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ORIGINAL CONTRIBUTION

Is a low blood level of vitamin B12 a cardiovascular and diabetes risk factor? A systematic review of cohort studies

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Abstract
Purpose To assess the prior hypothesis that low blood vitamin B12, partly through hyperhomocysteinemia and partly through direct effects, increases the risk of cardiovascular diseases and diabetes. As background, we also extracted all-cause mortality from the studies that met our criteria.
Methods A systematic review of prospective cohort studies identified through searching six electronic databases, screening of reference lists, and citation search. Included studies reported data on the association between vitamin B12 blood levels, or other appropriate surrogate biological markers e.g. holotranscobalamin or serum/urine methylmalonic acid, and fatal or non-fatal incident diabetes and cardiovascular events.
Results Seven studies were included. Studies differed regarding the population studied, length of follow-up, study outcomes, and data analysis—a narrative synthesis approach was performed to examine the results. Most studies met few of the quality assessment criteria which were adapted from the Scottish Intercollegiate Guidelines Network (SIGN). Only one high-quality study reported that low B12 increased the risk of incident cerebral ischaemia (RR = 1.76; 95% CI = 1.16–2.68). After controlling for homocysteine, the association persisted although weakened (RR = 1.57; 95% CI = 1.02–2.43), suggesting that the effects of low B12 were only partly mediated by homocysteine. In two studies, higher B12 levels were associated with a greater risk of total mortality (RR = 1.00; 95% CI = 1.00–1.00 and HR = 1.15; 95% CI = 1.08–1.22, respectively) and combined fatal and non-fatal coronary events (RR = 1.00; 95% CI = 1.00–1.00). No association between study outcomes and vitamin B12 levels was found in four other studies.
Conclusions Surprisingly, there is only very limited evidence that vitamin B12 deficiency predisposes to the risk of mortality and morbidity from either cardiovascular diseases or diabetes in adults. Current data do not support vitamin B12 supplementation to reduce the risk of cardiovascular diseases or diabetes.

Keywords Vitamin B12 · Cardiovascular diseases · Diabetes mellitus type 2 · Cohort studies · Aetiology

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Introduction

Vitamin B12 is an essential nutrient that functions as a coenzyme in two metabolic processes—the conversion of methylmalonyl-CoA to succinyl-CoA (by methylmelanoyl CoA mutase—MCM) and the remethylation of homocysteine to methionine (by methionine synthase—MS) [1]. Remethylation of homocysteine happens in the cell cytosol and requires vitamin B12 as a cofactor in the presence of 5-methyltetrahydrofolate (from folic acid) [1–3]. However,

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the actions of MCM, which happen in the mitochondria, only require B12 as a coenzyme and folic acid or its derivatives. Accumulation of methylmalonic acid is therefore a specific indicator if vitamin B12 deficiency. On the other hand, either vitamin B12 or folate deficiency can impair the re-methylation process and result in elevated homocysteine levels. Low vitamin B12 has emerged as the most common modifiable risk factor for raised homocysteine in countries implementing folic acid fortification of flour [4]. There is much evidence linking homocysteine with an increased risk of coronary heart disease (CHD) [5, 6] and stroke [5, 7], although the causality of the association and underlying pathophysiology remain to be established [2].

Vitamin B12 is found in animal products (or fortified foods). In low-income countries, reduced intake of these foods because of cultural reasons or low income is associated with low blood B12 levels [8, 9]. In high-income countries, vitamin B12 deficiency occurs commonly in the elderly due to decreased production of intrinsic factor (essential for B12 absorption) resulting in poor absorption [8]. Evidence from studying children born to Indian mothers with low vitamin B12 suggests subclinical B12 deficiency is associated with more adiposity and insulin resistance [10], and a recent Indian study [11] showed an association between low maternal vitamin B12 levels and gestational diabetes. It is plausible that MCM-dependent conversion of methylmalonyl-CoA is blocked (inside the mitochondria), which in turn can result in increased lipogenesis [12]. Therefore, it is very likely that the biological effects of B12 deficiency are partly through hyperhomocysteinemia and partly through its direct effects.

