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Review Article

Association between vitamin B_{12} intake and EURRECA's prioritized biomarkers of vitamin B_{12} in young populations: a systematic review

Iris Iglesia[1,*], Rosalie AM Dhonukshe-Rutten[2], Silvia Bel-Serrat[1], Esmée L Doets[2], Adrienne EJM Cavelaars[2], Pieter van 't Veer[2], Mariela Nissenshohn[3], Vassiliki Benetou[4], María Hermoso[5], Cristiana Berti[6], Lisette CPGM de Groot[2] and Luis A Moreno[1]

[1]GENUD (Growth, Exercise, Nutrition and Development) Research Group, Health Sciences Faculty, Cervantes Building, C/ Corona de Aragón, n° 42, 2nd floor, 50009 University of Zaragoza, Zaragoza, Spain: [2]Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands: [3]Department of Clinical Sciences, Las Palmas de Gran Canaria University, Las Palmas de Gran Canaria, Spain: [4]Department of Hygiene, Epidemiology and Medical Statistics, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece: [5]Division of Metabolic and Nutritional Medicine, Dr. von Hauner Children's Hospital, Ludwig Maximilians University of Munich Medical Centre, Munich, Germany: [6]Department of Clinical Sciences Hospital 'L Sacco' and Center for Fetal Research Giorgio Pardi, Unit of Obstetrics and Gynecology, University of Milan, Milan, Italy

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Abstract

Objective: To review evidence on the associations between vitamin B_{12} intake and its biomarkers, vitamin B_{12} intake and its functional health outcomes, and vitamin B_{12} biomarkers and functional health outcomes.

Design: A systematic review was conducted by searching electronic databases, until January 2012, using a standardized strategy developed in the EURRECA network. Relevant articles were screened and sorted based on title and abstract, then based on full text, and finally included if they met inclusion criteria. A total of sixteen articles were included in the review.

Setting: Articles covered four continents: America (*n* 4), Europe (*n* 8), Africa (*n* 1) and Asia (*n* 3).

Subjects: Population groups included healthy infants, children and adolescents, and pregnant and lactating women.

Results: From the total number of 5815 papers retrieved from the initial search, only sixteen were eligible according to the inclusion criteria: five for infants, five for children and adolescents, and six for pregnant and lactating women.

Conclusions: Only one main conclusion could be extracted from this scarce number of references: a positive association between vitamin B_{12} intake and serum vitamin B_{12} in the infant group. Other associations were not reported in the eligible papers or the results were not provided in a consistent manner. The low number of papers that could be included in our systematic review is probably due to the attention that is currently given to research on vitamin B_{12} in elderly people. Our observations in the current systematic review justify the idea of performing well-designed studies on vitamin B_{12} in young populations.

Keywords
Vitamin B_{12}
Intakes
Biomarkers
Young populations

Nutrition plays an important role in the programming of health across the lifespan, especially during the earliest periods, because of short- and long-term consequences in the absence of appropriate nutrition[1]. There are biological substances which keep homeostasis to prevent adverse health outcomes like vitamin B_{12}. In recent years, only a few studies have focused on the relationship between low vitamin B_{12} intake and cognitive function, megaloblastic anaemia or growth in young populations.

Across Europe, current reference values for vitamin B_{12} intake vary for infants from 0·3–0·5 to 1·5 μg/d depending on whether they are 3 or 9 months old, respectively[2],

*Corresponding author: Email iglesia@unizar.es

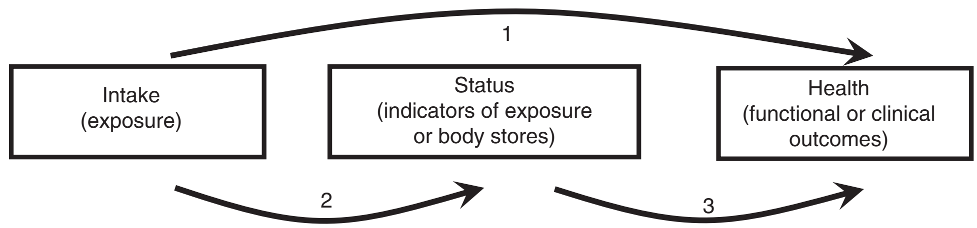

Fig. 1 Intake–status–health relationships relevant for deriving reference values: 1 = intake–health relationship; 2 = intake–status relationship; 3 = status–health relationship

from 0·8 to 3·0 μg/d for children and adolescents[3] and from 1·5 to 4·0 μg/d for pregnant and lactating women[4,5]. The range of ages, values and terminology used for recommendations differ between European countries. However, the underlying concepts could be equivalent to: the RDA (Recommended Dietary Allowance, which is the daily dietary intake level of a nutrient considered sufficient to meet the requirements of nearly all (97–98 %) healthy individuals in each life stage and gender group), the AI (Adequate Intake, which is an estimation of the lowest intake level that seems sufficient for almost all people in a group) and the acceptable range (which is defined as the range of intakes high enough to avoid deficiency and low enough to avoid toxic effects). For vulnerable population groups such as those represented herein, nutrient requirements are generally obtained from data extrapolated from the adult ANR (Average Nutrient Requirement, which is the estimated average or median requirement of a specific nutrient in a population)[6].

