Systematic Review on Daily Vitamin B12 Losses and Bioavailability for Deriving Recommendations on Vitamin B12 Intake with the Factorial Approach

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Abstract

Aims: To systematically review the literature on daily losses and bioavailability of vitamin B12. These estimates could be used for deriving recommendations on vitamin B12 intake for adults and elderly. **Methods:** We identified publications on daily vitamin B12 losses (July 2011) and publications on the bioavailability of vitamin B12 from foods or diets (June 2010) in MEDLINE, EMBASE and the Cochrane Library. **Results:** A pooled analysis of five studies (52 subjects) showed that 0.13 ± 0.03% of the total body store is lost per day. Absorption of vitamin B12 ranged from 4.5 (dose of 38 μg from consumption of liver) to 83% (dose of 3.0 μg from consumption of mutton meat). Data from eight studies including 83 subjects suggested that the amount of vitamin B12 absorbed from food (A_i) increased with increasing doses of vitamin B12 (D_i) as described by the equation: $\ln(A_i) = 0.7694 * \ln(D_i) - 0.9614$. **Conclusion:** Daily vitamin B12 losses in apparently healthy adults and elderly probably range from 1.4 to 5.1 μg. Vitamin B12 intakes needed to compensate for these losses seem to range from 3.8 to 20.7 μg. More evidence is needed on the relationships between biochemical markers of vitamin B12 status, vitamin B12 body store and long-term health outcomes to evaluate whether current recommendations on vitamin B12 intake (1.4–3 μg) need to be changed.

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Introduction

Current recommendations on vitamin B12 intake for adults and elderly vary from 1.4 to 3 μg per day between European countries. The variation in these recommendations results from different approaches used for estimating vitamin B12 requirements and from different assumptions regarding the interindividual variation in requirements [1].

One of the approaches used for estimating vitamin B12 requirements for adults and elderly in Europe (The Netherlands and France) is the factorial approach, which includes a summation of daily vitamin B12 losses that need to be compensated for by dietary intake of vitamin B12. Estimates of daily vitamin B12 losses were obtained from human studies measuring (1) the decrease in radioactivity after administration of labeled vitamin B12 or (2) vitamin B12 excretion in bile [2].

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To estimate vitamin B12 requirements, estimates of daily vitamin B12 losses need to be corrected for bioavailability of the vitamin from the usual diet. Bioavailability is defined as 'the efficiency with which a dietary component is used systematically through normal body functions' and expressed as a percentage of intake [3]. Currently, the bioavailability of vitamin B12 is generally assumed to be 40 or 50% for healthy adults with normal gastrointestinal functioning [2, 4–7]. This assumption is based on the absorption of labeled vitamin B12 from a few food products, including mutton and chicken meat, rainbow trout, eggs or fortified foods [8–12].

Recommended vitamin B12 intakes indicate the daily intake that is sufficient to fulfill requirements of nearly all apparently healthy individuals within a defined population. When the distribution of vitamin B12 requirements is known, the recommended vitamin B12 intake is derived as the average nutrient requirement (ANR) plus twice the SD of the ANR, with the ANR defined as the intake level that is sufficient to fulfill requirements in 50% of individuals in a defined population. As there is very limited evidence on the shape of this distribution, usually a standard assumption of a coefficient of variation (CV) of 10–20% is made.

Ideally, recommended vitamin B12 recommendations should be based on the relationship between vitamin B12 intake, a biochemical marker of vitamin B12 status, and a cutoff level to distinguish between an adequate and an inadequate vitamin B12 status. However, so far methods to derive recommendations based on dose-response evidence are still under development and there is no strong consensus on appropriate cutoff levels for defining health [13–15]. The factorial approach may serve as an alternative until more dose-response evidence becomes available. As shown previously, European recommendations on vitamin B12 intake for adults and elderly established using the factorial approach (The Netherlands and France) are based on a limited number of studies that were published between 1963 and 2001 (box 1) [1]. Assumptions underlying these recommendations were mainly focused on the prevention of severe deficiency symptoms, whereas long-term health outcomes were not taken into account. The aim of this article was to systematically review the available literature on daily vitamin B12 losses in apparently healthy adults and bioavailability of vitamin B12 from different food sources including estimates of interindividual variation. The results of this review could serve as an input for establishing vitamin B12 recommendations using the factorial approach.

Box 1. Calculations used for establishing vitamin B12 recommendations with the factorial approach

General formula
ANR = (sum of losses (feces, urine, skin, menstrual losses…) + needs for growth)/bioavailability factor

The Netherlands [6]
Assumption 1: daily vitamin B12 losses are maximally 0.2% of vitamin B12 stores [11, 16, 17]
Assumption 2: minimal body stores are 500 µg
Assumption 3: bioavailability is 50% [8–10, 12]
Assumption 4: CV = 20%
Daily losses = 0.2% * 500 µg = 1.0 µg
ANR = 1.0/0.5 = 2.0 µg
Recommended intake[a] = 2.0 * 1.4 = 2.8 µg

France [2]
Assumption 1: daily vitamin B12 excretion in bile is 1.4 µg [18]
Assumption 2: 40% of vitamin B12 excreted in bile is reabsorbed in the small intestine [19, 20]
Assumption 3: bioavailability is 40% [17]
Assumption 4: CV = 10%
Daily losses = 1.4 * 60% = 0.8 µg
ANR = 0.8/0.4 = 2.0 µg
Recommended intake[a] = 2.0 * 1.2 = 2.4 µg

[a] Recommended intake is derived as (1 + 2 * CV/100) * ANR.

