Studies of biomarker responses to intervention with vitamin B-12: a systematic review of randomized controlled trials

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ABSTRACT

Background: Mild vitamin B-12 deficiency is common among older adults, but evidence for setting dietary recommendations is limited because most studies have administered vitamin B-12 via nonoral routes or at doses several hundred times higher than current recommendations. Furthermore, different biomarkers of vitamin B-12 status have not been systematically reviewed.

Objective: The aim was to assess the effectiveness of biomarkers of vitamin B-12 status through a systematic review of published randomized controlled trials of oral vitamin B-12 supplementation.

Design: Methods included a structured search strategy on Ovid MEDLINE, EMBASE (Ovid), and Cochrane databases; formal inclusion and exclusion criteria; data extraction; validity assessment; and meta-analysis.

Results: Eight randomized controlled trials were included, and all studies measured serum and plasma total vitamin B-12, 3 studies measured methylmalonic acid, and 6 studies measured total homocysteine response. All 3 biomarkers were found to be effective measured methylmalonic acid, and 6 studies measured serum and plasma total vitamin B-12, 3 studies measured total homocysteine, which was measured in only one randomized controlled trial.

Conclusions: The available evidence suggests that plasma and serum concentrations of total vitamin B-12, methylmalonic acid, and total homocysteine are all effective biomarkers of a change in vitamin B-12 intake; however, because the available data were limited, it was not possible to examine fully the factors that could explain the substantial heterogeneity in total vitamin B-12. Future trials should include low-dose vitamin B-12 in adults across the entire age spectrum and measure the holotranscobalamin response to supplementation. Am J Clin Nutr 2009;89(suppl):1981S–96S.

INTRODUCTION

Vitamin B-12 (cobalamin) is found almost exclusively in foods of animal origin in the cofactor forms methyl-, hydroxy-, and deoxyadenosyl-cobalamin (1). Cyanocobalamin is the form typically used in supplements and fortified foods, although methylcobalamin is used in some non-European countries. All forms of vitamin B-12 are readily converted into the 2 biologically active forms, methyl- and deoxyadenosyl-cobalamin. Vitamin B-12 absorption is dependent on normal functioning of the gastrointestinal tract. Gastric acid and pepsin release vitamin B-12 from proteins in food in order for vitamin B-12 to bind with R proteins (2). In the duodenum, vitamin B-12 binds to gastric intrinsic factor once it is split from R proteins. The vitamin B-12 intrinsic factor complex then attaches to specific ideal receptors in the terminal ileum (3), although this pathway has limited capacity because the receptors become saturated at intakes of ~3 μg vitamin B-12 from a single meal (4). A small percentage (~1%) of high-dose vitamin B-12 can be absorbed passive diffusion, independent of intrinsic factor secretion or an intact small intestine (4).

The 2 biologically active forms of vitamin B-12 are required for the normal functioning of 2 mammalian enzymes. Adenosylcobalamin acts as a cofactor for methylmalonyl CoA mutase, which catalyzes the conversion of methylmalonyl CoA to succinyl CoA. Methylcobalamin acts as a cofactor for methionine synthase (which also requires folate), which is involved in the remethylation of homocysteine to methionine. Thus, in vitamin B-12 deficiency, the respective metabolites methylmalonic acid (MMA) and homocysteine accumulate. Both metabolites are sensitive metabolic markers of tissue deficiency, but whereas elevated MMA is specific to vitamin B-12 deficiency except in renal disease (5), plasma and serum total homocysteine concentration is influenced by many factors, most notably by variation in folate status (6), and therefore is a nonspecific marker of low and deficient vitamin B-12 status.

Prevention of low and deficient vitamin B-12 status is of public health importance because it is associated not only with classical deficiency symptoms, such as hematologic abnormalities and irreversible neurological complications, but also potentially with a number of common age-related problems such as cognitive...
decline (7, 8), cardiovascular disease (9), and bone fractures (10, 11). Although dietary intakes greatly exceed recommendations (the latter ranging from 1 to 2.8 μg/d for adults; 12–17) unless a strict vegan diet is followed (18, 19), mild vitamin B-12 deficiency (characterized by elevated metabolites and low or low/normal serum vitamin B-12 concentrations) is common in older people with a reported prevalence of between 10% and 38% (20–23), depending on the diagnostic criteria used. The most common cause of this mild deficiency is considered to be food-bound malabsorption caused by atrophic gastritis, an age-related condition resulting in hypochlorhydria. This in turn leads to diminished vitamin B-12 absorption because gastric acid is required for the release of vitamin B-12 from proteins in food (24). On this basis, the US Institute of Medicine (14) recommends that people aged ≥50 y consume most of their vitamin B-12 from fortified foods and supplements, but no such recommendation exists elsewhere. Emerging nutrient recommendations should be evidence based, but available evidence with respect to vitamin B-12 is somewhat limited in that most studies have administered vitamin B-12 via nonoral routes or have used doses of vitamin B-12 several hundred times higher than current dietary recommendations.

The primary aim of this study was to carry out a systematic review of randomized controlled trials (RCTs) of oral vitamin B-12 supplements to identify the biomarkers of status that reliably reflect change in vitamin B-12 intake.

METHODS

The methodology was based on the standard methodology developed for this set of reviews (25) and is summarized below; emphasizing any differences from the main methodology.

Inclusion criteria

To be included in this review, a study needed to fulfill all of the following criteria: 1) have an RCT design; 2) be conducted in humans; 3) include a placebo or untreated comparison group; 4) include supplementation with vitamin B-12, cobalamin, cyano-, hydroxy-, or methyl-cobalamin in the form of oral supplements, fortified foods, or through natural food sources; 5) report vitamin B-12 status at baseline and for at least one time point after supplementation; and 6) publish in English or other European language. Studies of factorial design were included if the other treatments (conadministered with vitamin B-12) were shown to have no effect on the biomarker of interest. No minimum duration of supplementation was defined because few studies measured the response of vitamin B-12 biomarkers to supplementation at more than one time point.

Exclusion criteria

Studies were excluded if they met any of the following criteria: 1) used nonoral routes of administration, 2) were conducted in patient groups with preexisting disease on the basis that the biomarker responses might not have been representative of the general population or the disease state might have confounded the outcomes, 3) administered vitamin B-12 in combination with other nutrients or other intervention if the independent effects of vitamin B-12 could not be identified, 4) did not report variance data, and 5) were acute studies (ie, typically one dose with measurement of hourly responses) using radioactive or nonradioactive forms of the vitamin.

Search strategy

Electronic searches

Ovid MEDLINE (www.ovid.com; 1950–week 2, September 2007), EMBASE (Ovid, 1980–week 38, 2007), and the Cochrane Library CENTRAL database (www.thecochranelibrary.com; inception to 19 September 2007) were searched for intervention studies of vitamin B-12 using text terms with appropriate truncation and relevant indexing terms. The search was in the form: vitamin B-12 terms AND intervention terms AND human studies. The full Ovid MEDLINE search strategy can be found in Supplemental Table S1 under “Supplemental data” in the online issue; the strategies for the other databases were based on this approach.