Here, we present a systematic review of cohort studies with the aim of investigating the possible role of vitamin B12 in blood in the aetiology of cardiovascular diseases (CVD) and diabetes in the adult population. From the relevant studies we extracted all-cause mortality to provide background to interpret the cause-specific findings. This follows Bhopal's sixth solution for inspiring epidemiology, where he recommends that cause-specific outcomes should be evaluated in the context of general outcomes, a public health approach [13]. Our prior hypothesis was that low blood vitamin B12 would be a risk factor, and higher levels protective, for these diseases. This hypothesis was generated by RSB and CSY from the observation that B12 deficiency, high rates of CVD, and very high diabetes prevalence co-exist in urbanised people originating from the Indian subcontinent i.e. South Asians. Two observations led us to focus on data from prospective epidemiological studies. First, although homocysteine is likely to be a major mediating factor of any vascular effects of low vitamin B12, some recent observations suggest an independent role for B12, possibly through lipogenesis which is a risk factor for diabetes and CVD. Second, despite much interest in homocysteine as a CVD risk factor, several clinical trials published to date have failed to confirm a benefit of homocysteine-lowering treatment with B vitamins on CVD events [14]. However, negative findings from clinical trials do not necessarily negate the role of vitamin B12 in the aetiology of CVD (so-called aetiology-treatment paradox). For example, available trials have not examined the effects of vitamin B12 per se but combinations of B vitamins and in non-physiological doses for the secondary prevention of CVD. Other potential shortcomings of trials in shedding light on any aetiological role of vitamin B12 include the lack knowledge of duration and amount of exposure to lead to an effect, the possibility that the effects of vitamin B12 need to be at a critical period of life (e.g. intrauterine or infancy) that cannot be mimicked in a trial, and that trials have been done in specific study populations (e.g. CVD patients rather than healthy populations).

Our aim was to identify all relevant observational follow-up studies examining the relationship between blood levels of vitamin B12 with incident CVD and diabetes-related morbidity and mortality in the adult general population.

Methods

We followed published guidelines (Meta-analysis of Observational Studies in Epidemiology) on reporting systematic reviews of observational studies although the current review is limited to English language-based publications [15]. We included original, population-based (all ages, both genders) cohort studies that provided at least a single baseline measure of serum vitamin B12 (cobalamin or holotranscobalamin) or other appropriate surrogate marker (serum or urine methylmalonic acid). Excluded studies included those: based on no original research, based non-human samples, using other types of study designs, lacking any of the study outcomes, based on hospital inpatients, and only reporting folate and/or homocysteine but not vitamin B12 (or relevant surrogate markers).

Studies were identified by computerised searches of MEDLINE, EMBASE, the Cumulative Index to Nursing and Allied Health Literature (CINAHL), Global Health, and the Commonwealth Agricultural Bureau (CAB) abstracts; all databases were searched from inception to February 2009. The personal bibliographic databases of two co-authors (CSY, RSB) were also searched. Reference lists of key publications were inspected. Articles that cited the retrieved studies were tracked using the Science Citation Index. With the assistance of an expert medical librarian, we applied a search strategy based on a combination of text-words and Medical Subject Headings (MeSH). The following common and chemical names were

used for the exposure of interest (vitamin B12, cobalamin, holotranscobalamin, HoloTC, methylmalonic acid/MMA), the outcome under study (atherosclerosis, myocardial infarction, coronary heart disease, ischaemic heart disease, stroke, peripheral arterial disease, cardiovascular diseases, diabetes type 2, and mortality), and the type of study, including cohort, prospective study, follow-up study, incidence study, relative risk, and risk ratio. A similar search string was adopted for each database.

All data searches were performed by SBR. Titles and abstracts of identified studies were independently screened for eligibility according to pre-specified inclusion criteria by two reviewers (SBR, PS)—agreement was 84%. Potentially relevant full-text articles were retrieved and further assessed. Disagreement was resolved by discussion. A third reviewer (RSB) was consulted where discrepancies remained. Data were subsequently extracted onto pre-designed forms by two reviewers (SBR, PS). For each study, we extracted information on the study site and participants, serum vitamin B12 (or other surrogate markers) measurement methods, follow-up length, confounding factors, all relevant study outcomes (all-cause mortality, diabetes mortality, CVD mortality, and non-fatal events attributed to diabetes, peripheral arterial disease, CHD and stroke) and results.

Studies were assessed for methodological quality using a pro forma for cohort studies that was adapted from the Scottish Intercollegiate Guidelines Network (SIGN) (www.sign.ac.uk). The quality assessment focused on appraising the internal and external validity of primary studies by evaluating specific methodological components of the study design, conduct and analysis of each study. The results of the quality assessment were used for descriptive purposes to provide an overall evaluation of the studies included in the review.

Substantial study heterogeneity was observed because of differences in vitamin B12 level thresholds and comparisons, study sample characteristics and outcomes, follow-up length, and data analyses performed (including measurement and adjustment for confounding factors). Additional sources of study heterogeneity arose from differences between studies in biological sample characteristics (plasma, serum) and assay techniques (microbial assay, radioimmunoassay). Therefore, a narrative synthesis approach was performed to examine the results instead of a meta-analysis.