Methods

This systematic review was conducted according to standardized methodology as developed within the scope of EURRECA (European Micronutrient Recommendations Aligned) Network of Excellence, which is shortly described below.

Search
We conducted two systematic literature searches to identify publications on (1) basal vitamin B12 losses (MEDLINE; 12 July 2010) and (2) the bioavailability of vitamin B12 from diets (MEDLINE, EMBASE and Cochrane Library; 15 June 2010). The first search included terms on vitamin B12 or labeled vitamin B12 in combination with terms on excretion routes, kinetics or maintenance, and the second search included terms on vitamin B12 in combination with terms on absorption. Both searches were restricted to studies in humans. To be able to use the same searches to identify publications for younger population groups, no terms regarding the age of the study population were added. The second search also included terms regarding folate to identify papers on folate bioavailability, as will be described elsewhere. The search strategies are included in online supplementary file 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000346968). Additional studies were identified by hand searching, i.e. screening of reference lists from all selected articles, review articles and re-

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ports including recommendations on micronutrient intakes. From July 2010 to July 2011, we checked database alerts to identify new relevant publications.

Since daily vitamin B12 losses are often reported as a proportion of the total body store of vitamin B12, we performed an additional search in MEDLINE (23 October 2012) to identify publications providing estimates of the total body store (online suppl. file 1). Search results were completed by hand searching as explained above.

Study Selection and Data Extraction

In general, studies on losses and bioavailability were eligible for inclusion when they had an experimental design and the study population included apparently healthy adults (age ≥18 years) or adult patients with diseases not associated with impaired vitamin B12 metabolism or absorption. Studies on losses were included when (1) the exposure was oral or parenteral administration of vitamin B12 and (2) the outcome was an estimate of daily vitamin B12 losses measured with whole body counting (WBC) or vitamin B12 excretion in bile. Studies on bioavailability were included when (1) the exposure concerned diets, meals or individual foods in- or extrinsically labeled with vitamin B12 and (2) the outcome was a measure of fractional vitamin B12 absorption.

Studies on total body stores were included when estimates of vitamin B12 stores were obtained from apparently healthy adults or patients with diseases not associated with impaired vitamin B12 metabolism or absorption.

Four reviewers screened all titles and abstracts of papers identified through the literature searches according to the inclusion criteria, and the full text of potentially relevant papers were evaluated against the same criteria (P.H.i.t.V., A.S., M.D. and E.L.D.). For the purpose of alignment and quality control, each reviewer screened and evaluated 10% of the total number of references in duplicate with another reviewer. Each study included was extracted by a single reviewer (P.H.i.t.V. or A.S.) and a second reviewer independently verified the extracted data (E.L.D.). Any disagreement during the selection and extraction process was resolved by discussion.

From all studies, we extracted general characteristics of the study design (duration, dose) and study population (number of participants, age, gender and ethnicity). In addition, from each study reporting on total vitamin B12 losses measured with WBC, we extracted the rate of loss indicating the mean decay in radioactivity per day as a percentage of the radioactivity measured after redistribution of the labeled vitamin B12 through different body compartments (i.e. at the time that a constant excretion rate was established). The underlying assumption is that labeled vitamin B12 is distributed throughout the body in a manner similar to nonlabeled vitamin B12. We did not merely extract estimates based on measurements in healthy subjects [11, 16, 22–24] but also on subjects with low serum concentrations of vitamin B12 [23] and patients with pernicious anemia (PA) [11, 16], because it appeared that the rate of loss does not significantly differ between these subpopulations [11, 16, 23]. One study reported the absolute mean loss of vitamin B12 per day and the total body content of vitamin B12 [24]. To allow comparison with the other studies, the rate of loss was calculated as (mean loss per day/total body content) * 100%.

From each study reporting on vitamin B12 excretion in bile, we extracted the rate of excretion, the vitamin B12 concentration of bile or the absolute vitamin B12 excreted in bile per day. The rate of excretion reflected the radioactivity measured in excreted bile as a percentage of the ingested dose minus total losses during the first 4 days (i.e. the period of redistribution of vitamin B12 throughout the body) after administration of labeled vitamin B12 [25, 26].

From studies on the bioavailability of vitamin B12, we extracted the percentage of the ingested dose that was absorbed. Furthermore, we extracted the absolute amount of vitamin B12 that was absorbed. In case this latter estimate was not reported, it was calculated as percent absorbed * ingested dose [9, 27–29].

To describe the relationship between the amount absorbed (A_i) and the dose administered (D_i) via the foods provided in the intervention studies, we performed linear regression with both variables transformed to their natural logarithm in order to achieve linearity of the regression.

As an upper limit of interindividual variation, we calculated CV for each outcome measure reported per study: SD/mean * 100%, ignoring the measurement errors and within-subject variation. When no SD was provided, we estimated it based on individual subject data $\{SD = \sqrt{[\Sigma(x_i - x_{mean}^2)/(N - 1)]}\}$ [8, 10, 11, 16, 22, 23, 30], on the standard error (SE: $SD = SE * \sqrt{n}$) [25, 26, 31] or we approximated it by the total range (SD ≈ 0.25 * total range) [11, 25, 32, 33].

Where applicable, amounts of vitamin B12 were converted from nanomoles to micrograms using the molar weight of vitamin B12 (1,355.38 g/mol) [18, 29].