Reference search

An additional Ovid MEDLINE search was conducted (1950–week 1, September 2007) for reviews of methods of assessing vitamin B-12 status. Eight of these reviews were collected in full text, and the reference lists were checked (26–33) for studies that appeared to be intervention studies not already assessed for inclusion. In addition, reference lists of the original articles included in this systematic review were also scrutinized for any potential articles not identified by the searches. Unpublished studies or unpublished data from existing studies were not included in this review.

Data collection

Titles and abstracts were screened for inclusion by a single reviewer, and duplicates were removed. Full-text articles were obtained for potentially suitable articles and for those where suitability was unclear from the screening stage. The full text of all articles collected was screened for inclusion using an inclusion/exclusion form by a single reviewer. Data for each included study were extracted onto a Microsoft Access (Microsoft Corp, Redmond, WA) database file by a single reviewer; all extracted data were then checked by a second reviewer. No disagreements occurred between the 2 reviewers. The complete duplication of the screening of articles for inclusion, exclusion, and data extraction, as suggested in the main methodology article (25) in this supplement, was not followed because of time constraints. The data extraction form was tested on a small number of articles by a single reviewer and discussed with the review team before beginning full data extraction.

Data extraction was as discussed in the main methodology article (25) with the following amendments. Corresponding authors of 3 papers were contacted to obtain mean (±SD) values because data in the original articles were presented as in the median and interquartile range (34, 35) or in the median and 5th and 95th percentiles (36).

Data synthesis

Primary and secondary measures of interest were as stated in the main methodology article (25), and data synthesis was carried out as described in the main article with the following
additional decisions and/or assumptions made for this review:
One article reported on 2 separate placebo-controlled trials (36),
which were treated as 2 independent studies in the analysis. In
cases in which studies differed in the units used for reporting
data, all data (mean ± SD) were converted to SI units. In cases
in which variance data were reported in a form other than ±SD
(on the basis that the data were skewed) and in cases in which
the SD could not be obtained from the authors, this value was
estimated from the reported interquartile range as follows: (top
interquartile range) − (bottom interquartile range) × 0.7413.
The median values were assumed to be similar to mean values
(35). If postsupplementation data were presented as the per-
centage change from baseline (37), these data were converted
to absolute change as follows: percentage change from baseline
(mean ± SD) × baseline mean value.
For one article, the data presented were assumed to be mean ±
SD (38). For the presentation of data, responses to intervention
were shown in the same plot whether they were presented as
change from baseline values (34, 36, 37) or absolute values
postintervention (35, 38–40). Where studies included ≥2 vita-
min B-12–treated groups and one common control group (34,
37), the lower dose administered was included only in the sec-
ondary analysis (ie, subgroup analysis by dose). However, for
the latter analysis, if both vitamin B-12 treatment arms fell
within the same range for dose, the groups were combined ac-
cording to the Cochrane formulas for combining groups (41).
Several studies measured the response at more than one time
point, and again the shorter study duration was included only in
the secondary analysis (ie, subgroup analysis by duration of

treatment; 35, 39).

RESULTS
The flow diagram outlining the search results for this review is
shown in Figure 1. After the removal of duplicates, 1597 titles
and abstracts were screened. Of these, the majority of articles
(1572) were excluded primarily because they were review ar-
ticles or intervention studies where vitamin B-12 was not ad-
ministered or studies where vitamin B-12 was not administered
orally or was administered in combination with other nutrients.
Twenty-five articles appeared to be potentially relevant and were
collected as full text articles to be assessed for inclusion.
Eighteen articles were subsequently excluded for the reasons
listed in Figure 1, which left 8 studies (reported in 7 pub-
lcations) that fulfilled the inclusion criteria.
Four biomarkers of vitamin B-12 status were identified, but one
biomarker, holotranscobalamin (holoTC), was measured in only
one study (Figure 1), so it was not possible to reliably evaluate its
effectiveness. The main focus of this systematic review, there-
fore, was on the assessment of responses of serum and plasma
total vitamin B-12 concentrations, MMA, and total homo-
cysteine as potential biomarkers of vitamin B-12 status, because
more than one study reported on each of these outcomes.

Serum and plasma total vitamin B-12 concentrations
Eight RCTs assessed this biomarker, and these studies in-
cluded 506 participants in studies ranging from 3 (38) to 113
participants per arm (34). One study was conducted in children
and adolescents (34), 3 were conducted in adults (35, 38, 40),
and 4 were in elderly populations (36, 37, 39). The majority of
studies were in populations of both sexes, with one involving
male participants only (40) and one in females only (35). All
studies selected participants on the basis of having low vitamin
B-12 status at baseline (34, 36–39) or likely to have a high
prevalence of vitamin B-12 deficiency (eg, vegetarians; 35, 40).
The study duration ranged from 4 wk (37) to one school year (ie,
three 3-mo periods each with a 1-mo break in between; 34). Five
studies administered vitamin B-12 in the form of cyanocobala-
imin (36, 37, 39, 40), 2 in the form of methylcobalamin (35, 38),
and one was food based (34). The dose ranged from physiologic
doses present in milk and meat (34) to pharmacologic doses of

FIGURE 1. Flow diagram of the study selection for the systematic review.
1500 µg (38), and all studies administered the supplements on a daily basis except for 2 studies in which the supplement was administered on a nondaily basis (every alternate day, 35; and 5 times/wk for one school year, 34). Although the biological sample (ie, plasma or serum) varied among studies, immunoassay methods were used in all studies to measure total vitamin B-12 concentrations. For further details, see Table 1.

All studies were randomized, with 5 providing details on the method of randomization (35, 36, 38, 39). The reasons for dropouts and exclusions were given in all publications, and there were no dropouts in one study (37). Compliance was checked in all but one study (38), and the results of the compliance check were reported in all but one (40). The dose delivered was checked by measuring levels in the supplements in 4 studies (36, 37, 39, 39). For further details, see Table 2.