In Western countries, the dietary intake of vitamin B_{12} among children, adolescents and adults is usually higher than the average requirement for vitamin B_{12}. For instance, the Spanish study EnKid showed that the 2–24-year-old population had a mean daily vitamin B_{12} intake of 8·2 μg (males) and 6·8 μg (females)[7]. However, data from the Framingham Offspring Study suggest that suboptimal vitamin B_{12} status occurs at intakes exceeding the recommended intakes[8] and raise the question of whether the current recommended intakes for vitamin B_{12} are adequate to promote a normal vitamin B_{12} status[9] and influence the occurrence of several health outcomes[8,10–12].

The preferred approach to define the requirement takes into account the level of intake at which functioning is optimal. This implies that both preventing deficiencies as well as reducing the risk of developing other chronic disorders have to be taken into account[13,14].

In order to provide up-to-date and evidence-based micronutrient reference values across Europe, it is important to assess the micronutrient status for different population groups[15] through its preferred biomarkers or functional health outcomes[16]. The use of a biomarker that reflects changes in micronutrient status can facilitate the understanding of the relationships between dietary micronutrient intake and status or health outcomes (Fig. 1). The best tools to provide this information are dose–response and repletion–depletion studies, but they are rarely carried out.

The aim of the present paper is to systematically review dose–response evidence from randomized controlled trials (RCT), prospective cohort and cross-sectional studies on the association of vitamin B_{12} with its main biomarkers, and also with its main health outcomes in infants, children, adolescents and pregnant and lactating women. The ultimate goal would be to provide micronutrient reference intake values for vitamin B_{12} in the aforementioned population groups.

Methods

The current systematic review on vitamin B_{12} in young populations and pregnant and lactating women was performed within the framework of EURRECA (www.eurreca.org) and has focused on one of the prioritized relationships set by the network[17] as illustrated in Fig. 1.

Search methods for identification of studies

To find the search strategy terms and the criteria for exclusion/inclusion papers, data on vitamin B_{12}[17] were first reviewed. A multiple-database searching in MEDLINE, Embase (both on Ovid) and the Cochrane Library CENTRAL was carried out until 17 February 2009. The general search strategy included terms on study designs in humans AND (intake or status) AND (vitamin B_{12}). The search terms included both MeSH terms and words to be found in the title or abstract. The initial search yielded 5815 references after exclusion of duplicates. Reference lists of six relevant review articles[18–23] were checked also to identify potentially relevant references that were not yet collected. This search did not yield any other references.

In January 2012 the search was repeated to retrieve other possible relevant papers. This search retrieved 596 new papers.

Criteria for the consideration of studies

Studies had to fulfill the following criteria to be included in the review:

1. Investigate the possible relationships between vitamin B_{12} intake, its biomarker levels or the selected health outcomes, following the structure available in Fig. 1;

2. Provide vitamin B_{12} from supplements, fortified foods or natural dietary sources;
3. Be observational studies (prospective cohort, nested case–control or cross-sectional, the latter for intake–status associations only) or intervention studies (only RCT);
4. Be performed in human subjects from birth to 18 years or pregnant or lactating women;
5. Include apparently healthy subjects.

Results on adults and the elderly in studying these relationships are reported elsewhere.

Accepted dietary assessment methods to include the paper were: (i) validated FFQ/dietary history; and (ii) 24 h recall/food records/diary measures for at least 2 d.

Serum/plasma vitamin B_{12}, methylmalonic acid (MMA) and holotranscobalamin (HoloTC)[24] were the biomarkers included as the most robust and sensitive biomarkers identified through earlier research activities in the EURRECA network[25,26].

The health outcomes chosen were those most relevant for the population group (based on public health reports and the scientific literature, i.e. current evidence of a relationship and the number of preliminary search hits from online databases) and not recently and thoroughly covered by a similar review. Health outcomes differed between population groups:

1. Neurodevelopment and megaloblastic anaemia for infants;
2. Megaloblastic anaemia, growth and cognitive function for children and adolescents;
3. Fetal malformations and fetal growth for fetuses;
4. Megaloblastic anaemia and pre-eclampsia for mothers.

Collection of papers

The results of the searches were combined in EndNote XII (Thompson Reuters). References were screened based on title and abstract. They were then sorted by population group: (i) infants, (ii) children and adolescents and (iii) pregnant and lactating women; and by relationship following the analytical model: (i) intake–health (I-H), (ii) intake–status (I-S), (iii) status–health (S-H) and (iv) intake–status–health (I-S-H).