If three or more studies were comparable with regard to exposure and outcome measures, we calculated summary estimates using random-effect meta-analysis. Applying the methods of DerSimonian and Laird [34], the between-study variance is estimated, which is then used to modify the weights for calculating the summary estimate. SEs were calculated as $SD/\sqrt{n}$. If no SD was reported and no individual data were available, we imputed an SE based on the pooled SD from the other studies included in the pooled analysis: $SD_{pooled} = \sqrt{\{\Sigma[(n_i - 1) * SD_i^2]/\Sigma(n_i - 1)\}}$ [24]. Heterogeneity between studies was evaluated using the I^2 statistic, which expresses the percentage of variation attributable to between-study heterogeneity rather than chance [35]. Meta-analyses were performed using STATA version 11.0 (College Station, Tex., USA), with statistical significance defined as a value of $p < 0.05$.

Results

Study Characteristics

In total, we identified 523 potentially relevant papers on daily vitamin B12 losses and 5,556 on bioavailability of vitamin B12 and folate (combined search). After evaluation of titles, abstracts and full texts, ten papers were included for vitamin B12 losses (five papers on daily vitamin B12 losses measured with WBC and five papers on daily vitamin B12 excretion in bile) and eight papers were included for bioavailability of vitamin B12 (fig. 1). All studies were performed in Western countries (USA, UK, Sweden, Norway or Finland). Unfortunately, information on age, sex and ethnicity of the study populations was not provided for most of the studies. Serum vitamin B12
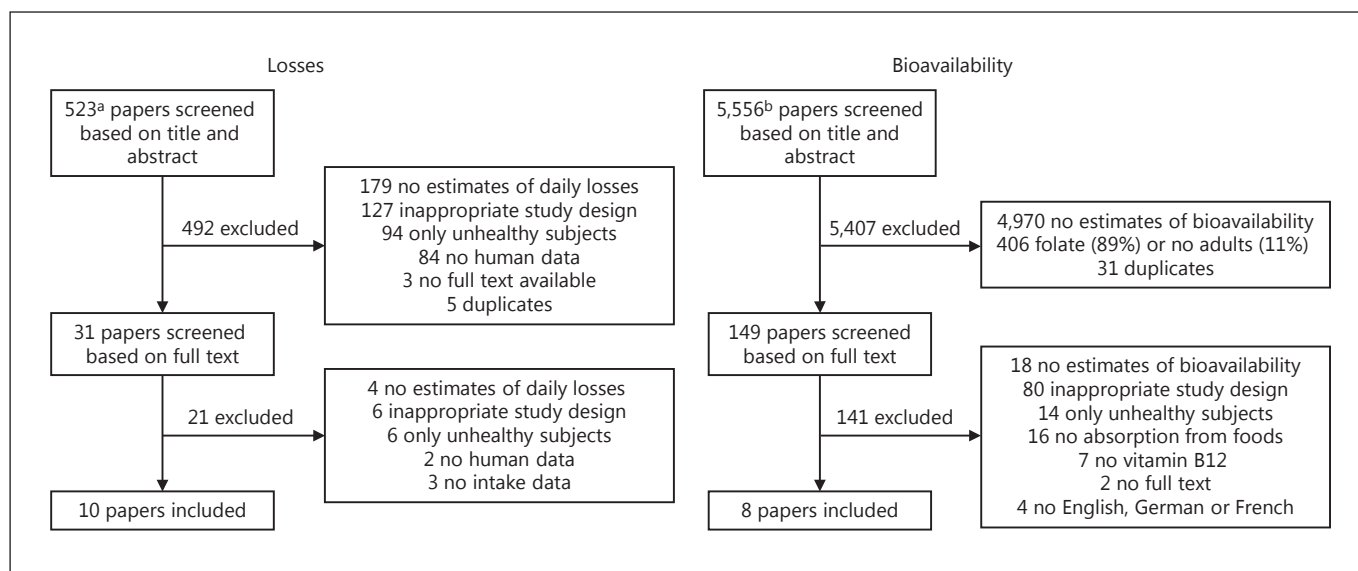
Fig. 1. Selection of studies for systematic review on daily vitamin B12 losses and bioavailability of vitamin B12. [a] 515 from MED-LINE, 8 identified from reference lists of selected articles and review articles. [b]5,551 from MEDLINE, EMBASE and Cochrane, 5 identified from reference lists of selected articles, review articles or recommendation reports. The multi-database search also included terms regarding folate in order to identify papers on folate bioavailability, as will be described elsewhere.

Table 1. Daily vitamin B12 losses measured with WBC

Reference	Population characteristics		Exposure		Outcome		
	n (% men)	health status, age, n	dose of vitamin B12 labeled with 57/58/60 Co, μg[a]	adminis-tration route	rate of loss, % per day[b]		
					mean	SD	CV
Boddy and Adams [23] (1968)	14 (50)	healthy, 42 years (n = 1) low vitamin B12, 34–74 years (n = 13)	5,000	i.v. or i.m.	*0.13*	*0.028*	21
Bozian et al. [16] (1963)	16 (69)	healthy, 25–41 years (n = 3) patients without PA, 40–65 years (n = 2) PA, 57–85 years (n = 11)	0.5–1.0	oral or i.v.	*0.14*	*0.039*	27
Adams and Boddy [22] (1968)	6 (100)	healthy, n.a. (n = 6)	0.1	i.v.	*0.17*	*0.017*	10
Heyssel et al. [11] (1966)	14 (n.a.)	healthy, n.a. (n = 3) PA, n.a. (n = 11)	?	i.m.	0.13	*0.02*	15
Reizenstein et al. [24] (1966)	2 (n.a.)	healthy, n.a. (n = 2)	0.5	i.v.	*0.04*	0.03[c]	n.a.

i.v. = Intravenous; i.m. = intramuscular; n.a. = not available. Values in italics are based on reported estimates or individual subject data.
[a] In the studies of Bozian et al. [16] and Adams and Boddy [22], the radioactivity of a dose of labeled vitamin B12 was 0.5 μCi. [b] Expressed as a percentage of the radioactivity measured after redistribution of the labeled vitamin B12 through different body compartments. [c] Pooled SD of other studies included in the pooled analysis.