The forest plot of serum and plasma total B-12 response to vitamin B-12 supplementation is shown in Figure 2. Concentrations of vitamin B-12 were significantly increased by intervention with vitamin B-12 [8 RCTs; weighted mean difference (WMD): 185 pmol/L; 95% CI: 107, 263 pmol/L; n = 506; P < 0.00001], although heterogeneity was significant (I²: 94.6%). Removal of weaker studies [on the basis that n was small and the CI was very large (38) or the supplement was administered on a nondaily basis (34, 35)] did not change the overall effect (not shown). Subgroup analysis was carried out to try to explain the heterogeneity, and the results are shown in Table 3. For the majority of secondary analyses, insufficient data were available to answer the questions posed. Adults and elderly people were the only age groups in which sufficient data were available to claim that serum and plasma total vitamin B-12 accurately reflects a change in intake. Cyanocobalamin was the most frequently used form of vitamin B-12 in the supplementation studies, and, as expected, it was found to be a suitable form for improving vitamin B-12 status. The response to methylcobalamin failed to reach statistical significance (P = 0.09), but this finding was based on 2 studies of small sample size. The use of very-low vitamin B-12 doses, in the range 0.75–10 µg, caused a significant increase in vitamin B-12 concentrations (P = 0.0004), and although the greatest overall effect was found at the highest B-12 doses (P < 0.00001), the number of studies using low-dose vitamin B-12 was too small to draw any firm conclusions in relation to dose. The test for heterogeneity remained high for most of the subgroup analysis (I² >50%) so population subgrouping does not appear to explain the heterogeneity seen.

Serum and plasma MMA

Three RCTs measured MMA concentrations, and these studies included 186 participants in studies ranging from 19 (37) to 53 participants per arm (39). All studies were conducted in elderly populations of both sexes, and the study duration was for either 12 (37) or 24 wk (39). All studies administered vitamin B-12 in the form of cyanocobalamin on a daily basis at pharmacologic doses of 1000 µg. Chromatographic methods [liquid chromatography–mass spectrometry (LC-MS)] were used in all studies. For further details on the characteristics and quality of included studies, see Tables 1 and 2.

The forest plot of MMA response to vitamin B-12 supplementation is shown in Figure 3. Concentrations of MMA were significantly lowered by intervention with vitamin B-12 (3 RCTs; WMD: −0.28 µmol/L; 95% CI: −0.35, −0.22 µmol/L; n = 186; P < 0.00001; I²: 0%). All 3 studies excluded subjects with impaired renal function. Subgroup analysis was not possible owing to the limited number of studies that measured this marker.

Serum and plasma total homocysteine concentrations

Six RCTs assessed this biomarker, and these studies included 282 participants in studies ranging from 10 participants per arm (37) to 54 participants per arm (39). Two studies were conducted in adults [one in males only (40); one in females only (35)] and 4 in elderly populations of both sexes (36, 37, 39). Study duration ranged from 4 (37) to 24 wk (39). Five studies administered vitamin B-12 in the form of cyanocobalamin (36, 37, 39, 40) and one in the form of methylcobalamin (35). All studies administered the supplements on a daily basis, which ranged from doses of 10 µg (37) to pharmacologic doses of 1000 µg (36, 39, 40) except for one study in which the supplement was administered every alternate day (35). Five of the 6 studies used chromatographic methods (HPLC or gas chromatography–mass spectrometry), and all measured total homocysteine in plasma except for one study in which serum was used (37). For further details on the characteristics and quality of the included studies, see Tables 1 and 2.

The forest plot of serum and plasma total homocysteine response to vitamin B-12 supplementation is shown in Figure 4. Concentrations of serum and plasma total homocysteine were significantly lowered by intervention with vitamin B-12 (6 RCTs; WMD: −3.3 µmol/L; 95% CI: −4.5, −2.2 µmol/L; n = 282; P < 0.00001; I²: 0%). Results of the subgroup analysis are shown in Table 4. For the majority of secondary analyses, insufficient data were available to answer the questions posed. The group of older people was the only age group in which sufficient data were available to state that the serum and plasma total homocysteine response accurately reflects a change in vitamin B-12 status. Cyanocobalamin was the most frequently used form of vitamin B-12 in the supplementation studies and, as expected, it was found to be a suitable form for lowering serum and plasma total homocysteine concentrations. There was a trend toward lower total homocysteine concentrations with increasing intakes of vitamin B-12, although this relation reached significance only in the groups receiving the higher doses of vitamin B-12 (≥100 µg). However, the number of studies using low-dose vitamin B-12 was too small to draw any firm conclusions.

Plasma holoTC

The effectiveness of holoTC as a biomarker of vitamin B-12 status could not be evaluated as it was measured in only one study. The outcome as reported in the original publication was that holoTC responded significantly to supplementation with high-dose vitamin B-12 (1000 µg/d) for 24 wk in elderly subjects with mild vitamin B-12 deficiency (39).