Results

Study characteristics

Seven studies met all inclusion criteria (Fig. 1). Details of the study populations and methods are presented in Table 1. Four investigations used a prospective cohort study design where study subjects were followed from baseline until the onset of either non-fatal events or death, or were lost to follow-up [16–19]. Three studies used a case–cohort design which consists of a sub-cohort sampled at the beginning of the study and followed over time, and a case sample that is ascertained through the course of the study [20–22]. Specifically, case status was based either on the occurrence of fatal and non-fatal incident CHD [21], only fatal CHD [20], or incident stroke [22]. Although no study provided data on incidence of diabetes, one investigation [18] reported on combined diabetes/nephropathy mortality. Moreover, all studies determined baseline levels of vitamin B12 from blood samples using widely available radioimmunological or microbiological assay methods. Average length of follow-up from baseline ranged from 3.3 [21] to 29 [17] years. Study populations were from the UK [16], Australia [17], the US [18, 19, 21], the Netherlands [20], and Germany [21]. All studies included both men and women, although the proportion of each sex varied considerable between samples. The mean age at baseline ranged from 48.4 [17] to 79.0 [19] years.

Study quality assessment

Table 2 shows that the included studies differed substantially in methodological quality i.e. the sample type and characteristics, participation rate, study outcomes, exposure assessment, and measurement and control of confounding factors. One study [22] met most of the quality assessment criteria used, four [16, 17, 20, 21] met some, and two [18, 19] only few of them. One study [17] reported a clear research question, specifying the direction of the association under study. All studies sufficiently described sampling methods. All but one study [18] clearly reported the participation rates. In two studies [20, 22] was the study outcome measured blind to baseline B12 levels. All studies used standard methods for assessing B12 levels at baseline. One study [19] undertook repeated B12 measurements during follow-up. Three studies [17–19] did not assess use of vitamin B12 supplements. One study [22] controlled for potential confounding effects of three major factors i.e. baseline diabetes, blood cholesterol levels, and folate levels/status. One study [18] investigated whether the influence of vitamin B12 levels on the study outcomes existed independently of serum homocysteine levels.

Vitamin B12 levels and all-cause mortality

Four studies provided data on the relationship between baseline vitamin B12 levels and the risk of all-cause mortality, so producing contextual, supplementary information