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Ann Nutr Metab 2013;62:311–322
DOI: 10.1159/000346968

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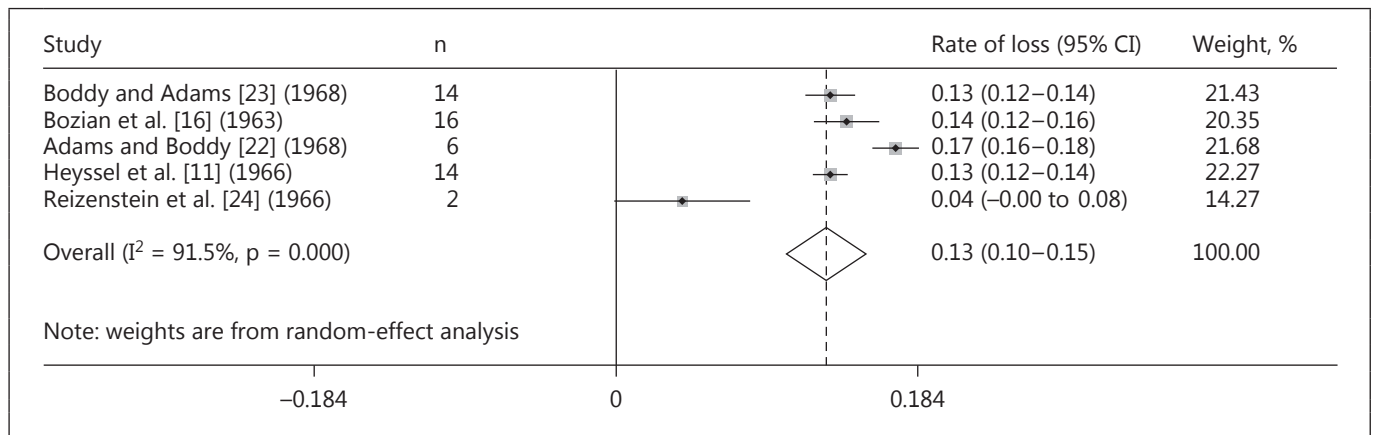
Fig. 2. Pooled analysis of the daily rate of loss of vitamin B12 as a percentage of total body stores.

concentrations were reported to be normal in all but one study that included subjects with low serum concentrations [23], but only five studies [8–10, 27, 28, 36] actually reported concentrations (137–546 pmol/l).

The searches on vitamin B12 body stores yielded 176 hits, of which 4 could be included based on our inclusion criteria and availability of abstracts or full texts.

Vitamin B12 Losses Measured with WBC

Table 1 presents estimates of daily vitamin B12 losses measured with WBC expressed as the percentage of total body stores lost per day. Estimates were based on measurements in healthy subjects [11, 16, 22–24], subjects with low serum concentrations of vitamin B12 [23] and PA patients [11, 16]. Absolute amounts of vitamin B12 lost per day varied in these subpopulations, but the rate of loss was a fixed percentage of the total body store of vitamin B12, regardless of the store size: the larger the body store, the larger the losses [16, 24]. Across studies, the rate of loss varied from 0.04 (SD not available) to 0.17% per day (SD = 0.017). In 1966, Reizenstein et al. [24] observed the lowest rate (0.04%), which was based on observations in only 2 individuals. This result deviated from the other four studies that reported a rate of loss in the range of 0.13–0.17% per day (SD_{pooled} = 0.03%). A meta-analysis of all five studies showed a summary estimate for the rate of loss of 0.13% per day with high heterogeneity between studies (I^2 = 91.5%, p < 0.0001; fig. 2). When the estimate of the rate of loss from the study by Reizenstein et al. [24] was omitted from the meta-analysis, the summary estimate only slightly changed to 0.14% per day (95% confidence interval: 0.12–0.16), and the observed heterogeneity between studies remained large (I^2 = 87.6%, p < 0.0001).

Vitamin B12 Excretion in Bile

Studies reporting on vitamin B12 excretion in bile included healthy subjects or patients with normal vitamin B12 metabolism subjected to duodenal lavage [25, 26], patients with postoperative drain in common bile duct [25, 26, 30, 37], patients undergoing surgery for lithiasis of the bile duct [18], patients undergoing cholecystectomy [30] or autopsy results [30]. Results of the studies included are shown in table 2. Two studies [25, 26] reported on the rate of excretion, indicating similar amounts of vitamin B12 excreted in bile per day as a percentage of total body stores (rate of loss 1.1 and 1.5%, respectively). One study measured the absolute vitamin B12 excretion in bile and the concentrations of the vitamin in bile [18], and two studies only measured concentrations of vitamin B12 in bile. Halsted et al. [37] observed no excretion of vitamin B12 in bile in 1 subject, whereas the two other studies showed mean vitamin B12 concentrations in bile of 3,248 [30] and 2,955 pg/ml [18].

Total Vitamin B12 Body Store

Estimates of the total body store provided by the studies included ranged from 780 to 11,100 μg, with average values between 1,060 (n = 4) [33] and 3,900 μg (n = 4–22) [25] (table 3). For one study [33], the health status of the subjects was not clear and the average total body store was lower compared to the other studies (1,060 vs. 2,528–3,900 μg).