DISCUSSION

From the potential 25 articles, the current systematic review identified 8 RCTs that reported the response of 4 different biomarkers to supplementation with vitamin B-12. Sufficient data
<table>
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<tr>
<th>Study</th>
<th>Population</th>
<th>Intervention and control</th>
<th>Outcomes reported</th>
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<td><strong>Studies using serum/plasma total</strong></td>
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<td><strong>vitamin B-12 as a biomarker</strong></td>
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<tr>
<td>Dhounushe-Rutten et al, 2005 (36)</td>
<td>Country: Netherlands</td>
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<td></td>
<td>Age range: ≥70 y. Mean (±SD): intervention group—82 (5.4) y; control group—82 (4.7) y.</td>
<td>Intervention: 1000 µg/d cyanocobalamin or placebo capsule. Latest time point: 12 wk No. in intervention at latest time point: 19 No. in control at latest time point: 24</td>
<td>Analytic method: Automated chemiluminescent immunoassay analyzer (Access 2; Beckman Coulter, Mijdrecht, Netherlands). The interassay CV was 6.3%.</td>
<td>Study aim: to compare high-dose vitamin B-12 supplementation provided as a capsule or added to a milk product on cobalamin status in mildly deficient elderly subjects. Comments: vitamin B-12 supplements permitted if &lt;50 µg/d.</td>
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<td>Sex: males (n = 15) and females (n = 33).</td>
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<td>Participants: elderly subjects living in sheltered housing accommodation identified with a mild-cobalamin deficiency (serum cobalamin 100–300 pmol/L and plasma methylmalonic acid &gt;0.30 µmol/L).</td>
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<td>No. included: 48</td>
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<tr>
<td>Dhounushe-Rutten et al, 2005 (36)</td>
<td>As above (country and participants)</td>
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<td>Age range: ≥70 y. Mean (±SD): intervention group—81 (5.6) y; control group—82 (3.7) y.</td>
<td>Intervention: 1000 µg/d cyanocobalamin added to a milk drink or placebo milk drink. Latest time point: 12 wk No. in intervention at latest time point: 19 No. in control at latest time point: 19</td>
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<td>Sex: males (n = 15) and females (n = 26).</td>
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<td>No. included: 41</td>
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<td>Eussen et al, 2006 (39)</td>
<td>Country: Netherlands</td>
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<td>Age range: ≥70 y. Mean (±SD): intervention group—82 (5) y; control group—82 (5) y.</td>
<td>Intervention: 1000 µg/d cyanocobalamin or placebo capsule. Latest time point: 24 wk No. in intervention at latest time point: 52 No. in control at latest time point: 54</td>
<td>Analytic method: IMMULITE 2000 (manufacturer details not provided) cobalamin method</td>
<td>Study aim: to examine whether vitamin B-12 alone or in combination with folic acid has any beneficial effects on cognitive function. Comments: vitamin B-12 supplements permitted if &lt;50 µg/d. Subjects also sampled at 12 wk. Another group given vitamin B-12 and folic acid were excluded from the data extraction.</td>
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<td>Sex: males (n = 29) and females (n = 100).</td>
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<td>Participants: elderly subjects either free-living or living in care facility homes with mild deficiency (serum vitamin B-12 concentration between 100–200 pmol/L or between 200–300 pmol/L, a plasma methylmalonic acid concentration &gt;0.32 µmol/L and a serum creatinine concentration &lt;120 µmol/L)</td>
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<td>No. included: 129</td>
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<td>Study</td>
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<td>Siekmann et al, 2003 (34)</td>
<td>Country: Kenya</td>
<td>Intervention: food based: milk (0.96–1.16 µg vitamin B-12) or meat (0.75–0.91 µg vitamin B-12) added to a local dish or a local dish alone given 5 times/wk. Latest time point: one school year (ie, 3 3-mo periods each with 1-mo break in between). No. in milk intervention at latest time point: 113 No. in meat intervention at latest time point: 103 No. in control at latest time point: 103</td>
<td>Analytic method: radioimmunoassay (ICN Diagnostics, Costa Mesa, CA). The CV for plasma vitamin B-12 was 5% using commercial controls. Study aim: to examine whether increased intake of animal source foods (specifically milk and meat) improves micronutrient status and anemia. Comments: vitamin B-12 status differed at baseline among the treatment groups. Baseline summary status measures given as median and interquartile range values and postsupplementation data given as mean ± SD change values.</td>
<td>Analytic method: radioimmunoassay (ICN Diagnostics, Costa Mesa, CA). The CV for plasma vitamin B-12 was 5% using commercial controls. Study aim: to examine whether increased intake of animal source foods (specifically milk and meat) improves micronutrient status and anemia. Comments: vitamin B-12 status differed at baseline among the treatment groups. Baseline summary status measures given as median and interquartile range values and postsupplementation data given as mean ± SD change values.</td>
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<td>Seal et al, 2002 (37)</td>
<td>Country: Australia</td>
<td>Intervention: 10 µg/d, 50 µg/d cyanocobalamin or placebo in liquid form. Latest time point: 4 wk No. in intervention at latest time point: 17 No. in control at latest time point: 17</td>
<td>Analytic method: solid-phase, no-boil, dual-count radioassay (Diagnostics Products Corporation, Los Angeles, CA). Study aim: to determine the minimum daily dose of vitamin B-12 required to normalize status in older people. Comments: many suffered from chronic disease. Baseline summary status measures given as absolute values and postsupplementation data given as percentage change values.</td>
<td>Analytic method: solid-phase, no-boil, dual-count radioassay (Diagnostics Products Corporation, Los Angeles, CA). Study aim: to determine the minimum daily dose of vitamin B-12 required to normalize status in older people. Comments: many suffered from chronic disease. Baseline summary status measures given as absolute values and postsupplementation data given as percentage change values.</td>
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<td>Ubbink et al, 1994 (40)</td>
<td>Country: South Africa</td>
<td>Intervention: 400 µg/d cyanocobalamin or placebo capsule. Latest time point: 6 wk No. in intervention at latest time point: 17 No. in control at latest time point: 17</td>
<td>Analytic method: radioimmunoassay (Simul TRAC-SNB; Becton Dickinson, Orangeburg, NY). Study aim: to examine the independent and combined effects of folate, vitamin B-12, and vitamin B-6 on plasma homocysteine levels. Comments: capsules administered after dinner. Other groups given vitamin B-6, folic acid, or B-vitamin complex were excluded from the data extraction.</td>
<td>Analytic method: radioimmunoassay (Simul TRAC-SNB; Becton Dickinson, Orangeburg, NY). Study aim: to examine the independent and combined effects of folate, vitamin B-12, and vitamin B-6 on plasma homocysteine levels. Comments: capsules administered after dinner. Other groups given vitamin B-6, folic acid, or B-vitamin complex were excluded from the data extraction.</td>
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<td>Yajnik et al, 2007 (35)</td>
<td>Country: India Age range: 20–50 y. Median (25th, 75th centile): intervention group—31 (23, 37) y; control group—29 (20, 35) y. Sex: females Participants: healthy, lacto-vegetarian women recruited from hospital staff. No. included: 42</td>
<td>Intervention: 2 × 2 factorial design, 500 μg/d methylcobalamin or placebo capsule and green leafy vegetables or control meal provided every alternate day. Latest time point: 6 wk No. in intervention at latest time point: 20 No. in control at latest time point: 20</td>
<td>Analytic method: radioimmunoassay. Intra- and interbatch CV was &lt;10%. Study aim: to determine whether vitamin B-12 supplementation reduces plasma total homocysteine concentration. Comments: subjects also sampled at 2 wk.</td>
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<td>Zaqqa et al, 2005 (38)</td>
<td>Country: Jordan Age with mean (±SD): intervention group—40.2 (7.1) y; control group—46.4 (5.2) y. Sex: males (n = 13) and females (n = 5). Participants: outpatients with normal cardiac tests but cobalamin deficiency (serum vitamin B-12 &lt;180 pmol/L). No. included: 18</td>
<td>Intervention: 1500 μg/d meccobalamin or placebo capsule. Latest time point: 12 wk No. in intervention at latest time point: 5 No. in control at latest time point: 3</td>
<td>Analytic method: microparticle enzyme immunoassay (Abbot Imx, Abbot Diagnostics; manufacturer location not provided). Study aim: to determine whether vitamin B-12 supplements improve chest pain. Comments: have assumed that data are presented as mean ± SD.</td>
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<td>Studies using plasma/serum MMA as a biomarker</td>
<td>Dhonukshe-Rutten et al, 2005 (36)</td>
<td>Country: Netherlands Age range: ≥70 y. Mean (±SD): intervention group—82 (5.4) y; control group—82 (4.7) y. Sex: males (n = 15) and females (n = 33). Participants: elderly subjects living in sheltered housing accommodation identified with a mild cobalamin deficiency (serum cobalamin 100–300 pmol/L and plasma methylmalonic acid &gt;0.30 μmol/L). No. included: 48</td>
<td>Intervention: 1000 μg/d cyanocobalamin or placebo capsule. Latest time point: 12 wk No. in intervention at latest time point: 19 No. in control at latest time point: 24</td>
<td>Analytic method: liquid chromatography mass spectrometry method. The interassay CV was 5%. Study aim: to compare high-dose vitamin B-12 supplementation provided as a capsule or added to a milk product on cobalamin status in mildly deficient elderly subjects.</td>
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<tr>
<td>Dhonukshe-Rutten et al, 2005 (36)</td>
<td>As above (country and participants) Age range: ≥70 y. Mean (±SD): intervention group—81 (5.6) y; control group—82 (3.7) y. Sex: males (n = 15) and females (n = 26). No. included: 41</td>
<td>Intervention: 1000 μg/d cyanocobalamin added to a milk drink or placebo milk drink. Latest time point: 12 wk No. in intervention at latest time point: 19 No. in control at latest time point: 19</td>
<td>As above</td>
<td>As above</td>
</tr>
</tbody>
</table>