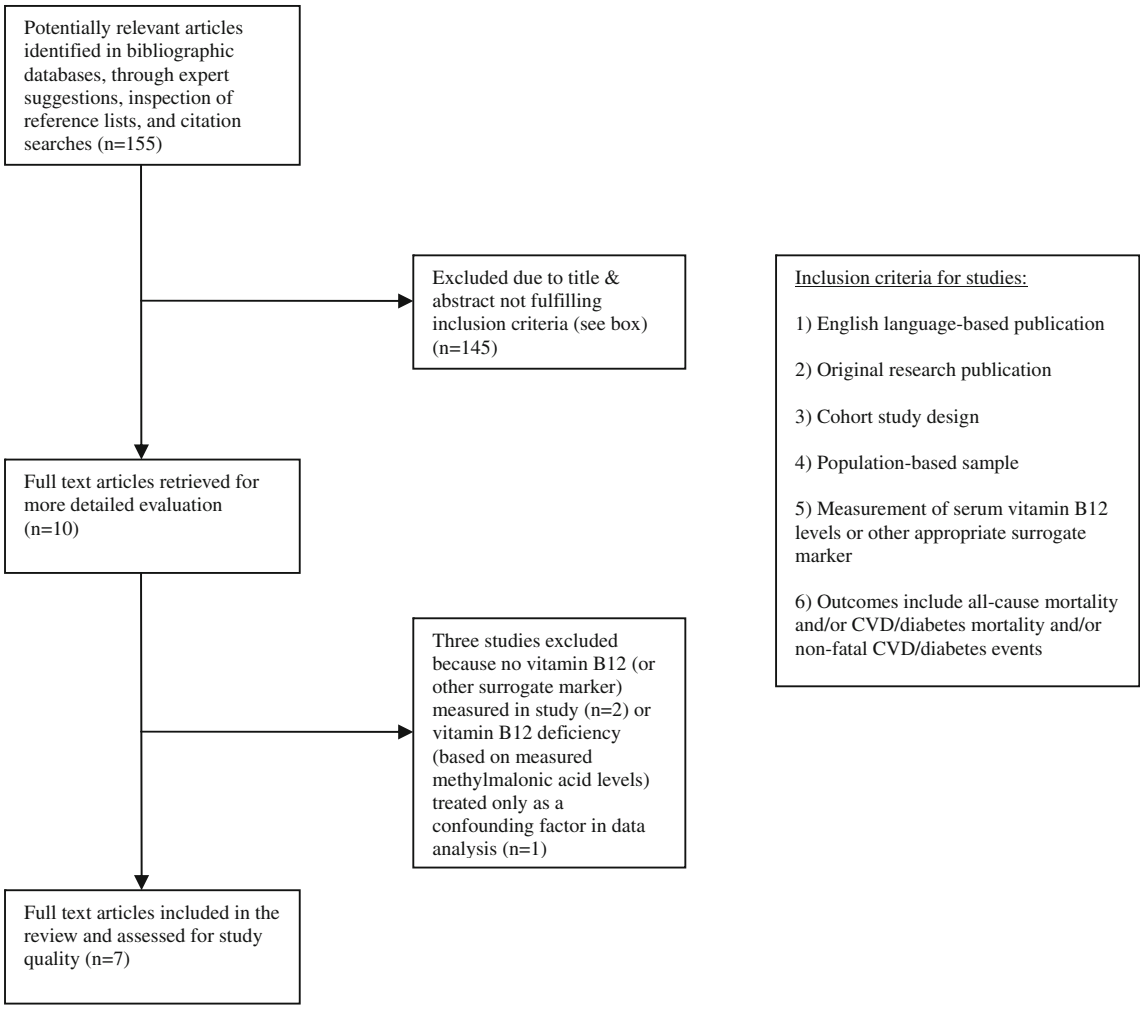

Fig. 1 Flow diagram of studies included in the review

(Table 3). In one study [19], higher vitamin B12 levels were associated with greater total mortality after controlling for confounding factors, including history of previous myocardial infarction (MI) or stroke, and baseline blood pressure (however, folate levels/status was not adjusted for in the multivariate analyses). Similarly, vitamin B12 levels were positively associated with increased all-cause mortality in multivariate analyses adjusting for folate and homocysteine [18]. In contrast, two other studies found neither low [17] nor high [16] levels of vitamin B12 to be associated with mortality from all causes once multiple confounding factors were controlled for.

Vitamin B12 levels and cause-specific mortality

Six studies examined the relationship between vitamin B12 levels and mortality from CVD and diabetes (Table 3). In one study [19], incident CHD (based on combining fatal and non-fatal MI as well as other fatal heart disease) was associated with raised B12 levels after controlling for multiple confounding factors (but not folate levels/status). The risk of incident stroke (fatal and non-fatal combined), incident MI (fatal and non-fatal combined), and incident CVD (fatal and non-fatal combined) was not increased. A second study [18] found no association between raised B12 levels and CVD mortality, but the risk of mortality from diabetes/nephropathy was increased. Other four investigations showed no relationship between increased B12 levels and fatal and non-fatal CHD events combined [21], low B12 and fatal CHD and overall CVD mortality [17], high B12 levels and CHD mortality [20], or high B12 levels and overall CVD mortality [16], after controlling for multiple confounding factors (all multivariate models adjusted for folate levels/status).

Vitamin B12 levels and non-fatal events

Only one study investigated the association between baseline vitamin B12 blood levels and non-fatal events [22]. After statistically controlling for multiple confounding

