with dementia and elevated plasma homocysteine. *Int J Geriatr Psychiatry* **16**, 609–614.
10. Osimani A, Berger A, Friedman J, *et al.* (2005) Neuropsychology of vitamin B_{12} deficiency in elderly dementia patients and control subjects. *J Geriatr Psychiatry Neurol* **18**, 33–38.
11. Reynolds E (2006) Vitamin B_{12}, folic acid, and the nervous system. *Lancet Neurol* **5**, 949–960.
12. Morrison LD, Smith DD & Kish SJ (1996) Brain *S*-adenosylmethionine levels are severely decreased in Alzheimer's disease. *J Neurochem* **67**, 1328–1331.
13. McCracken C, Hudson P, Ellis R, *et al.* (2006) Methylmalonic acid and cognitive function in the Medical Research Council Cognitive Function and Ageing Study. *Am J Clin Nutr* **84**, 1406–1411.
14. Wald DS, Kasturiratne A & Simmonds M (2011) Serum homocysteine and dementia: meta-analysis of eight cohort studies including 8669 participants. *Alzheimer's dement* **7**, 412–417.
15. Werder SF (2010) Cobalamin deficiency, hyperhomocysteinemia, and dementia. *Neuropsychiatr Dis Treat* **6**, 159–195.
16. Van Dam F & Van Gool WA (2009) Hyperhomocysteinemia and Alzheimer's disease: a systematic review. *Arch Gerontol Geriatr* **48**, 425–430.
17. Ellinson M, Thomas J & Patterson A (2004) A critical evaluation of the relationship between serum vitamin B, folate and total homocysteine with cognitive impairment in the elderly. *J Hum Nutr Diet* **17**, 371–383 (quiz 85-7).
18. Ho RCM, Cheung MWL, Fu E, *et al.* (2011) Is high homocysteine level a risk factor for cognitive decline in elderly? A systematic review, meta-analysis, and meta-regression. *Am J Geriatr Psychiatry* **19**, 607–617.

19. Balk E, Chung M, Raman G, *et al.* (2006) *Vitamins and Berries and Age-related Neurodegenerative Disorders*. Evidence Report/Technology Assessment no. 134, AHRQ Publication no. 06-E008. Rockville, MD: Agency for Healthcare Research and Quality.
20. Vogel T, Dali-Youcef N, Kaltenbach G, *et al.* (2009) Homocysteine, vitamin B_{12}, folate and cognitive functions: a systematic and critical review of the literature. *Int J Clin Pract* **63**, 1061–1067.
21. Raman G, Tatsioni A, Chung M, *et al.* (2007) Heterogeneity and lack of good quality studies limit association between folate, vitamins B-6 and B-12, and cognitive function. *J Nutr* **137**, 1789–1794.
22. Rosenberg IH (2008) Effects of folate and vitamin B_{12} on cognitive function in adults and the elderly. *Food Nutr Bull* **29**, S132–S142.
23. Liberati A, Altman DG, Tetzlaff J, *et al.* (2009) The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *J Clin Epidemiol* **62**, e1–e34.
24. American Dietetic Association (2012) Evidence Analysis Manual: Steps in the Evidence Analysis Process. http://www.adaevidencelibrary.com/topic.cfm?cat=1155 (accessed January 2012).
25. National Institute for Health and Clinical Excellence (2009) The Guidelines Manual. http://www.nice.org.uk/aboutnice/howwework/developingniceclinicalguidelines/clinicalguidelinedevelopmentmethods/GuidelinesManual2009.jsp?domedia=1&mid=5F238D80-19B9-E0B5-D4CB1191544B5D45 (accessed March 2010).
26. Clarke R, Birks J, Nexo E, *et al.* (2007) Low vitamin B-12 status and risk of cognitive decline in older adults. *Am J Clin Nutr* **86**, 1384–1391.
27. Tangney CC, Tang Y, Evans DA, *et al.* (2009) Biochemical indicators of vitamin B_{12} and folate insufficiency and cognitive decline. *Neurology* **72**, 361–367.
28. Hooshmand B, Solomon A, Kareholt I, *et al.* (2010) Homocysteine and holotranscobalamin and the risk of Alzheimer disease: a longitudinal study. *Neurology* **75**, 1408–1414.