(Continued)
<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Intervention and control</th>
<th>Outcomes reported</th>
<th>Methodology</th>
<th>Study aim: to examine whether vitamin B-12 alone or in combination with folic acid has any beneficial effects on cognitive function. Comments: vitamin B-12 supplements permitted if &lt;50 μg/d. Subjects also sampled at 12 wk. Another group given vitamin B-12 and folic acid were excluded from the data extraction.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eussen et al, 2006 (39)</td>
<td>Country: Netherlands</td>
<td>Intervention: 1000 μg/d cyanocobalamin or placebo capsule. Latest time point: 24 wk No. in intervention at latest time point: 52 No. in control at latest time point: 53</td>
<td>Analytic method: liquid chromatography electrospray ionization tandem mass spectrometry system.</td>
<td>Study aim: to examine whether vitamin B-12 alone or in combination with folic acid has any beneficial effects on cognitive function. Comments: vitamin B-12 supplements permitted if &lt;50 μg/d. Subjects also sampled at 12 wk. Another group given vitamin B-12 and folic acid were excluded from the data extraction.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age range: ≥70 y. Mean (±SD): intervention group—82 (5.5) y; control group 82—(5) y. Sex: males (n = 29) and females (n = 100). Participants: elderly subjects either free-living or living in care facility homes with mild deficiency (serum vitamin B-12 concentration between 100 and 200 pmol/L or between 200 and 300 pmol/L, a plasma methylmalonic acid concentration &gt;0.32 μmol/L and a serum creatinine concentration &lt;120 μmol/L) No. included: 129</td>
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</tr>
<tr>
<td>Dhonukshe-Rutten et al, 2005 (36)</td>
<td>Country: Netherlands</td>
<td>Intervention: 1000 μg/d cyanocobalamin or placebo capsule. Latest time point: 12 wk No. in intervention at latest time point: 19 No. in control at latest time point: 24</td>
<td>Analytic method: HPLC with fluorimetric detection. The interassay CV was 7%.</td>
<td>Study aim: to compare high-dose vitamin B-12 supplementation provided as a capsule or added to a milk product on cobalamin status in mildly deficient elderly subjects. Comments: vitamin B-12 supplements permitted if &lt;50 μg/d.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age range: ≥70 y. Mean (±SD): intervention group—82 (5.4) y; control group 82—(4.7) y. Sex: males (n = 15) and females (n = 33). Participants: elderly subjects living in sheltered housing accommodation identified with a mild-cobalamin deficiency (serum cobalamin 100–300 pmol/L and plasma methylmalonic acid &gt;0.30 μmol/L). No. included: 41</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dhonukshe-Rutten et al, 2005 (36)</td>
<td>As above (country and participants) Age range: ≥70 y. Mean (±SD): intervention group—81 (5.6) y; control group 82—(3.7) y. Sex: males (n = 15) and females (n = 26). No. included: 41</td>
<td>Intervention: 1000 μg/d cyanocobalamin added to a milk drink or placebo milk drink. Latest time point: 12 wk No. in intervention at latest time point: 19 No. in control at latest time point: 19</td>
<td>As above</td>
<td>As above</td>
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<td>(Continued)</td>
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### TABLE 1 (Continued)

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Intervention and control</th>
<th>Outcomes reported</th>
<th>Methodology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eussen et al, 2006 (39)</td>
<td>Country: Netherlands</td>
<td>Age range: ≥70 y. Mean (±SD): intervention group—82 (5) y; control group—82 (5) y.</td>
<td>Intervention: 1000 µg/d cyanocobalamin or placebo capsule. Latest time point: 24 wk No. in intervention at latest time point: 52 No. in control at latest time point: 54</td>
<td>Analytic method: based on methylchloroformate derivatization and gas-chromatography-mass spectrometry as described by Windelberg et al, 2005 (reference 33 of the original publication). Study aim: to examine whether vitamin B-12 alone or in combination with folic acid has any beneficial effects on cognitive function. Comments: vitamin B-12 supplements permitted if &lt;50 µg/d. Subjects also sampled at 12 wk. Another group given vitamin B-12 and folic acid was excluded from the data extraction.</td>
</tr>
<tr>
<td>Seal et al, 2002 (37)</td>
<td>Country: Australia</td>
<td>Mean age: 81.4 y. Variance not reported. Sex: males (n = 14) and females (n = 17). Participants: inpatients from 2 geriatric hospitals identified from routine clinical assessment as having subnormal vitamin B-12 status (between 100 and 150 pmol/L) and mean vitamin B-12 intake of 2.02 µg/d. No. included: 129</td>
<td>Intervention: 10 µg/d, 50 µg/d cyanocobalamin or placebo in liquid form. Latest time point: 4 wk No. in 10 µg/d intervention at latest time point: 10 No. in 50 µg/d intervention at latest time point: 10 No. in control at latest time point: 11</td>
<td>Analytic method: HPLC Study aim: to determine the minimum daily dose of vitamin B-12 required to normalize status in older people. Comments: Many suffered from chronic disease. Baseline summary status measures given as absolute values and postsupplementation data given as percentage change values.</td>
</tr>
<tr>
<td>Ubbink et al, 1994 (40)</td>
<td>Country: South Africa</td>
<td>Age range: 20–73 y. Mean (±SD): intervention group—35.0 (12.5) y; control group—40.6 (14.5) y.</td>
<td>Intervention: 400 µg/d cyanocobalamin or placebo capsule. Latest time point: 6 wk No. in intervention at latest time point: 17 No. in control at latest time point: 17</td>
<td>Analytic method: derivatized with ammonium 7-fluoro 2-oxa-1,3 diazole-4-sulfonate and determined by HPLC as described by Ubbink et al, 1991b (cited in the original publication). Study aim: to examine the independent and combined effects of folic acid, vitamin B-12, and vitamin B-6 on plasma homocysteine levels. Comments: capsules administered after dinner. Other groups given vitamin B-6, folic acid, or B-vitamin complex were excluded from the data extraction.</td>
</tr>
</tbody>
</table>
### TABLE 1 (Continued)