Table 1 Methodological overview of studies included in the review

Reference	Study (country)	Study design/ population and size	Age in years at baseline	Follow-up in years	Vitamin B12 measurement method/baseline levels	Study outcome ascertainment methods
Zeitlin et al. 1997 [19]	The Bronx aging study (USA)	Cohort study/488 community-resident volunteers (90% white; 64% women) from northern and eastern Bronx, New York	Mean 79; range 75–85	10	Radioimmunoassay kit/ 381 pmol/l (mean for total sample)	Annual medical examinations, review of hospital records, underlying cause of death extracted from death certificates
Folsom et al. 1998 [21]	The atherosclerosis risk in communities study (USA)	Case–cohort study/232 incident CHD cases (75% men; 25% African American) and 537 subjects comprising a reference cohort sample	Range 45–64	3.3 (median)	Radioimmunoassay kit/ 271 pmol/l (mean for cases)—286 pmol/l (mean for non-cases)	Annual questionnaires, three-year medical examinations, surveillance of hospitalisations, underlying cause of death extracted from death certificates
de Bree et al. 2003 [20]	The monitoring project on CVD risk factors (Netherlands)	Case–cohort study/103 incident fatal CHD cases (75% men) and 630 randomly selected reference subjects (48% men)	Mean 51.6 (cases) and 41.5 (non-cases)	10.3 (mean)	Radioimmunoassay kit/ 284 pmol/l (mean for cases)—303 pmol/l (mean for non-cases)	Underlying cause of death extracted from death certificates
Hung et al. 2003 [17]	Longitudinal study of Busselton (Western Australia)	Cohort study/1772 men and 1,904 women listed on electoral rolls in the town of Busselton	Mean 48.4 (men) and 47.9 (women)	29	Automated microbiological assay system/268 pmol/l (mean for total sample)	Underlying cause of death extracted from death certificates
Looker et al. 2007 [18]	Longitudinal study of type 2 diabetes (USA)	Cohort study/396 community-living diabetic Pima Indians (64.9% women) from Gila River Indian Community, Central Arizona	Mean 54.1; range 40–82.8	15.7 (median)	Radioimmunoassay kit/ 358 pmol/l (mean for total sample)	Underlying cause of death extracted from death certificates, review of available clinical and autopsy records
Weikert et al. 2007 [22]	European prospective investigation into cancer and nutrition-Potsdam study (Germany)	Case–cohort study/188 incident ischaemic stroke and TIA cases (44.7% men) and 779 randomly selected reference subjects (39.4% men)	Mean 56.3 (cases) and 49.7 (non-cases)	6 (mean)	Commercial test kit/ 270 pmol/l (median for total sample)	Self-completed questionnaire on stroke and TIA—subsequent validation was based on a medical records review
Dangour et al. 2008 [16]	Medical research council trial of assessment and management of older people in the community (UK)	Cohort study/853 community-living men (43.6%) and women, registered with a general practitioner in the UK	Mean age 78.6; range 75–84	7.6 (median)	Radioimmunoassay kit/ 277 pmol/l (mean from total sample)	Underlying cause of death extracted from death certificates

Table 2 Quality assessment of studies included in the review

Quality assessment criteria[a]	Zeitlin et al. 1997 [19]	Folsom et al. 1998 [21]	de Bree et al. 2003 [20]	Hung et al. 2003 [17]	Looker et al. 2007 [18]	Weikert et al. 2007 [22]	Dangour et al. 2008 [16]
Research question/ hypothesis clearly defined and stated?	No	No	No	Yes	No	No	Yes
Source population described?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Participation rate reported?	Yes	Yes	Yes	Yes	No	Yes	Yes
Evaluation whether study outcomes were present at the time of enrolment?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Comparison between full participants and those lost to follow-up by exposure status?	No	No	No	No	No	No	No
Study outcomes clearly defined?	No	Yes	Yes	Yes	Yes	Yes	Yes
Assessment of study outcomes made blind to exposure status?	No	No	Yes	No	No	Yes	No
Where blinding was not possible, there is some recognition that knowledge of exposure status could have influenced the assessment of outcome?	No	No	No	No	No	No	No
The measure of assessment of exposure is reliable?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Evidence from other sources is used to demonstrate that the method of outcome assessment is valid and reliable?	No	No	No	No	Yes	Yes	No
Exposure level is assessed more than once?	No	No	No	No	No	No	No
The main potential confounders are identified and taken into account in the design and analysis?	Yes	Yes	No	Yes	Yes	Yes	Yes
Confidence intervals provided for sample estimates?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Overall assessment	Few criteria fulfilled–unfulfilled criteria are *likely/very likely* to alter the conclusions	Some criteria fulfilled–unfulfilled criteria *unlikely* to alter the conclusions	Some criteria fulfilled–unfulfilled criteria *unlikely* to alter the conclusions	Some criteria fulfilled–unfulfilled criteria *unlikely* to alter the conclusions	Few criteria fulfilled–unfulfilled criteria are *likely/very likely* to alter the conclusions	Most criteria fulfilled–unfulfilled criteria *very unlikely* to alter the conclusions	Some criteria fulfilled–unfulfilled criteria *unlikely* to alter the conclusions

[a] Adapted from the Scottish Intercollegiate Guidelines Network (SIGN) (www.sign.ac.uk)

factors, including baseline diabetes and cholesterol levels, the risk of cerebral ischaemic events was associated with lower vitamin B12 blood values (Table 3). When serum homocysteine levels were further adjusted for, the strength of the association was slightly reduced, suggesting that the association was partly mediated by homocysteine.