Selection of studies

Once papers were screened based on title and abstract and sorted by population group, those selected were again screened based on full text by obtaining them electronically, as photocopies or reprints, according to the predefined criteria. The reasons for exclusion and the name of the reviewer were registered in the EndNote library. One hundred and seventeen potentially relevant references were considered for inclusion based on full text review; characteristics of the 101 references excluded are shown in Table 1. Figure 2 shows the flowchart of the selection steps for the populations reviewed herein. If language expertise existed in the review team, articles written in languages other than English could be included.

Data extraction

Data from papers identified as relevant were extracted to characterize studies and to facilitate meta-analysis. Data were entered into an Access database specifically developed for EURRECA.

Quality check controls

For alignment and quality control, at the start of each step two independent reviewers screened 10 % of the references in duplicate. Any discrepancies at this step were discussed before proceeding with the rest of the references.

Assessment of risk of bias in included studies

To exclude major sources of bias, internal validity of the relevant studies was assessed. The criteria used were adapted from the Cochrane Handbook[27]. The criteria for RCT were based on: method of sequence generation and allocation; blinding; potential funding bias; number of participants at start; drop-outs and reasons for dropping out; dose check; dietary intake data reported; and similarity of most and least exposed groups at baseline. For longitudinal studies the criteria were based on: drop-outs adequate and outcome data complete; funding; lack of other potential threats to validity; control for confounders; and assessment of exposure adequacy. For cross-sectional studies the criteria were based on: funding; lack of other potential threats to validity, such as those related to the specific study design used or related to differences in baseline characteristics of participants; confounders; and assessment of exposure adequacy.

Results

The systematic search retrieved sixteen relevant papers. Table 2 summarizes the characteristics and results of these studies.

Infants

Two out of five selected papers were RCT[28,29] and three were observational studies (one cross-sectional[30] and two longitudinal studies[31,32]). In all these studies the association between intake and status (I-S) was reported, except for one longitudinal study[32]. In both RCT, the intervention groups[28,29] received vitamin B_{12} through intramuscular injection: once per month during the first 4 months (100 μg/month) in one study[29] and in the other[28] the injected amount was only once (400 μg). In the RCT from Worthington-White *et al.*[29], serum levels were significantly increased after the intervention (either with or without folate supplementation) at each point of the measurements. In that study, the dose–response association between injected vitamin B_{12} and levels of biomarkers was not estimated.