Table 2. Daily vitamin B12 excretion in bile

First author	n	Population characteristics health status, age, n	Exposure		Outcome			
			dose of vitamin B12 labeled with 57/58/60 Co, µg[a]	administration route	outcome measure	result		
						mean	SD	CV
Reizenstein [26] (1959)	14	healthy, 20–34 years, n.a. patients with irrelevant diseases[b], 16–69 years, n.a. patients with postoperative drain in common bile duct, n.a.	0.3–0.6	i.m.	rate of loss, %/day[c]	1.5	*1.2*	77
Grasbeck [25] (1958)	7	healthy, n.a. (n = 2) patients with postoperative T-tube drain common bile duct, n.a. (n = 5)	0.5	i.m.	rate of loss, %/day[c]	1.1	*0.8*	72
el Kolthy [18] (1991)	8	patients undergoing surgery for lithiasis of the bile duct, 62–70 years (n = 8)	?	oral	absolute loss, µg/day concentration in bile, pg/ml	1.4 2,955	0.60 1,003	42 34
Ardeman [30] (1965)	6	drain in bile duct, n.a. (n = 4) cholecystectomy, n.a. (n = 1) autopsy, n.a. (n = 1)	none	–	concentration in bile, pg/ml	*3,248*	*4,314*	133
Halsted [37] (1956)	1	T-tube in common bile duct, n.a. (n = 1)	2.0	oral	concentration in bile, pg/ml	0	–	–

i.m. = Intramuscular; n.a. = not available. Values in italics are based on reported estimates or individual subject data.
[a] The radioactivity of a dose of labeled vitamin B12 ranged from 0.1 to 1.2 µCi. [b] Diagnosis were asthenia and achlorhydria, allergic bronchial asthma, diabetes mellitus and acute or subacute nephritis. All patients showed normal serum vitamin B12 concentrations and normal vitamin B12 absorption tests.
[c] Expressed as percent of total body stores.

Table 3. Total body stores of vitamin B12

First author	Study population		Outcome				
	n	health status	outcome measure	average stores, µg	range of stores, µg	SD	CV %
Grasbeck [25] (1958)	4–22	autopsies	sum of vitamin B12 content in liver, kidneys, heart, spleen, brain, muscles	3,900	790–11,100	2,578	66
Reizenstein [24] (1966)	n.a.	healthy controls	whole body content	3,030	n.a.	n.a.	n.a.
Adams [32] (1970)[a]	18	patients for surgery	whole body content	2,528	960–5,984	1,256	50
Bessent [33] (1980)[a]	4	n.a. control/treated PA patients	whole body content	1,060	780–1,350	143	13

n.a. = Not available. [a] Based on abstract only.

Bioavailability of Vitamin B12

We did not identify any study that assessed bioavailability of vitamin B12 from specific diets. All studies rather addressed absorption of vitamin B12 from a specific food product alone or in combination with a meal free of vitamin B12. Study populations included healthy subjects and subjects with a disease not affecting vitamin B12 absorption. Table 4 shows quantitative estimates of absorption obtained by the fecal excretion method [38] or WBC based on observations in 2–13 subjects per study [11]. Overall, absorption ranged from 4.5% at a dose of 38 µg from liver to 83% at a dose of 3.03 µg from mutton meat [11]. CVs for absorption estimates within studies ranged from 11 to 84%, whereas SDs were more similar ranging from 1.7 [11] to 22% [29], with most estimates in the range of 5.2–14%.

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Table 4. Bioavailability of vitamin B12 from different food products

First author	Population characteristics		Exposure		Outcome			
	n	health status, age	food product	dose of vitamin B12 labeled with 57/58/60 Co, µg[a]	% absorbed			mean amount absorbed, µg
					mean	SD	CV	
Fecal excretion method								
Doscherholmen [28] (1973)	11	healthy, 23–55 years	scrambled whole egg	0.56	28	–	–	*0.15*
Doscherholmen [9] (1975)	5	healthy	scrambled egg yolk	0.5	36	–	–	*0.18*
	6	healthy	scrambled whole egg	0.56	28			*0.15*
	4	healthy	boiled egg	0.51–0.94	24			*0.12–0.23*
	4	healthy	fried egg	0.51–0.94	24			*0.12–0.23*
Doscherholmen [27] (1976)	13	healthy	scrambled egg white	0.30–0.62	36	–	–	*0.11–0.22*
Doscherholmen [16] (1978)[c]	3	healthy	cooked chicken meat (100, 200 or 300 g)	0.42–0.64	*65*	*8.4*	*13*	*0.35*
	3			0.84–1.28	*63*	*13.9*	*22*	*0.68*
	3			1.26–1.92	*61*	*13.1*	*22*	*1.0*
Doscherholmen [8] (1981)[c]	2	healthy	cooked rainbow trout (50, 100, 200 or 300 g)	1.95–2.18	*42*	*5.9*	*14*	*0.87*
	3			3.90	*38*	*8.1*	*22*	*1.5*
	3			7.80–10.90	*41*	*7.5*	*18*	*3.9*
	3			11.7–15.6	*30*	*5.2*	*17*	*4.0*
Reizenstein [31] (1959)	11	healthy or irrelevant diseases[b], 17–38 years	raw pig/calf liver	22	30	*14*	47	6.0
Kittang [26] (1985)[c]	6	healthy, mean 31 years	boiled/fried rabbit liver	0.50	49	22	45	*0.24*
	6			1.0	34	10	29	*0.34*
	6			1.5	26	8.4	32	*0.39*
	6			3.0	10	8.4	84	*0.31*
Whole body counting								
Heyssel [11] (1966)	3	healthy young	mutton lean meat	0.95	65	*11*	17	*0.62*
	2	healthy young	mutton lean meat	3.03	83	*9.2*	11	*2.5*
	2	healthy young	mutton lean meat	5.11	52	*16*	32	*2.7*
	6	healthy young	mutton liver pâté	38	11	*6.0*	55	*4.1*
	4	healthy old	mutton liver pâté	38	4.5	*1.7*	38	*1.7*

Values in italics are based on reported estimates or individual subject data.
[a] In the studies of Doscherholmen (1973, 1975, 1981), radioactivity of a dose of labeled vitamin B12 ranged from 0.05 to 1.02 µCi. [b] Irrelevant diseases included hypoplastic anemia and mediastinal tuberculosis. [c] Repeated measures in same subjects.