Table 3 Results from the studies included in the review

Reference	Confounding factors	Study outcomes (no of events)	Effect measure	Study outcome and size of effects
Zeitlin et al. 1997 [19]	Age, sex, history of MI, history of stroke, SBP, DBP	1: All-cause mortality (137) 2: Incident stroke: fatal/non-fatal (31) 3: Incident MI: fatal/non-fatal (58) 4: Incident CHD: fatal/non-fatal MI/ other fatal cardiac disease (70) 5: Incident CVD: fatal/non-fatal MI/ stroke/other fatal cardiac disease (101)	Hazard ratio (HR) for each increase in B12 levels by 1.0 pg/ml	1: HR = 1.00; 95% CI = 1.00–1.00; $p < 0.01$ 2: HR = 1.00; 95%CI = 0.99–1.00; $p > 0.05$ 3: HR = 1.00; 95% CI = 0.99–1.00; $p > 0.05$ 4: HR = 1.00; 95% CI = 1.00–1.00; $p < 0.05$ 5: HR = 1.00; 95% CI = 1.00–1.00; $p > 0.05$
Folsom et al. 1998 [21]	Age, sex, race, field centre, total cholesterol, HDL cholesterol, hypertension, DM, smoking status	1: CHD incidence: definite/probable MI or silent MI between examinations by ECG or definite CHD death or coronary revascularisation (232)	Relative risk (RR) relative to 1st quintile of vitamin B12 levels	1: RR (*highest quintile*) = 0.81; 95% CI = 0.40–1.81; p for trend = 0.13
de Bree et al. 2003 [20]	Age, study centre, creatinine, hypertension, smoking, HDL cholesterol, total cholesterol	1: CHD mortality: ICD9 410–414; ICD10 I20–I25 (103)	Relative risk (RR) relative to 1st tertile of vitamin B12 levels	1: men: RR (*third tertile*) = 0.95; 95% CI = 0.42–2.33; p for trend = 0.8; women: RR (*third tertile*) = 0.99; 95% CI = 0.34–3.75; p for trend = 0.9
Hung et al. 2003 [17]	Age, SBP, DBP, BMI, serum cholesterol, white cell count, smoking, menopause, treatment for DM and hypertension, alcohol intake, history of CHD, stroke, and intermittent claudication	1: All-cause mortality (1,202) 2: CVD mortality: ICD9: 410–459 (644) 3: CHD mortality: ICD9: 410–414 (372)	Hazard ratio (HR) relative to the 4th (*highest*) quartile of vitamin B12 levels	1: men: HR (*first quartile*) = 0.99; 95% CI = 0.86–1.27; p for trend = 0.65; women: HR (*first quartile*) = 1.04; 95% CI = 0.88–1.39; P for trend = 0.86 2: men: HR (*first quartile*) = 1.14; 95% CI = 0.80–1.51; P for trend = 0.24; women: HR (*first quartile*) = 0.88; 95% CI = 0.62–1.23; P for trend = 0.50 3: men: HR (*first quartile*) = 1.09; 95% CI = 0.74–1.65; p for trend = 0.30; women: HR (*first quartile*) = 0.74; 95% CI = 0.56–1.17; p for trend = 0.37
Looker et al. 2007 [18]	Age, sex, DM duration, homocysteine, folate, serum creatinine	1: All-cause mortality (221) 2: CVD mortality: ICD9 400–459 (76) 3: Diabetes/nephropathy mortality: ICD9 250, 580–587 (36)	Hazard ratio (HR) for a 100 pg/ml difference in vitamin B12 levels	1: HR = 1.15; 95% CI = 1.18–1.29; $p < 0.05$ 2: HR = 1.05; 95% CI = 0.90–1.21; $p > 0.05$ 3: HR = 1.28; 95% CI = 1.12–1.53; $p < 0.05$
Weikert et al. 2007 [22]	Age, sex, smoking status, DM, hypertension, cholesterol/HDL ratio, education, alcohol use, BMI, PA, total energy intake	1: Incident cerebral ischaemia: ICD10 I63.0–I63.9 or TIA: ICD10 G45.0–G45.9 (188)	Relative risk (RR) relative to the 3rd (*highest*) tertile of vitamin B12 levels	1: RR = 1.57; 95% CI = 1.04–2.45
Dangour et al. 2008 [16]	Age, sex, DM, history of CVD and cancer, smoking, alcohol, PA, homocysteine, folate	1: All-cause mortality (429) 2: CVD mortality: ICD9 390–459/ ICD10 I1–99 (185)	Hazard ratio (HR) relative to the 1st tertile of vitamin B12 levels	1: HR (*highest tertile*) = 1.02; 95% CI = 0.86–1.37; p for trend = 0.92 2: HR (*highest tertile*) = 0.95; 95% CI = 0.68–1.59; p for trend = 0.78