Table 1 Characteristics of excluded studies

Reference	Main reason for exclusion
Monsen *et al.* (2003)[58]	Only data on biomarkers
Monsen *et al.* (2006)[59]	Does not address any relationships of interest
Casanueva *et al.* (2006)[60]	Type of intervention: multivitamin supplement
Choudhry *et al.* (1972)[61]	Study design: intervention but not RCT
Cikot *et al.* (2001)[62]	Only data on biomarkers
Couto *et al.* (2007)[63]	Only data on biomarkers
Cornel *et al.* (2005)[64]	Irrelevant micronutrient
Czeizel and Dudas (1992)[65]	Type of intervention: multi-vitamin supplement
Czeizel and Medveczky (2003)[66]	Does not address any relationships of interest
Dagnelie *et al.* (1989)[67]	Does not address any relationships of interest
Dawson *et al.* (2000)[68]	Study design: intervention but not RCT
van Dusseldorp *et al.* (1999)[69]	Study design: case–control study
Eilander *et al.* (2010)[70]	Study design: cross-sectional study investigating S-H relationship
Gomber *et al.* (2003)[71]	Study design: cross-sectional study investigating S-H relationship
Gomber *et al.* (1998)[72]	Study design: cross-sectional study investigating S-H relationship
Gordon and Carson (1976)[73]	Study design: case report
Graham *et al.* (1992)[74]	Population group: infants did not meet the inclusion criteria (unhealthy)
Haggarty *et al.* (2006)[75]	Irrelevant health outcome
Haiden *et al.* (2006)[76]	Type of intervention: multi-vitamin supplement
Haiden *et al.* (2006)[77]	Type of intervention: multi-vitamin supplement
Hay *et al.* (2010)[78]	Relationship assessed: S-S
Hininger *et al.* (2004)[79]	Type of intervention: multi-vitamin supplement
Hjelt and Krasilnikoff (1990)[80]	Population group: infants did not meet the inclusion criteria (unhealthy)
Huemer *et al.* (2005)[81]	Study design: case report
Järvenpää *et al.* (2007)[82]	Does not address any relationships of interest
Johnson *et al.* (2002)[83]	Does not address any relationships of interest
Knight *et al.* (1994)[84]	Only data on biomarkers
Kuschel and Harding (2004)[19]	Study design: systematic review
Levy *et al.* (1992)[85]	Type of intervention: multi-vitamin supplement
López de Romaña *et al.* (2005)[86]	Type of intervention: multi-vitamin supplement
Lovblad *et al.* (1997)[87]	Irrelevant health outcome
Lundgren and Blennow (1999)[88]	Study design: case report
Makedos *et al.* (2007)[89]	Study design: case–control
Mamlok *et al.* (1986)[90]	Study design: case report
Martin *et al.* (2004)[91]	Does not address any relationships of interest
Mathan *et al.* (1979)[92]	Does not address any relationships of interest
Mathews (1996)[20]	Does not address any relationships of interest
Maurage *et al.* (1995)[93]	Does not address any relationships of interest
Masalha *et al.* (2008)[94]	Study design: cross-sectional study investigating I-H relationship
McCoy *et al.* (1984)[95]	Only data on intakes
McGrath *et al.* (2006)[96]	Population group: mothers did not meet the inclusion criteria (unhealthy)
McNulty *et al.* (1996)[97]	Only data on intakes
Mena *et al.* (2001)[98]	Irrelevant health outcome
Merialdi *et al.* (2004)[99]	Does not address any relationships of interest
Metcalf *et al.* (1994)[100]	Does not address any relationships of interest
Metz *et al.* (1965)[101]	Study design: intervention but not RCT
Mills *et al.* (2005)[102]	Type of intervention: multi-vitamin supplement
Minet *et al.* (2000)[103]	Does not address any relationships of interest
Miyake *et al.* (2006)[104]	Irrelevant health outcome
Molloy *et al.* (1985)[105]	Irrelevant health outcome
Molloy *et al.* (2005)[106]	Study design: cross-sectional study investigating S-H relationship
Monagle and Tauro (1997)[107]	Study design: description of several cases
Moran (2007)[108]	Only data on biomarkers
Morkbak *et al.* (2007)[109]	Study design: editor letter
Msolla and Kinabo (1997)[110]	Irrelevant biomarkers
Murphy *et al.* (2007)[111]	Study design: S-S
Mwanda and Dave (1999)[112]	Study design: intervention but not RCT
Neiger *et al.* (1993)[113]	Population group: mothers did not meet the inclusion criteria (unhealthy)
Nelen *et al.* (2000)[114]	Does not address any relationships of interest
Neri *et al.* (2005)[115]	Irrelevant health outcome
Neuhouser *et al.* (1998)[116]	Does not address any relationships of interest
Neumann and Harrison (1994)[117]	Irrelevant health outcome
Niebyl and Goodwin (2002)[118]	Irrelevant health outcome
Nikolaus and Nikolaus (1979)[119]	Study design: intervention but not RCT
Osganian *et al.* (1999)[120]	Only data on biomarkers
Patel and Lovelady (1998)[121]	Study design: intervention but not RCT
Ratan *et al.* (2008)[122]	Does not address any relationships of interest
Ray and Laskin (1999)[23]	Does not address any relationships of interest

Table 1 *Continued*

Reference	Main reason for exclusion
Ray and Blom (2003)[53]	Irrelevant health outcome
Ronnenberg *et al.* (2000)[123]	Irrelevant population group
Ronnenberg *et al.* (2002)[124]	Irrelevant biomarkers
Ronnenberg *et al.* (2002)[125]	Study design: case–control
Ronnenberg *et al.* (2007)[126]	Does not address any relationships of interest
Rumbold *et al.* (2005)[127]	Type of intervention: multi-vitamin supplement
Sachdeva and Mann (1994)[128]	Does not address any relationships of interest
Scatliff *et al.* (2011)[129]	Population group: children did not meet the inclusion criteria (unhealthy)
Schneede *et al.* (1994)[130]	Only data on biomarkers
Shih *et al.* (1976)[131]	Study design: editor letter
Siekmann *et al.* (2003)[132]	The intervention was realized with meat or milk
Singla *et al.* (1982)[133]	Type of intervention: multi-vitamin supplement
Sivakumar *et al.* (2006)[134]	Only data on biomarkers
Smith Fawzi *et al.* (2007)[135]	Irrelevant health outcome
Sneed *et al.* (1981)[136]	Study design: intervention but not RCT
Sohrabvand *et al.* (2006)[137]	Irrelevant health outcome
Steegers-Theunissen *et al.* (1995)[138]	Does not address any relationships of interest
Steen *et al.* (1998)[139]	Irrelevant health outcome
Strand *et al.* (2007)[140]	Does not address any relationships of interest
Suarez *et al.* (2003)[141]	Irrelevant health outcome
Thomas *et al.* (2008)[142]	Does not address any relationships of interest
Thompson *et al.* (2009)[143]	Irrelevant health outcome
Thoradeniya *et al.* (2006)[144]	Only data on biomarkers
Thurlow *et al.* (2005)[145]	Study design: cross-sectional study investigating S-H relationship
Valman (1972)[146]	Study design: case report
Veena *et al.* (2010)[147]	Study design: maternal S and children's H at 10 years old
Verkleij-Hagoort *et al.* (2008)[148]	Study design: case–control
Villamor *et al.* (2008)[149]	Study design: data from I is referred to dietary patterns, not to proper amounts of micronutrient
Vinod Kumar and Rajagopalan (2008)[150]	Type of intervention: multi-vitamin supplement
Vujkovic *et al.* (2007)[151]	Study design: data from I is referred to dietary patterns, not to proper amounts of micronutrient
Vujkovic *et al.* (2009)[152]	Study design: data from I is referred to dietary patterns, not to proper amounts of micronutrient
Wald *et al.* (1996)[153]	Irrelevant health outcome
Wright (1995)[154]	Study design: case–control