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Intervention and control</th>
<th>Outcomes reported</th>
<th>Methodology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yajnik et al, 2007 (35)</td>
<td>Country: India</td>
<td>Age range: 20–50 y. Median (25th, 75th centile): intervention group—31 (23, 37) y; control group—29 (20, 35) y. Sex: females</td>
<td>Intervention: 2 × 2 factorial design, 500 µg/d methylcobalamin or placebo capsule and green leafy vegetables or control meal provided every alternate day. Latest time point: 6 wk</td>
<td>Analytic method: immunofluorescence polarization assay on an AxSYM system (Abbott, IL). Intra- and interbatch CV was &lt;10%. Study aim: to determine whether vitamin B-12 supplementation reduces plasma total homocysteine concentration. Comments: subjects also sampled at 2 wk.</td>
</tr>
<tr>
<td>Eussen et al, 2006 (39)</td>
<td>Country: Netherlands</td>
<td>Age range: ≥70 y. Mean (±SD): intervention group—82 (5) y; control group—82 (5) y. Sex: males (n = 29) and females (n = 100). Participants: elderly subjects either free-living or living in care facility homes with mild deficiency (serum vitamin B-12 concentration between 100 and 200 pmol/L or between 200 and 300 pmol/L, a plasma methylmalonic acid concentration &gt;0.32 µmol/L, and a serum creatinine concentration &lt;120 µmol/L).</td>
<td>Intervention: 1000 µg/d cyanocobalamin or placebo capsule. Latest time point: 24 wk</td>
<td>Analytic method: AXIS-Shield radioimmunoassay method described by Ulleland et al, 2002 (reference 34 of the original publication). Study aim: to examine whether vitamin B-12 alone or in combination with folic acid has any beneficial effects on cognitive function. Comments: vitamin B-12 supplements permitted if &lt;50 µg/d. Subjects also sampled at 12 wk. Another group given vitamin B-12 and folic acid were excluded from the data extraction.</td>
</tr>
</tbody>
</table>

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1 MMA, methylmalonic acid; holoTC, holotranscobalamin.
### TABLE 2
Validity of included studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Randomization</th>
<th>Dropouts</th>
<th>Compliance</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dhonukshe-Rutten et al,</td>
<td>Double-blind study. Matched by age, sex, and methylmalonic acid concentration with priority given to the latter.</td>
<td>Intervention, $n = 4$; control, $n = 1$. Withdrew for health reasons.</td>
<td>Subjects provided with 7-d pillboxes and diary to record whether the capsule was taken. Compliance assessed by reviewing the diary and counting any remaining capsules; 90% consumed &gt;90% of their supplements.</td>
<td>The mean ($\pm$SD) cobalamin concentration of several capsules was 936 ± 34 $\mu$g (expected value 1000 $\mu$g).</td>
</tr>
<tr>
<td>2005 (36)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dhonukshe-Rutten et al,</td>
<td>As above</td>
<td>Intervention, $n = 1$; control, $n = 2$. Withdrew for health reasons or excluded as had normal B-12 status.</td>
<td>Subjects provided with 500-ml containers of milk along with a 125-ml cup and a diary to record whether the milk was consumed. Subjects recorded how much of the 500 ml was left at the end of each 4-d period. Compliance assessed by reviewing the diary; 90% consumed &gt;90% of their milk.</td>
<td>Cobalamin-fortified milk contained 7000 $\mu$g/L cobalamin (expected value 8000 $\mu$g/L) and the placebo milk contained 3.7 $\mu$g/L.</td>
</tr>
<tr>
<td>2005 (36)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eussen et al, 2006 (39)</td>
<td>Double-blind study. Stratified by methylmalonic acid concentration at the screening visit (&lt; and &gt; 0.45 $\mu$mol/L), age (&lt; and &gt; 80 y), sex, and MMSE score (&lt; and &gt; 24 points).</td>
<td>Intervention, $n = 10$; control, $n = 8$. Withdrew mostly because of illness.</td>
<td>Subjects provided with a diary to record whether the capsule was taken. Compliance assessed by reviewing the diary and counting any remaining capsules. For institutionalized subjects, nurses monitored daily capsule intake. Mean compliance was 99% and 4 subjects had a compliance of between 80–90%.</td>
<td>The mean ($\pm$SD) vitamin B-12 content of capsules was 986 ± 3.4 $\mu$g (expected value 1000 $\mu$g).</td>
</tr>
<tr>
<td>Siekmann et al, 2003 (34)</td>
<td>Randomly assigned by school (3 schools per group) to treatment groups. Randomization was carried out without stratification by school size.</td>
<td>Statistical analysis was by intention to treat so did not exclude the 17 children who did not like the taste of milk or meat or the 17 children who changed schools.</td>
<td>Consumption of foods was observed and leftovers weighed and recorded. Of those who attended school, 99.4% ate all of the food provided. Children were present on 84.9% of all school days in the meat group and on 84.7% of all school days in the milk group.</td>
<td>None mentioned</td>
</tr>
<tr>
<td>Seal et al, 2002 (37)</td>
<td>Blind to laboratory staff.</td>
<td>There were none.</td>
<td>Unused medicine returned and volume measured and compared with the expected dose consumption. No difference between the treatment groups.</td>
<td>Samples of the formulations were analyzed for vitamin B-12 content before the study and after storage for 3-mo at room temperature or at 4°C. Concentrations did not fall by &gt;5%.</td>
</tr>
<tr>
<td>Ubbink et al, 1994 (40)</td>
<td>Double-blind study</td>
<td>Intervention, $n = 3$; control, $n = 3$. Withdrew or excluded due to lack of compliance.</td>
<td>Blood samples collected at 3 wk and analyzed for B-12 concentrations. Results of check not reported.</td>
<td>None mentioned</td>
</tr>
</tbody>
</table>

(Continued)
were available to show that serum and plasma concentrations of total vitamin B-12, MMA, and total homocysteine respond to intervention with vitamin B-12 in adult populations selected on the basis of having mild vitamin B-12 deficiency or being at risk of deficiency. The evidence, however, was insufficient to establish the effectiveness of holoTC as a biomarker of vitamin B-12 status. In the case of total vitamin B-12 response, substantial heterogeneity was observed among the studies, but the limited number of available studies prevented detailed subgroup analysis being undertaken to fully explain this variability.

For the current review, relatively few studies were identified because much of the older literature on vitamin B-12 involved studies of clinical and biochemical responses to nonoral routes of administration, different pharmacologic doses, or different forms of the vitamin in patients with diagnosed vitamin B-12 deficiency. Only in recent years have studies involved healthy populations in light of the evidence that mild vitamin B-12 deficiency has a high prevalence among elderly people (19) and that this condition is associated with a number of chronic diseases such as cognitive decline (7, 8). Furthermore, studies show that vitamin B-12 becomes the main nutritional determinant of serum and plasma total homocysteine once folate status is optimized (42–45) and that lowering total homocysteine might be beneficial in the prevention of stroke (46). Several recent systematic reviews that have addressed important issues such as the role of vitamin B-12 in cognitive function and the effectiveness of different routes of administration (47–49) have been published, but to our knowledge none have addressed the questions being asked here.