BMI, body mass index; *CHD,* coronary heart disease; *CVD*, cardiovascular disease; *DM*, diabetes mellitus; *DBP,* diastolic blood pressure; *ECG,* electrocardiograph; *HDL*, high-density lipoprotein; *ICD*, International Classification of Diseases; *MI*, myocardial infarction; *PA*, physical activity; *SBP*, systolic blood pressure; *TIA*, transient ischaemic attack

Subsequent analyses of vitamin B status showed that the risk of cerebral ischaemia was specifically associated in individuals with either low vitamin B12/normal folate or low vitamin B12/low folate. Neither association remained when homocysteine was further controlled for in these analyses.

Discussion

Main findings and interpretation

This is the first systematic review investigating the association between blood levels of vitamin B12 and the risk of CVD and diabetes (and extracting data on all-cause mortality to help interpret the results). While our results highlight much heterogeneity in the findings of the few studies available, overall we were unable to support our prior hypothesis: that low blood vitamin B12 increases the risk of cardiovascular diseases and diabetes.

The only evidence in favour of this came from a high-quality study by Weikert et al. [22]. This study reported a relationship between low plasma vitamin B12 concentrations and increased risk of cerebral ischaemia, even after adjustment for baseline disease and cholesterol levels. After controlling for serum homocysteine, the relative risk of cerebral ischaemia was reduced only by about 10%, suggesting that the effects of vitamin B12 were only partly mediated by homocysteine. Although the association between raised homocysteine levels and the risk of stroke has been described previously [5, 7], the independent cerebrovascular effects of low vitamin B12 levels are harder to explain. For example, one possibility is that low vitamin B12 increases levels of circulating proinflammatory proteins that have been found to be associated with an increased risk of ischaemic stroke [23, 24]. Such changes can be observed in classical vitamin B12 deficiency but it is currently unknown whether they occur when vitamin B12 status is low to normal [25]. Alternatively, the effects of low vitamin B12 may be mediated by some other biochemical factors [4]. The health effects of low vitamin B12 may also depend on the folate status of the study population [25]. For example, the risk of cerebral ischaemia was higher in subjects with both low vitamin B12 and low folate compared to those with only low vitamin B12 levels [22].

In contrast to Weikert et al. [22], Looker and Zeitlin's studies, albeit lower quality on our assessment (see Table 2), showed an effect of vitamin B12 in the *opposite* direction to what we expected: increased vitamin B12 levels were positively associated with increased total mortality [18, 19], fatal and non-fatal cardiac disease combined [19], and mortality from diabetes and nephropathy combined [18]. In attempting to explain these discrepancies, it is important to note that these studies included the most diseased [18] and the oldest [19] study populations. Moreover, these studies reported the highest average baseline vitamin B12 concentrations, 381 pmol/l [18] and 358 pmol/l [19], respectively. Neither study assessed vitamin B12 supplementation intake which is a likely explanation for these high levels. Also, neither study excluded subjects with co-morbidity, including malignancy or liver disease, which is relevant because raised vitamin B12 levels in the elderly may be a sign of a life-limiting chronic disease [26]. The relatively high average vitamin B12 levels in the two studies may be attributed to B12 supplementation intake in an undetermined proportion of either diabetic patients [18] or very old subjects [19] with chronic co-morbidities. This could explain why a positive association was found between vitamin B12 levels and mortality risk.

Excess B12 intake might also increase the risk of disease directly. For example, there is evidence that excess vitamin B12 may be hepatotoxic [19]. Also, vitamin B12 has been reported to be an acute phase reactant, increasing in response to inflammation [27]. Alternatively, vitamin B12 may be a marker for the consumption of animal source foods. Potentially protective benefits of vitamin B12 may be outweighed by the high saturated fat content of these foods.

Neither Looker nor Zeitlin's study [18, 19] controlled for potential effects of cholesterol on the risk of mortality and morbidity but the studies that reported either an inverse [22] or no association [16, 17, 20, 21] between vitamin B12 levels and increased risk all adjusted for serum cholesterol levels in the multivariate analyses.