For specific food groups and doses, absorption ranged from 24 to 36% for egg products (dose 0.3–0.94 µg), from 52 to 83% for lean meat (dose 0.54–5.11 µg), from 30 to 42% for fish (dose 2.1–13.1 µg) and from 4.5 to 49% for liver products (dose 0.5–38 µg). Four studies measured absorption from a food product with varying vitamin B12 contents. In two of four studies, percent absorption decreased with increasing vitamin B12 content [10, 29], whereas the other two did not show such a clear trend [8, 11].

The percentages of absorption mentioned above suggest very heterogeneous absorption of vitamin B12 from different sources. However, when expressed as dose-response relationship, it appeared that the dose taken, rather than the food source, as such influences the amount absorbed. Overall, the amount of vitamin B12 absorbed (A_i) increased with increasing vitamin B12 doses (D_i), and the relationship was estimated as $\ln(A_i) = 0.7694 * \ln(D_i) - 0.9614$, as illustrated in figure 3. Since we applied a natural logarithmic transformation on both the vitamin B12 dose and the amount of vitamin B12 absorbed, the regression coefficient of 0.7694 means that for every doubling in vitamin B12 dose, the difference in absorbed amount is $2^{0.7694}$ (= 1.70), which is 70%.

Fig. 3. Absorbed amounts of vitamin B12 for different doses and food groups. Both the dose and the absorbed amount were transformed to their natural logarithm in order to achieve linearity of the regression. Values of 0, 1, 2, 3 and 4 on the x-axis correspond to labeled vitamin B12 doses of 1.0, 2.7, 7.4, 20.1 and 54.6 µg, respectively. Values of –2, –1, 0, 1 and 2 on the y-axis correspond to absorbed vitamin B12 of 0.1, 0.4, 1.0, 2.7 and 7.4 µg, respectively.
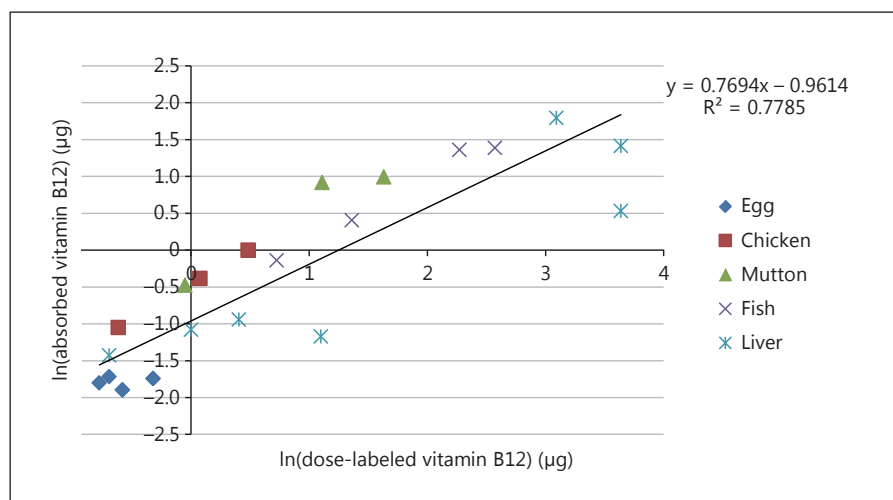
Discussion

Main Findings

In Europe, ANRs for vitamin B12 established with the factorial approach are currently based on the study selection, as summarized in this review. This systematic review indicates that the total amount of vitamin B12 lost per day is 0.13% (CV = 23%) of the total body store, with mean values between 1.1 and 3.9 mg. Observations of the rate of vitamin B12 excretion in bile were similar (1–1.5% of the total body store per day, CV = 72–77%), whereas the absolute amount of vitamin B12 excreted in bile per day and vitamin B12 concentrations in bile varied largely between and within studies (CV within studies = 34–133%). Estimates of vitamin B12 absorption varied between 4.5 and 83% (CV 11–84%) based on studies with varying vitamin B12 contents and different types of food products. The relationship between the amount absorbed (A_i) and the dose of vitamin B12 ingested (D_i) was estimated as $\ln(A_i) = 0.7694 * \ln(D_i) – 0.9614$.