RCT, randomized controlled trial; S, status; H, health; I, intake.

In the RCT from Bjorke-Monsen *et al.*[28], the intervention was the strongest predictor of changes for all blood indices (regression coefficient = 183 for serum vitamin B_{12} and regression coefficient = −0·70 for MMA). Four months after delivery, the median (range) of serum vitamin B_{12} was 421 (291–497) pmol/l and 240 (162–337) pmol/l for the intervention and placebo groups, respectively; corresponding values for MMA were 0·2 (0·15–0·43) pmol/l and 0·51 (0·23–1·55) pmol/l.

In the Guatemalan cross-sectional study[30], mean intake of vitamin B_{12} was 3·1 μg/d for mothers and 2·2 μg/d for infants at the age of 12 months and the accompanying mean (SD) plasma vitamin B_{12} concentration in mothers and infants was 114·4 (9·2) g/l and 262·2 (163·5) pmol/l, respectively. The plasma vitamin B_{12} concentrations of the infants were correlated with maternal concentrations and they were also positively associated with infant B_{12} intake from complementary foods ($r = 0·16$, $P < 0·0001$).

In the longitudinal study by Hay *et al.*[31], the results were divided between breast-fed (*n* 104) and non-breast-fed (*n* 115) infants: the mean intake of vitamin B_{12} was 1·4 (95 % CI 1·3, 1·6) μg/d for breast-fed infants excluding the intake from breast milk and 2·4 (95 % CI 2·1, 2·6) μg/d for the non-breast-fed infants. In that study, the selected biomarkers were measured at the age of 12 months. Mean (95 % CI) serum vitamin B_{12}, HoloTC and MMA were 343 (319, 369) pmol/l, 54 (49, 60) pmol/l and 0·22 (0·20, 0·25) μmol/l, respectively, for breast-fed infants, and 397 (372, 424) pmol/l, 76 (70, 83) pmol/l and 0·20 (0·19, 0·22) μmol/l, respectively, for non-breast-fed infants. Infants who were breast-fed at the age of 12 months had significantly lower serum vitamin B_{12} and HoloTC and higher MMA than those who were not breast-fed at the same age. In that study, total vitamin B_{12} intake from complementary foods was positively associated with serum vitamin B_{12} ($r = 0·15$ and $P = 0·030$) and HoloTC ($r = 0·25$ and $P = 0·001$).

The longitudinal study by Dagnelie *et al.*[32] was the only one studying the relationship between vitamin B_{12} intake and health, specifically psychomotor development, in spite of the status also being stated in the paper. However, they were not related with intakes or health outcomes. The results were divided between infants following a specified macrobiotic diet and those following an omnivorous one.