Serum and plasma total vitamin B-12 concentrations increased significantly in response to intervention with vitamin B-12 over supplementation periods of at least 4 wk, although the response varied considerably among studies that measured total vitamin B-12. Likewise, significant decreases in both total homocysteine and MMA concentrations were observed with vitamin B-12 intervention. However, the latter biomarker response was based on 3 studies carried out in the same country with similar population groups, so the conclusions may not necessarily be applicable to other populations. Other inherent weaknesses were that too few trials, mostly of small sample size, were identified, and these varied considerably in terms of the dose and form of supplement administered, study duration, primary outcome, methodologic factors (eg, serum or plasma, fasting or nonfasting

<table>
<thead>
<tr>
<th>Study</th>
<th>Randomization</th>
<th>Dropouts</th>
<th>Compliance</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yajnik et al, 2007 (35)</td>
<td>Randomly allocated to groups after minimization for age and plasma B-12 concentration (above and below the median).</td>
<td>Intervention, n = 2. Withdrew because moved away and pregnancy.</td>
<td>Fed under supervision</td>
<td>None mentioned</td>
</tr>
<tr>
<td>Zaqqa et al, 2005 (38)</td>
<td>Computer-generated random numbers.</td>
<td>One patient lost to follow-up. Postsupplement concentrations only measured in a subset of subjects.</td>
<td>None mentioned</td>
<td>None mentioned</td>
</tr>
</tbody>
</table>

\(^{1}\text{MMSE, Mini-Mental State Examination.}\)
samples), and population studied. Few of the studies recruited from the general population but instead focused on subjects recruited from particular locations (eg, a hospital setting or a school), so the findings may not be applicable to all subgroups. Study populations varied from apparently healthy subjects with mild vitamin B-12 deficiency or at risk of deficiency to subjects attending outpatient clinics, which could therefore confound the study outcomes. Furthermore, several studies permitted the inclusion of participants taking vitamin B-12 supplements at the time of recruitment as long as the intake was at low doses. Although all trials were randomized, few provided details on the method of randomization used. Probably of greatest importance is the fact that only 1 of the 8 trials administered vitamin B-12 with food (40). This might be a major limitation in the extent of the response observed because the presence of food is recognized as being necessary to stimulate the normal vitamin B-12 absorptive mechanisms (4). Subgroup analysis was undertaken to explore the factors that might have contributed to the variability in total vitamin B-12 response among the studies, but the above limitations (particularly the small number of published RCTs) mean that our conclusions should be interpreted with caution.

### TABLE 3
Results of the subgroup analysis for serum and plasma total vitamin B-12 concentration

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Mean effect, WMD (95% CI)</th>
<th>P value</th>
<th>RCTs included</th>
<th>I²</th>
<th>Biomarker useful?</th>
</tr>
</thead>
<tbody>
<tr>
<td>All studies (primary outcome)</td>
<td>185 (107, 263)</td>
<td>P &lt; 0.00001</td>
<td>8 (506)</td>
<td>94.6</td>
<td>Yes</td>
</tr>
<tr>
<td>Infants</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Unclear</td>
</tr>
<tr>
<td>Children and adolescents</td>
<td>79 (61, 97)</td>
<td>P &lt; 0.00001</td>
<td>1 (216)</td>
<td>NA</td>
<td>Unclear</td>
</tr>
<tr>
<td>Pregnancy and lactation</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Unclear</td>
</tr>
<tr>
<td>Adults</td>
<td>132 (52, 212)</td>
<td>P = 0.001</td>
<td>3 (82)</td>
<td>47.1</td>
<td>Yes</td>
</tr>
<tr>
<td>Postmenopausal women</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Unclear</td>
</tr>
<tr>
<td>Elderly people</td>
<td>232 (115, 350)</td>
<td>P = 0.0001</td>
<td>4 (208)</td>
<td>94.5</td>
<td>Yes</td>
</tr>
<tr>
<td>Low-income and immigrant groups</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Unclear</td>
</tr>
<tr>
<td>Males</td>
<td>168 (71, 265)</td>
<td>P = 0.0007</td>
<td>1 (34)</td>
<td>NA</td>
<td>Unclear</td>
</tr>
<tr>
<td>Females</td>
<td>88 (41, 135)</td>
<td>P = 0.0003</td>
<td>1 (40)</td>
<td>NA</td>
<td>Unclear</td>
</tr>
<tr>
<td>Vitamin B-12 status at baseline</td>
<td></td>
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</tr>
<tr>
<td>Low</td>
<td>185 (107, 263)</td>
<td>P &lt; 0.00001</td>
<td>8 (506)</td>
<td>94.6</td>
<td>Yes</td>
</tr>
<tr>
<td>Moderate</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Unclear</td>
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<tr>
<td>High</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Unclear</td>
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<tr>
<td>Micronutrient type</td>
<td></td>
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</tr>
<tr>
<td>1: cyanocobalamin</td>
<td>220 (120, 321)</td>
<td>P &lt; 0.0001</td>
<td>5 (242)</td>
<td>92.8</td>
<td>Yes</td>
</tr>
<tr>
<td>2: methylcobalamin</td>
<td>137 (~23, 296)</td>
<td>P = 0.09</td>
<td>2 (48)</td>
<td>49.4</td>
<td>Unclear but likely to be useful</td>
</tr>
<tr>
<td>3: food-based</td>
<td>33 (10, 56)</td>
<td>P = 0.005</td>
<td>1 (319)</td>
<td>NA</td>
<td>Unclear</td>
</tr>
<tr>
<td>Dose</td>
<td></td>
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</tr>
<tr>
<td>1: ≤ 10 µg</td>
<td>35 (16, 55)</td>
<td>P = 0.0004</td>
<td>2 (340)</td>
<td>0</td>
<td>Unclear but likely to be useful</td>
</tr>
<tr>
<td>2: 11–50 µg</td>
<td>68 (17, 120)</td>
<td>P = 0.01</td>
<td>1 (21)</td>
<td>NA</td>
<td>Unclear</td>
</tr>
<tr>
<td>3: 100–500 µg</td>
<td>116 (41, 191)</td>
<td>P = 0.002</td>
<td>2 (74)</td>
<td>52.6</td>
<td>Unclear but likely to be useful</td>
</tr>
<tr>
<td>4: &gt; 1000 µg</td>
<td>286 (223, 349)</td>
<td>P &lt; 0.00001</td>
<td>3 (187)</td>
<td>73.2</td>
<td>Yes</td>
</tr>
</tbody>
</table>

1 WMD, weighted mean difference; RCTs, randomized controlled trials; N/A, no available data; NA, not applicable (ie, only one available study).