In several studies, no effects of either low vitamin B12 blood levels on fatal CHD and total CVD mortality [17] or of raised B12 levels on combined fatal and non-fatal coronary events [20], CHD mortality [20] and total CVD mortality [16] were observed after multiple confounding factors were controlled for. Mortality as an outcome may not be specific enough to capture any potential adverse vascular effects of low vitamin B12 levels. Even cause-specific mortality is affected by many other factors than individual risk factors for a particular disease, including access to and level of medical care. The difference between these results and those reported above by Weikert et al. [22] might partly stem from the use of different study outcomes (total and cause-specific mortality versus non-fatal cerebral ischaemic events) or from the possibility of residual confounding by unmeasured factors in the latter study. The findings by Weikert et al. [22] need replicating.

The influence of low vitamin B12 levels, if any, on the risk of developing diabetes and CVD may occur earlier in life. The importance of intrauterine conditions for the development of disease in later life is well established

through various foetal programming stimuli, including nutritional factors [12]. Maternal nutrition is thought to have major effects on foetal growth and programming, and in animal experiments, the possible role of epigenetics in foetal programming has been highlighted [28]. Micronutrients such as vitamin B12 and folate seem to play a role in these processes. For example, in pregnant mothers in the Pune Maternal Nutrition Study, low vitamin B12 status coupled with normal to high folate status predicted both adiposity and insulin resistance in 6-year-old Indian offsprings [10]. In consequence, the authors argued, low maternal vitamin B12 and high folate status may contribute to the current epidemic of adiposity and type 2 diabetes on the Indian subcontinent. If this is true, the associations between B12 levels in adults and the risk of CVD are likely to provide conflicting results especially if the risk of CVD (effects size) with low B12 levels is small compared to other traditional risk factors. It is therefore imperative that the role of B12 before and during pregnancy needs to be investigated further.

Limitations of the review

The present review included only published English-language studies. It is possible that either non-published data or data in other languages exist which we did not identify. The present review included only prospective cohort studies. We are aware of several other published reports from investigations which also measured vitamin B12 levels albeit using different study designs i.e. cross-sectional surveys and case–control studies of patients with either diabetes or CVD. When determining the potential causality of an association, the total evidence needs to be assessed, including from clinical trials. Our present purpose, however, was to review available data on the potential aetiological role of vitamin B12 in diabetes and CVD, knowing that substantial heterogeneity existed between these studies alone. In our opinion, adding results from other types of studies, including cross-sectional surveys and case–control studies, which in fact may be more prone to important study biases, will not reduce the uncertainty about this association. Moreover, because of this apparent heterogeneity of the studies reviewed here we did not perform a statistical synthesis (meta-analysis) of the results. This is frequently done in systematic reviews of observational studies despite being a highly contentious issue [29]. Our view, corroborated by an independent expert (see acknowledgements), is that a meta-analysis of the current studies (and the absence of individual patient data) would have been unhelpful and potentially misleading given that the outcome of that data synthesis might have been a more precise but potentially spurious estimate of the association examined in this review.

Future research

Our recommendations for future research include the following: any prospective studies of vitamin B12 levels and risk of diabetes and CVD in adults should consider alternative biomarkers given that vitamin B12 blood/plasma levels may not be the best indicator of the concentration of B12 in cells or tissues; for example, plasma/urine methylmalonic acid, blood holotranscobalamin, transcobalamin saturation, and the ratio of holotranscobalamin to total vitamin B12 should be considered [25]. Whether there is a linear or threshold effect of low vitamin B12 on disease outcomes also remains to be determined. Moreover, all the studies reviewed here included samples from populations from high-income countries who generally have a good vitamin B12 status—future studies should sample populations at high risk of vitamin B12 deficiency, including those in low-income countries and vegetarian populations. In addition, non-fatal disease outcomes need to be considered. As indicated earlier, mortality may neither be specific nor appropriate in studies of younger subjects. More thought needs to be given to potential confounding factors in future studies. Adjustment for better measured confounders may also reduce the likelihood of residual confounding. Importantly, the lack of association between MTHFR genotypes and CVD [30] challenges a causal role for homocysteine in CVD but does not negate an independent role for vitamin B12 deficiency—this needs to be tested by specific genetic markers, for example, polymorphisms in the TCN2 [31], in Mendelian randomisation studies. Finally, more life-course research is needed of the relationship between levels of vitamin B12 measured in early life and adult disease. Both diabetes and CVD should be included as key outcomes in such studies. As a first step, we are currently assessing the feasibility of preparing a systematic review of this association.

Conclusions

The present review has highlighted much heterogeneity in the results of available cohort studies of the association between blood levels of vitamin B12 and the risk of diabetes and CVD in adulthood. Few relevant studies were identified. Existing investigations differ substantially in study methodology and quality. Overall, the review lends little support to our prior hypothesis that high vitamin B12 would be associated with reduced CVD and diabetes.

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