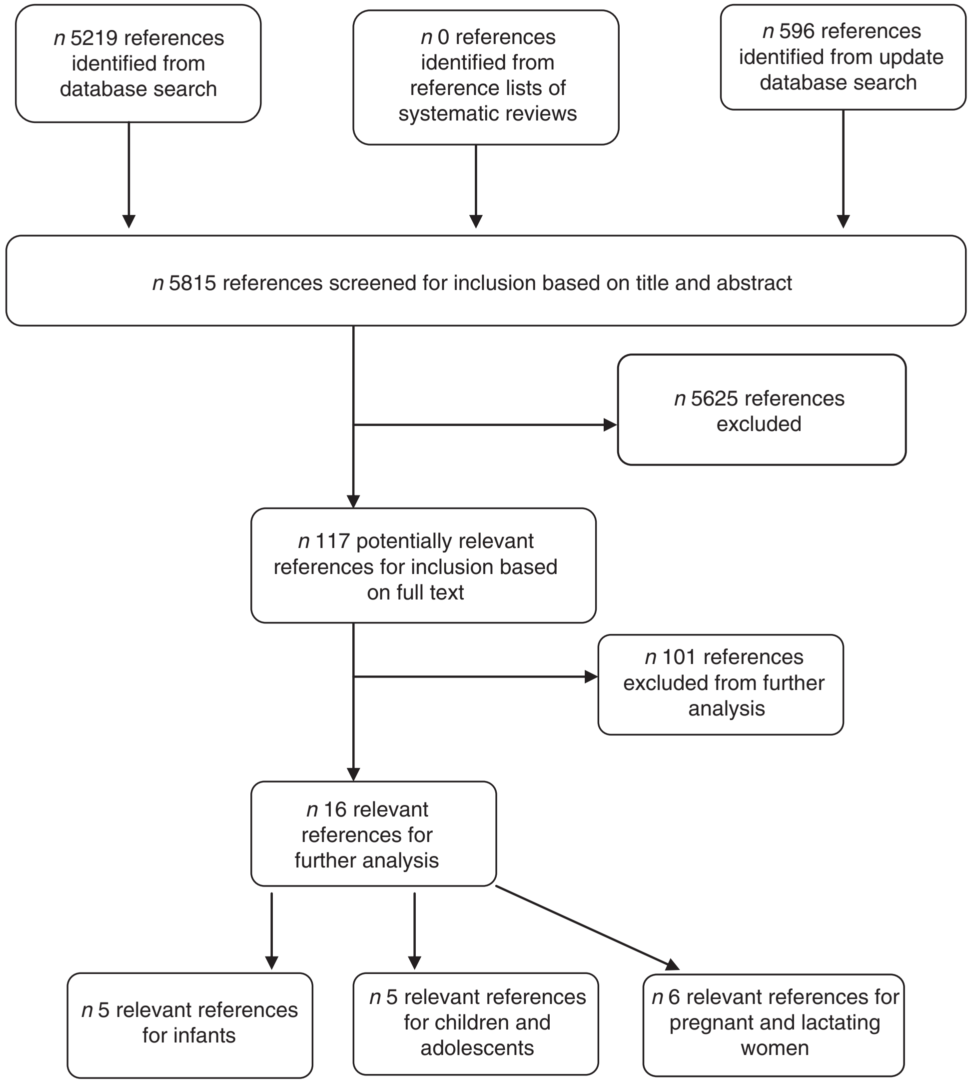

Fig. 2 Selection of studies for the current systematic review

Mean vitamin B_{12} intakes were significantly higher in the omnivorous group (2·9 (SD 1·3) μg/d) in comparison to the macrobiotic group (0·3 (SD 0·2) μg/d; $P < 0·001$). These differences could be also be shown in the scores obtained in the psychomotor development test in the areas of gross motor development (for which the mean difference in standard deviations between feeding groups was −0·48) and speech and language development (for which the mean difference in standard deviations between feeding groups was −0·42), with a P value of 0·04 and 0·03, respectively. Despite these differences in health outcomes obtained between feeding groups, the authors did not study an association between vitamin B_{12} intakes and differences in scores in psychomotor tests; for this reason, these results cannot be attributed only to the obtained difference in vitamin B_{12} intakes.

The results of the four studies evaluating the I-S relationship showed that the status of vitamin B_{12} biomarkers is significantly and positively associated with vitamin B_{12} consumption (ingested or injected). The strength of this association was stated in almost all of the studies, with the exception of one RCT[29] in which the regression coefficient was not given. The limited availability of I-H data in infants did not allow for drawing any conclusions.

Children and adolescents

For the children and adolescents group, we identified four cross-sectional studies[33–35,37] and one RCT[36]. Two out of three cross-sectional studies were conducted with children[33,37], one study[34] was carried out among adolescents and one[35] included both children and adolescents. In three cross-sectional studies[33–35], vitamin B_{12} intake and plasma vitamin B_{12} was described. However, searching for an association between intake and status was not the purpose of the studies. Only in the study by Hay *et al.*[37], performed in Norwegian children, was vitamin B_{12} intake shown to be significantly and positively associated ($r = 0·21$, $P < 0·05$) with HoloTC. In that study, serum

Table 2 Main characteristics of the studies selected in the systematic review by study population group