Implications of Our Findings for Estimates of Vitamin B12 Requirements

Interindividual Variation in Requirements

Most studies reporting on daily vitamin B12 losses or absorption are old, include a small number of subjects and details on the study subjects are very limited. Moreover, we observed a large variation between and within studies, especially among studies reporting on vitamin B12 excretion in bile. Estimating vitamin B12 requirements with the factorial approach, as in the Netherlands, requires assumptions regarding the rate of loss, the total body store and bioavailability (box 1). Each assumption is a component of uncertainty and following the simple error-propagation formula for independent measurements, for any given value of the body store, the CV of the ANR for vitamin B12 can be estimated as: $\sqrt{(CV^2_{\text{rate of loss}} + CV^2_{\text{bioavailability}})}$. Based on the observations of within-study variation for the rate of loss (CV = 23%, based on the pooled SD) and bioavailability (CV = 8–81%), this would imply a CV for the ANR of at least 24%. These calculations suggest that the CV exceeds the 20% currently assumed for estimating recommended intakes of vitamin B12 in the Netherlands. However, it must be noted that the CVs used in these calculations are based on studies with few subjects, which may result in an overestimation of the interindividual variation. Moreover, there is some evidence that lower body stores of vitamin B12 go together with higher absorption [26], as for many other nutrients. If bioavailability of vitamin B12 depends on the total body store, the CV of vitamin B12 requirements may be less than predicted with the error-propagation formula.

The French recommended intake of vitamin B12 is derived as the ANR multiplied by 1.2 (2 times a CV of 10%). However, this review showed that measures of vitamin B12 excretion in bile vary largely between and within studies (CV: 34–133%). So, considering the additional assumptions required for estimating vitamin B12 requirements based on daily losses in bile (% reabsorption from the intestine, % bioavailability), it seems likely that a CV of 10% underestimates the interindividual variation and uncertainty in requirements.

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Table 5. Summary of vitamin B12 requirements estimated with the factorial approach based on different assumptions

Approach	Studies n	Measure	Assumption	Total losses μg	Requirements μg[a]
Daily losses (based on WBC)	5	rate of loss 0.13%	body stores: 1.1–3.9 mg	1.4–5.1	8.7–20.7
Daily losses (based on excretion in bile)	2	rate of loss 1.1–1.5%	body stores: 1.1–3.9 mg 67% reabsorption	3.8–19.3	14.5–117.7
	1	bile concentrations 3,248 pg/ml	daily output of bile: 493 ml 67% reabsorption	0.5	1.1
	1	absolute amounts in bile 1.4 μg	67% reabsorption	0.5	0.9

[a] ln(the amount absorbed) = 0.7694 * ln(intake) − 0.9614, assuming that total losses should be compensated by three meals a day containing equal amounts of vitamin B12.

Total Vitamin B12 Losses Measured with WBC

Current recommendations based on the factorial approach include the assumption that compensation of daily losses will be sufficient to maintain the vitamin B12 body store and thereby prevent deficiency disorders. Estimates of the body store (500 μg) were based on observations in patients suffering from malabsorption that remained both hematologically normal (no megaloblastic changes of the bone marrow, no anemia and no macrocytosis) and serum vitamin B12 concentrations at 96–148 pmol/l. The selection of the hematological status as a criterion for adequacy may be debatable for several reasons. Although the biological role of vitamin B12 in blood cell formation is well defined [39–41], it has been estimated that 19–28% of patients with PA do not have anemia, and 17–33% have a normal mean cell volume [42, 43]. Secondly, potentially irreversible neurologic disorders due to vitamin B12 deficiency may occur in the absence of anemia or an elevated mean cell volume [44–46]. It is even suggested that the occurrence of neurological complications is inversely correlated with the degree of anemia; patients who are less anemic show more prominent neurological complications and vice versa [47, 48]. Moreover, recommendations on micronutrient intake are designed for apparently healthy populations, and therefore it may be more appropriate to use estimates of body stores based on apparently healthy subjects for estimating daily losses. Our review on total body stores of vitamin B12 showed that in adults mean total body stores are between 1.1 and 3.9 mg. This is in line with reported mean values on vitamin B12 liver content (0.7–1.0 μg/g liver) [49–53] and liver weights [men: 1,677 g (range: 670–2,900), women: 1,475 g (range 508–3,081) [54]] assuming that half of vitamin B12 in the human body is stored in the liver [39]. Based on these stores and a rate of loss of 0.13% of the store per day, total losses would range from 1.4 to 5.1 μg per day under the assumption that the size of the total body store of vitamin B12 is the major determinant of the amount of vitamin B12 lost per day (table 5).

Vitamin B12 Excretion in Bile

The absolute amount excreted in bile per day was 1.4 μg in one study. Based on the reported mean rate of loss of 1.1 or 1.5% of the total body store and assuming that body stores range from 1.1 to 3.9 mg, as also referred to above, daily vitamin B12 excretion in bile may range from 12 to 59 μg. Daily biliary excretion of vitamin B12 derived from the reported concentration of the vitamin in bile was 1.6 μg per day, assuming a daily output of bile of 493 ml [18]. So, using the French assumption that 40% of vitamin B12 excreted in bile is reabsorbed in the small intestine (box 1), total losses of vitamin B12 range from 0.8 to 35 μg per day. It must be noted, however, that in two studies included in this review, excretion of vitamin B12 was measured both in bile and in feces, and results showed that losses in feces were about one third (28–33%) of the amount of vitamin B12 excreted in bile [25, 26]. This would imply that more than two thirds of the vitamin B12 excreted in bile are reabsorbed in the small intestine and that daily losses range from 0.5 to 19 μg per day (table 5).

Bioavailability of Vitamin B12

Absorption of vitamin B12 from food is influenced by the type of food source and its vitamin B12 content, as

shown in this review and in other publications [4, 7]. Studies included in our review measured amounts absorbed up to 6 μg from a single meal. This is not consistent with previous data showing that the average maximum amount of vitamin B12 that can be absorbed from amounts usually consumed with a meal is about 1.5 μg due to saturation of the vitamin B12 intrinsic factor receptors, which are key factors in the absorption of vitamin B12 from the ileum [39]. Although passive diffusion of 1% of the ingested dose also takes place, this only becomes relevant when high doses are ingested, e.g. via supplements.