29. Kivipelto M, Annerbo S, Hultdin J, *et al.* (2009) Homocysteine and holo-transcobalamin and the risk of dementia and Alzheimer's disease: a prospective study. *Eur J Neurol* **16**, 808–813.
30. Crystal HA, Ortof E, Frishman WH, *et al.* (1994) Serum vitamin B_{12} levels and incidence of dementia in a healthy elderly population: a report from the Bronx Longitudinal Aging Study. *J Am Geriatr Soc* **42**, 933–936.
31. Wang HX, Wahlin A, Basun H, *et al.* (2001) Vitamin B(12) and folate in relation to the development of Alzheimer's disease. *Neurology* **56**, 1188–1194.
32. Ravaglia G, Forti P, Maioli F, *et al.* (2005) Homocysteine and folate as risk factors for dementia and Alzheimer disease. *Am J Clin Nutr* **82**, 636–643.
33. Annerbo S, Wahlund L & Lökk J (2005) The relation between homocysteine levels and development of Alzheimer's disease in mild cognitive impairment patients. *Dement Geriatr Cogn Disord* **20**, 209–214.
34. Annerbo S, Wahlund L & Lökk J (2006) The significance of thyroid-stimulating hormone and homocysteine in the development of Alzheimer's disease in mild cognitive impairment: a 6-year follow-up study. *Am J Alzheimers Dis Other Demen* **21**, 182–188.
35. Eussen SJPM, Ferry M, Hininger I, *et al.* (2002) Five year changes in mental health and associations with vitamin B_{12}/folate status of elderly Europeans. *J Nutr Health Aging* **6**, 43–50.
36. Huang CW, Chang WN, Lui CC, *et al.* (2010) Impacts of hyper-homocysteinemia and white matter hyper-intensity in Alzheimer's disease patients with normal creatinine: an MRI-based study with longitudinal follow-up. *Curr Alzheimer Res* **7**, 527–533.
37. La Rue A, Koehler KM, Wayne SJ, *et al.* (1997) Nutritional status and cognitive functioning in a normally aging sample: a 6-y reassessment. *Am J Clin Nutr* **65**, 20–29.
38. Tu MC, Huang CW, Chen NC, *et al.* (2010) Hyperhomocysteinemia in Alzheimer dementia patients and cognitive decline after 6 months follow-up period. *Acta Neurol* **19**, 168–177.
39. Kim J-M, Kim S-W, Shin I-S, *et al.* (2008) Folate, vitamin b(12), and homocysteine as risk factors for cognitive decline in the elderly. *Psychiatry Investig* **5**, 36–40.
40. Haan MN, Miller JW, Aiello AE, *et al.* (2007) Homocysteine B vitamins, and the incidence of dementia and cognitive impairment: results from the Sacramento Area Latino Study on Aging. *Am J Clin Nutr* **85**, 511–517.
41. Kado DM, Karlamangla AS, Huang M-H, *et al.* (2005) Homocysteine versus the vitamins folate, B6, and B12 as predictors of cognitive function and decline in older high-functioning adults: MacArthur Studies of Successful Aging. *Am J Med* **118**, 161–167.
42. Elias MF, Sullivan LM, D'Agostino RB, *et al.* (2005) Homocysteine and cognitive performance in the Framingham offspring study: age is important. *Am J Epidemiol* **162**, 644–653.
43. Teunissen CE, Blom AHJ, Van Boxtel MPJ, *et al.* (2003) Homocysteine: a marker for cognitive performance? A longitudinal follow-up study. *J Nutr Health Aging* **7**, 153–159.
44. McCaddon A, Hudson P, Davies G, *et al.* (2001) Homocysteine and cognitive decline in healthy elderly. *Dement Geriatr Cogn Disord* **12**, 309–313.
45. van den Kommer TN, Dik MG, Comijs HC, *et al.* (2010) Homocysteine and inflammation: predictors of cognitive decline in older persons? *Neurobiol Aging* **31**, 1700–1709.
46. Kang JH, Irizarry MC & Grodstein F (2006) Prospective study of plasma folate, vitamin B_{12}, and cognitive function and decline. *Epidemiology* **17**, 650–657.