Group	Study	Country	Population (characteristics, *n*)	Objectives	Design	Intake	Status	Health outcome	Results	Conclusion
Infants	Dagnelie and van Staveren (1994)[32]	Netherlands	4–18-month-years old macrobiotic infants (*n* 53) and omnivorous control subjects (*n* 57) were assessed in three cohorts: one cohort aged 4–10 months, the second cohort aged 8–14 months and the third cohort aged 12–18 months	To associate macrobiotic diets in infants with lower scores in psychomotor development in comparison with omnivorous diet infants	Population-based, mixed-longitudinal cohort study, in which omnivorous group was frequency-matched with the macrobiotic group for month of birth, sex, parity, education of the parents and region of residence	Mean (SD) vitamin B_{12} intake (μg/d) was significantly different in the macrobiotic group (*n* 49), 0·3 (SD 0·2), and the omnivorous group (*n* 57), 2·9 (SD 1·3), $P<0{\cdot}001$	Plasma vitamin B_{12} concentrations were 149 pmol/l in macrobiotic infants and 404 pmol/l in omnivorous infants ($P<0{\cdot}001$)	Differences in psychomotor development of macrobiotic infants relative to omnivorous infants (means of differences in SD): −0·48 for gross motor development and −0·42 for speech and language development	The psychomotor checklist revealed that the macrobiotic group was significantly slower in gross motor ($P=0{\cdot}04$) and in speech and language development ($P=0{\cdot}03$)	The macrobiotic group had worst scores regarding psychomotor development. However, no association with lower intakes of vitamin B_{12} was looked for
	Worthington-White *et al.* (1994)[29]	USA	184 premature infants (<1800 g at birth and <36 weeks' gestation)	To investigate if IM injection of vitamin B_{12} has effects on vitamin B_{12} biomarkers	Single-centre, randomized, placebo-controlled trial. Study groups: folate+vitamin B_{12} supplementation; folate only; vitamin B_{12} only; and no additional supplementation	Control group: vitamin B_{12} 15 μg/l/d administered IM+0·07 pmol/l administered by formula fed as tolerated. Supplemented group: the same as control group+100 μg vitamin B_{12} IM per month for 4 months	Mean (SEM) serum vitamin B_{12} (pmol/l) in supplemented groups[29] was 640 (100) at baseline, 1150 (130)* at 1–2 weeks, 990 (110)* at 3–4 weeks, 830 (100)† at 6–8 weeks, 910 (150)† at 10–12 weeks and 1010 (230)† at 6 months. In the control group[28] the corresponding amounts were: 830 (200), 640 (60)†, 500 (60)*, 270 (50)‡, 380 (60)† and 810 (180). *†‡Significantly different from values at birth: *$P<0{\cdot}01$; †$P<0{\cdot}05$; ‡$P<0{\cdot}005$	Not stated	Not stated	In vitamin B_{12}-supplemented patients, an increase in serum B_{12} concentrations was serologically demonstrated in comparison with those who did not receive any supplementation
	Jones *et al.* (2007)[30]	Guatemala	304 infants and their mothers	To examine predictors of deficient plasma vitamin B_{12} concentrations	Cross-sectional study. A door-to-door census was conducted and SES, anthropometry, dietary intake of vitamin B_{12} and micronutrient status were measured at 12 months of age	Estimated mean intake of vitamin B_{12} in mothers was 3·1 μg/d and 2·2 μg/d in infants using a semi-quantitative FFQ	Mean (SD) plasma vitamin B_{12} in infants was 262·2 (163·5) pmol/l and in mothers 114·4 (9·2) g/l	Not stated	Infant intake of B_{12} is a predictor of infant plasma B_{12} ($r=0{\cdot}16$, $P<0{\cdot}0001$; *n* 270)	Infant intake from complementary foods was positively associated with infant plasma vitamin B_{12}
	Bjorke-Monsen *et al.* (2008)[28]	Norway	107 healthy, term, 6-week-old (±2 weeks) infants	To investigate if IM injection of vitamin B_{12} has effects on vitamin B_{12} biomarkers	RCT. Intervention group: *n* 54; control group: *n* 53	An IM injection of 400 μg of hydroxycobalamin after blood sampling at the first visit	In the intervention group, median (range) serum B_{12} (pmol/l) was 172 (128–250) at 6 weeks and 421 (291–497) at 4 months; median (range) MMA (pmol/l) was 0·58 (0·28–0·97) at 6 weeks and 0·20 (0·15–0·43) at 4 months	Not stated	Regression coefficient = 183 ($P<0{\cdot}001$) for serum vitamin B_{12} and regression coefficient = −0·70 ($P<0{\cdot}001$) for MMA, between injected vitamin B_{12} and vitamin B_{12} status	At 4 months, vitamin B_{12} intervention was by far the strongest predictor of infant vitamin B_{12} status