2 To claim whether a biomarker was useful, the following terms indicate the conditions needed to be met: “yes,” forest plot showed a significant effect (P < 0.05) based on ≥ 3 studies and ≥ 50 participants between the intervention and control arms; “unclear but likely to be useful,” forest plot showed a significant effect (P < 0.05), but the result was based on 2 studies; “unclear,” insufficient data were available.

FIGURE 3. Effect of supplementation with vitamin B-12 on plasma methylmalonic acid concentration (µmol/L). Mean (±SD) values show change from baseline (36) or are absolute values postintervention (39). RCT, randomized controlled trials; WMD, weighted mean difference.
Two potential factors that could determine response to any nutrient supplementation are the age and sex of the study population. In the case of vitamin B-12, age is considered to be the far more critical factor and, in fact, most RCTs included in this review involved older adults selected on the basis of having mild vitamin B-12 deficiency, presumably (although not explicitly) a result of food-bound vitamin B-12 malabsorption. Furthermore, although we did not specifically exclude studies involving food-bound vitamin B-12 malabsorption.

### TABLE 4
Results of the subgroup analysis for plasma and serum total homocysteine concentration

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Mean effect, WMD (95% CI)</th>
<th>P value</th>
<th>RCTs included</th>
<th>Biomarker useful?</th>
</tr>
</thead>
<tbody>
<tr>
<td>All studies (primary outcome)</td>
<td>-3.3 (−4.5, −2.2)</td>
<td>P &lt; 0.00001</td>
<td>6 (282)</td>
<td>0 Yes</td>
</tr>
<tr>
<td>Infants</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A Unclear</td>
</tr>
<tr>
<td>Children and adolescents</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A Unclear</td>
</tr>
<tr>
<td>Pregnancy and lactation</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A Unclear</td>
</tr>
<tr>
<td>Adults</td>
<td>-5.5 (−9.7, −1.3)</td>
<td>P = 0.01</td>
<td>2 (74)</td>
<td>0 Unclear but likely to be useful</td>
</tr>
<tr>
<td>Postmenopausal women</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A Unclear</td>
</tr>
<tr>
<td>Elderly people</td>
<td>-3.1 (−4.3, −2.0)</td>
<td>P &lt; 0.00001</td>
<td>4 (208)</td>
<td>0 Yes</td>
</tr>
<tr>
<td>Low-income and immigrant groups</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A Unclear</td>
</tr>
<tr>
<td>Males</td>
<td>-4.7 (−17.4, 8.0)</td>
<td>P = 0.47</td>
<td>1 (34)</td>
<td>NA Unclear</td>
</tr>
<tr>
<td>Females</td>
<td>-5.6 (−10.1, −1.2)</td>
<td>P = 0.01</td>
<td>1 (40)</td>
<td>NA Unclear</td>
</tr>
<tr>
<td>Vitamin B-12 status at baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>-3.3 (−4.5, −2.2)</td>
<td>P &lt; 0.00001</td>
<td>6 (282)</td>
<td>0 Yes</td>
</tr>
<tr>
<td>Moderate</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A Unclear</td>
</tr>
<tr>
<td>High</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A Unclear</td>
</tr>
<tr>
<td>Micronutrient type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1: cyanocobalamin</td>
<td>-3.2 (−4.3, −2.0)</td>
<td>P &lt; 0.00001</td>
<td>5 (242)</td>
<td>0 Yes</td>
</tr>
<tr>
<td>2: methylcobalamin</td>
<td>-5.6 (−10.1, −1.2)</td>
<td>P = 0.01</td>
<td>1 (40)</td>
<td>NA Unclear</td>
</tr>
<tr>
<td>3: food based</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A Unclear</td>
</tr>
<tr>
<td>Dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1: ≤10 µg</td>
<td>-1.9 (−8.0, 4.2)</td>
<td>P = 0.54</td>
<td>1 (21)</td>
<td>NA Unclear</td>
</tr>
<tr>
<td>2: 11–50 µg</td>
<td>-2.2 (−6.7, 2.3)</td>
<td>P = 0.34</td>
<td>1 (21)</td>
<td>NA Unclear</td>
</tr>
<tr>
<td>3: 100–500 µg</td>
<td>-5.5 (−9.7, −1.3)</td>
<td>P = 0.01</td>
<td>2 (74)</td>
<td>0 Unclear but likely to be useful</td>
</tr>
<tr>
<td>4: ≥1000 µg</td>
<td>-3.2 (−4.4, −2.0)</td>
<td>P &lt; 0.00001</td>
<td>3 (187)</td>
<td>0 Yes</td>
</tr>
<tr>
<td>Analytic method</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1: HPLC</td>
<td>-3.1 (−4.5, −1.7)</td>
<td>P &lt; 0.0001</td>
<td>4 (136)</td>
<td>0 Yes</td>
</tr>
<tr>
<td>2: GC-MS</td>
<td>-3.3 (−5.6, −1.1)</td>
<td>P = 0.004</td>
<td>1 (106)</td>
<td>0 Unclear</td>
</tr>
<tr>
<td>3: Immunoassay</td>
<td>-5.6 (−10.1, −1.2)</td>
<td>P = 0.01</td>
<td>1 (40)</td>
<td>0 Unclear</td>
</tr>
</tbody>
</table>

1 WMD, weighted mean difference; RCTs, randomized control trials; GC-MS, gas chromatography–mass spectrometry; N/A, no available data.; NA, not applicable (ie, only one available study).

2 To claim whether a biomarker was useful, the following terms indicate the conditions needed to be met: “yes,” forest plot showed a significant effect (P < 0.05) based on ≥3 studies and ≥50 participants between the intervention and control arms; “unclear but likely to be useful,” forest plot showed a significant effect (P < 0.05), but the result was based on 2 studies; “unclear,” insufficient data were available.
A SYSTEMATIC REVIEW OF BIOMARKERS OF VITAMIN B-12 STATUS

This review highlights a number of gaps in the field of vitamin B-12 research. Well-designed RCTs of sufficient size and with varying doses (including doses equivalent to dietary intakes) and duration of supplementation are required in adults across the entire age spectrum. Such studies will establish the optimal dose and duration of treatment with vitamin B-12 that are necessary to normalize status in adults and will be of relevance to those tasked with revising dietary recommendations. Furthermore, studies should include holoTC as an outcome measure to determine the efficacy of this biomarker and make use of the new automated immunoassays available for measuring biological samples. (Other articles in this supplement to the Journal include references 25 and 56–62.)

We thank the authors of the main methodology article (25) for running the electronic searches and for their assistance and guidance with all aspects of data collection and analysis. We also thank the authors of the original articles, in particular those who were approached for additional information.

The authors’ responsibilities were as follows—LH: responsible for assessing studies for inclusion, data extraction, conducting meta-analyses, and writing of the manuscript; HM: responsible for checking the data extraction, and writing of the manuscript; and JJS: involved in the discussion of the results and writing of the manuscript. None of the authors had any conflicts of interest.

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