In general, absorption of 50% from the diet was assumed for estimating vitamin B12 requirements. Although two studies observed vitamin B12 absorption >50% (chicken or mutton meat), absorption from other food products was <50% at doses of 0.5–5 mg. In addition, usual vitamin B12 intake among adults and elderly people across Europe ranges from 3.5 to 9.3 μg per day [55]. Assuming that each day three meals contribute to vitamin B12 intake, and that these three meals contain equal amounts of vitamin B12 (1.2–3.1 μg per meal), the regression equation derived in this review [ln(A_i) = 0.7694 * ln(D_i) – 0.9614] could be used to predict the bioavailability as follows:

ln(bioavailability) =
ln(A/D) = ln(A) – ln(D) = (0.7694 – 1) * ln(D) – 0.9614

and bioavailability =
$(1.2–3.1 \ \mu g)^{(0.7694 - 1)} * (e^{-0.9614})$, which amounts to 29–37%.

This suggests that the generally assumed 50% overestimates real absorption, potentially leading to an underestimation of the requirements. Moreover, as it is suggested that there is a maximum amount of vitamin B12 that can be absorbed per meal, it does not seem right to report on the percent absorption of a specific food without referring to the actual dose ingested [4].

Unfortunately, the regression equation was based on absorption data from a limited number of food products. For example, no estimates of vitamin B12 absorption from foods such as dairy products were included that are widely consumed in Europe. Results from some observational studies suggest that absorption from dairy products, especially milk, may be higher than that from other dietary sources [56, 57]; however, so far, these results are not verified by experimental studies.

Vitamin B12 Requirements

Based on the estimates of daily vitamin B12 losses and the regression equation derived in this review, the dietary vitamin B12 intake needed to compensate daily losses would range from 3.8 to 20.7 μg per day based on WBC measurements or from 0.9 to 118 μg per day based on excretion in bile (table 5) assuming that three meals a day equally contribute to vitamin B12 intake. These intake ranges indicate the daily intake necessary to maintain the body store in apparently healthy adults and elderly people rather than the intake level at which requirements of 50% of individuals in a population are fulfilled since it is unknown what stores reflect which state of deficiency or sufficiency [13]. Therefore, the intake ranges reflect the recommended vitamin B12 intake rather than the ANR.

As vitamin B12 requirements based on the excretion in bile vary largely depending on the total body stores assumed, these estimates seem less adequate for deriving recommended vitamin B12 intakes. Although vitamin B12 requirements based on total losses measured with WBC vary with a factor of five depending on the assumed total body stores, these estimates are in line with findings from five studies that showed that plasma concentrations of markers for vitamin B12 status (total cobalamin, methylmalonic acid and holotranscobalamin II) leveled off at daily intakes between 4 and 10 μg per day [56–60]. Daily intakes between 4 and 10 μg per day will therefore likely be adequate for the prevention of subclinical deficiencies in vitamin B12 that may be associated with potential health risks, including neurologic and cognitive risks [61]. However, more evidence is needed on the relationship between plasma concentrations of markers for vitamin B12 status, vitamin B12 body stores and long-term health outcomes to define whether it is beneficial to maintain maximal vitamin B12 status and stores of 1.1–3.9 mg.

It must be noted that all studies included in our review were based on subjects with normal absorption. At the age of ≥60 years, the prevalence of food-bound malabsorption increases, which is a primary cause for vitamin B12 deficiency affecting about 20% of the elderly in industrialized countries [62]. Food-bound malabsorption is characterized by the inability to release vitamin B12 from food or from intestinal transport proteins. Therefore, this vitamin B12 cannot or only partly bind to the intrinsic factor, and so absorption of vitamin B12 does not take place or only to a lesser extent. Absorption of crystalline vitamin B12 is usually not affected in subjects with food-bound malabsorption, and therefore most European countries recommend elderly people to consume a high proportion of their vitamin B12 intake as fortified foods and supplements [1]. In two studies investigating absorption from bread, squash or milk fortified with 0.25–0.5 μg vitamin B12, an absorption between 55 and 74% was observed [12, 36].

Conclusions

This review showed that daily vitamin B12 losses in apparently healthy adults and elderly probably range from 1.4 to 5.1 µg. Based on the relationship between the ingested dose and the amount absorbed, vitamin B12 intakes needed to compensate for these daily losses seem to range from 3.8 to 20.7 µg in apparently healthy adults and elderly people, which is 1.4–8.6 times higher than the amount needed to prevent deficiency. Current recommendations on vitamin B12 intake of 1.4–3.0 µg may be inadequate to maintain body stores of 1.1–3.9 mg and optimal plasma concentrations of markers of vitamin B12 status. However, evidence on daily vitamin B12 losses and bioavailability could only be derived from relatively old studies published between 1958 and 1991 and can hardly be updated because this requires invasive methods or the use of isotopes that do not comply with current ethical standards. In view of this, establishing recommended vitamin B12 intakes with the factorial approach can be based on evidence on the rate of loss (a relatively accurate and precise estimate) and bioavailability (large variability and uncertainty), together with an estimate of the re-quired total body stores (crude estimate). However, the available evidence is rather scarce and resulting estimates have considerable uncertainty. The commonly used CV of 10–20% seems too low to cover uncertainty in all these underlying assumptions and may be at least 24%. More evidence is needed on the relationship between plasma concentrations of markers for the vitamin B12 status, vitamin B12 body stores and long-term health outcomes to evaluate whether the currently recommended intake of vitamin B12 intake need to be changed.

Acknowledgments

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