47. Tucker KL, Qiao N, Scott T, *et al.* (2005) High homocysteine and low B vitamins predict cognitive decline in aging men: the Veterans Affairs Normative Aging Study. *Am J Clin Nutr* **82**, 627–635.
48. Mooijaart SP, Gussekloo J, Frolich M, *et al.* (2005) Homocysteine, vitamin B-12, and folic acid and the risk of cognitive decline in old age: the Leiden 85-Plus study. *Am J Clin Nutr* **82**, 866–871.
49. Nurk E, Refsum H, Tell GS, *et al.* (2005) Plasma total homocysteine and memory in the elderly: the Hordaland Homocysteine Study. *Ann Neurol* **58**, 847–857.
50. Garcia A, Haron Y, Pulman K, *et al.* (2004) Increases in homocysteine are related to worsening of stroop scores in healthy elderly persons: a prospective follow-up study. *J Gerontol A Biol Sci Med Sci* **59**, 1323–1327.
51. Dufouil C, Alperovitch A, Ducros V, *et al.* (2003) Homocysteine, white matter hyperintensities, and cognition in healthy elderly people. *Ann Neurol* **53**, 214–221.
52. Zylberstein DE, Lissner L, Bjorkelund C, *et al.* (2011) Midlife homocysteine and late-life dementia in women. A prospective population study. *Neurobiol Aging* **32**, 380–386.
53. Kim JM, Stewart R, Kim SW, *et al.* (2008) Changes in folate, vitamin B_{12} and homocysteine associated with incident dementia. *J Neurol Neurosurg Psychiatry* **79**, 864–868.
54. Seshadri S, Beiser A, Selhub J, *et al.* (2002) Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N Engl J Med* **346**, 476–483.

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55. Luchsinger JA, Tang MX, Shea S, *et al.* (2004) Plasma homocysteine levels and risk of Alzheimer disease. *Neurology* **62**, 1972–1976.
56. Bowirrat A, Friedland RP, Farrer L, *et al.* (2002) Genetic and environmental risk factors for Alzheimer's disease in Israeli Arabs. *J Mol Neurosci* **19**, 239–245.
57. Oulhaj A, Refsum H, Beaumont H, *et al.* (2010) Homocysteine as a predictor of cognitive decline in Alzheimer's disease. *Int J Geriatr Psychiatry* **25**, 82–90.
58. Small BJ & Backman L (1998) Predictors of longitudinal changes in memory, visuospatial, and verbal functioning in very old demented adults. *Dement Geriatr Cogn Disord* **9**, 258–266.
59. Small BJ, Viitanen M, Winblad B, *et al.* (1997) Cognitive changes in very old persons with dementia: the influence of demographic, psychometric, and biological variables. *J Clin Exp Neuropsychol* **19**, 245–260.
60. Ravaglia G, Forti P, Maioli F, *et al.* (2006) Conversion of mild cognitive impairment to dementia. Predictive role of mild cognitive impairment subtypes and vascular risk factors. *Dement Geriatr Cogn Disord* **21**, 51–58.
61. O'Leary F & Samman S (2010) Vitamin B_{12} in health and disease. *Nutrients* **2**, 299–316.
62. Green R (2011) Indicators for assessing folate and vitamin B-12 status and for monitoring the efficacy of intervention strategies. *Am J Clin Nutr* **94**, 666S–672S.
63. Willis CD, Elshaug AG, Milverton JL, *et al.* (2011) Diagnostic performance of serum cobalamin tests: a systematic review and meta-analysis. *Pathology* **43**, 472–481.
64. Hooshmand B, Solomon A, Kareholt I, *et al.* (2012) Associations between serum homocysteine, holotranscobalamin, folate and cognition in the elderly: a longitudinal study. *J Intern Med* **271**, 204–212.
65. Folstein MF, Folstein SE & McHugh PR (1975) "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* **12**, 189–198.
66. Schafer JH, Glass TA, Bolla KI, *et al.* (2005) Homocysteine and cognitive function in a population-based study of older adults. *J Am Geriatr Soc* **53**, 381–388.
67. Smith A, Smith SM, de Jager CA, *et al.* (2010) Homocysteine-lowering by B vitamins slows the rate of accelerated brain atrophy in mild cognitive impairment: a randomized controlled trial. *PLoS One* **5**, 1–10.
68. Vogiatzoglou A, Refsum H, Johnston C, *et al.* (2008) Vitamin B-12 status and rate of brain volume loss in community-dwelling elderly. *Neurology* **71**, 826–832.
69. de Lau LML, Smith AD, Refsum H, *et al.* (2009) Plasma vitamin B_{12} status and cerebral white-matter lesions. *J Neurol Neurosurg Psychiatry* **80**, 149–157.
70. Payton A (2009) The impact of genetic research on our understanding of normal cognitive ageing: 1995 to 2009. *Neuropsychol Rev* **19**, 451–477.
71. Raber J, Huang Y & Ashford JW (2004) ApoE genotype accounts for the vast majority of AD risk and AD pathology. *Neurobiol Aging* **25**, 641–650.
72. Bunce D, Kivipelto M & Wahlin A (2004) Utilization of cognitive support in episodic free recall as a function of apolipoprotein E and vitamin B_{12} or folate among adults aged 75 years and older. *Neuropsychology* **18**, 362–370.
73. Bunce D, Kivipelto M & Wahlin A (2005) Apolipoprotein E, B vitamins, and cognitive function in older adults. *J Gerontol: Series B: Psychol Sci Soc Sci* **60**, P41–P48.
74. Brown B, Huang MH, Karlamangla A, *et al.* (2011) Do the effects of APOE-epsilon4 on cognitive function and decline depend upon vitamin status? MacArthur Studies of Successful Aging. *J Nutr Health Aging* **15**, 196–201.
75. Selhub J, Morris MS & Jacques PF (2007) In vitamin B_{12} deficiency, higher serum folate is associated with increased total homocysteine and methylmalonic acid concentrations. *Proc Natl Acad Sci U S A* **104**, 19995–20000.
76. Morris MS, Jacques PF, Rosenberg IH, *et al.* (2007) Folate and vitamin B-12 status in relation to anemia, macrocytosis, and cognitive impairment in older Americans in the age of folic acid fortification. *Am J Clin Nutr* **85**, 193–200.
77. Smith AD, Kim Y-I & Refsum H (2008) Is folic acid good for everyone? *Am J Clin Nutr* **87**, 517–533.
78. Carmel R, Green R, Rosenblatt DS, *et al.* (2003) Update on cobalamin, folate, and homocysteine. *Hematology Am Soc Hematol Educ Program* 62–81.

Appendix: Medline search protocol

1. vitamin B?12.mp. or exp Vitamin B 12/
2. vitamin B?12 deficiency.mp. or exp Vitamin B 12 Deficiency/
3. transcobalamin.mp. or exp Transcobalamins/
4. exp homocysteine/ or exp s-adenosylhomocysteine/
5. homocysteine.mp.
6. homocystine.mp.
7. exp Homocystine/
8. exp Hyperhomocysteinemia/
9. hyperhomocysteinemia.mp.
10. hyperhomocysteinaemia.mp.
11. methylmalonic acid.mp.
12. methylmalonate.mp.
13. holotranscobalamin.mp.
14. cognition/ or exp awareness/ or exp comprehension/
15. cognition.mp.
16. cognit*.mp. [mp = title, original title, abstract, name of substance word, subject heading word, unique identifier]
17. dementia.mp. or exp Dementia/
18. exp Dementia, Vascular/ or exp Dementia, Multi-Infarct/ or Dementia/ or exp Frontotemporal Dementia/
19. memory/ or memory, short-term/ or mental recall/or "recognition (psychology)"/ or "retention (psychology)"/
20. memory.mp. [mp = title, original title, abstract, name of substance word, subject heading word, unique identifier]
21. alzheimer's disease.mp. or exp Alzheimers Disease/
22. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13
23. 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21
24. dietary supplements.mp. or Dietary Supplements/
25. 22 or 24
26. 23 and 25
27. limit 26 to (english language and humans)
28. limit 27 to "middle aged (45 plus years)"

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