Table 2 *Continued*

Group	Study	Country	Population (characteristics, *n*)	Objectives	Design	Intake	Status	Health outcome	Results	Conclusion
	Hay *et al.* (2008)[31]	Norway	364 mothers and their healthy children	To investigate if different levels of intake of vitamin B_{12} have effects on vitamin B_{12} biomarkers	In a longitudinal study, serum vitamin B_{12}, HoloTC and MMA were measured at birth and at 6, 9, 12, 18 and 24 months	Mean (95 % CI) daily intake of vitamin B_{12} at 12 months, excluding intake from breast milk, in breast-fed (*n* 104) and non-breast-fed (*n* 115) infants was 1·4 (1·3, 1·6) μg and 2·4 (2·1, 2·6) μg, respectively ($P<0·001$), measured by using question-naires and 7 d weighed-food records at 12 months	Mean (95 % CI) serum vitamin B_{12}, HoloTC and MMA (pmol/l) were 343 (319, 369; *n* 85), 54 (49, 60; *n* 78) and 0·22 (0·20, 0·25; *n* 86), respectively, for breast-feeding infants receiving complementary foods. Corresponding values were 397 (372, 424; *n* 127), 76 (70, 83; *n* 117) and 0·20 (0·19, 0·22; *n* 125) for non-breast-fed infants at 12 months	Not stated	Partial *r* values considering total vitamin B_{12} intake were 0·15 ($P=0·030$) for serum vitamin B_{12} and 0·25 ($P=0·001$) for HoloTC	Vitamin B_{12} intake at 12 months was significantly associated with both serum vitamin B_{12} and HoloTC
Children and adole-scents	Papoutsakis *et al.* (2006)[33]	Greece	186 sixth-grade students (99 females and 87 males aged 10·8–13·5 years)	To describe the intake and the status of vitamin B_{12} in children	Cross-sectional study by face-to-face interview. B_{12} was measured in plasma and dietary intake data were collected by two non-consecutive 24 h recalls	Mean (95 % CI) for vitamin B_{12} intake was 3·2 (2·7, 3·8) μg/d in females and 3·7 (3·1, 4·5) μg/d in males ($P=0·024$)	Mean (95 % CI) for plasma vitamin B_{12} (pmol/l) was 411 (388, 435) for females (*n* 99) and 383 (360, 406) for males (*n* 87)	Not stated	Not stated	Not stated
	Gewa *et al.* (2009)[36]	Kenya	520 children (270 boys and 250 girls) with a mean age of 7·4 years, belonging to twelve selected schools randomized to one of four feeding groups during 24 months	To evaluate the relationship between dietary vitamin B_{12} and gains in cognitive test scores	A 2-year longitudinal, randomized controlled feeding intervention study using animal-source foods, in which dietary nutrient values were obtained from nineteen 24 h recalls (at least once per month), and a cognitive battery test repeated once per term	As there were no significant differences between boys and girls regarding vitamin B_{12} intakes, mean intake for the entire group was 0·64 (SD 0·38) μg	Not stated	As there were no significant differences between boys and girls regarding Digit span-forward test, mean (SD) score in this part of the cognitive test for the entire group was 2·79 (1·12)	A child with a daily high intake of vitamin B_{12} gained a significant 0·24 more points in the Digit Span-forward test than one with a low intake level	These results demonstrate the importance of improved intake of vitamin B_{12} contained in animal-source foods on cognitive function among school-aged children
	Hay *et al.* (2011)[37]	Norway	178 children from 2-year-olds (68 girls and 87 boys)	To examine vitamin B_{12} intake in relation to serum vitamin B_{12} status in 2-year-olds	Cross-sectional study by face-to-face interview. B_{12} was measured in plasma and dietary intake data were collected by 7 d weighed records (seven consecutive days). Information on supplement use was also taken	Mean vitamin B_{12} intake without gender differences was 3·1 μg/d	Median serum vitamin B_{12} was 407 pmol/l for the total population, median HoloTC was 93 and 106 pmol/l for boys and girls, and MMA was 0·16 and 0·14 μmol/l for boys and girls, respectively (significant differences between boys and girls for HoloTC and MMA; *n* 155)	Not stated	Significant correlation was found for vitamin B_{12} intake and HoloTC ($r=0·21$, $P<0·05$)	HoloTC was more significantly associated with vitamin B_{12} intake than other biomarkers

Table 2 *Continued*

Group	Study	Country	Population (characteristics, *n*)	Objectives	Design	Intake	Status	Health outcome	Results	Conclusion
	Steluti *et al.* (2011)[34]	Brazil	99 adolescents (58·6 % were girls) whose mean age was 17·6 years	To report vitamin B_{12} intakes and serum concentrations in Brazilian adolescents	Cross-sectional study by face-to-face interview. B_{12} was measured in plasma and dietary intake data were collected by 3 d records (three non-consecutive days)	Mean (95 % CI) vitamin B_{12} intake was 4·45 (4·28, 4·64) μg/d	Mean (SD) serum vitamin B_{12} was 397·5 (188·4) pg/ml	Not stated	Not stated	Not stated
	Yeung *et al.* (2011)[35]	USA	Non-pregnant population aged 1–18 years (*n* 7161)	To report vitamin B_{12} intakes and serum concentrations in US children and adolescents	Cross-sectional study. B_{12} was measured in plasma and dietary intake data by two 24 h recalls on non-consecutive days (the first in person and the second by telephone). Information on supplement use was also taken	Results are shown by FA consumption group and sociodemo-graphic characteristics	Mean serum vitamin B_{12} (*n* 5895) and MMA (*n* 2436) results are shown by FA consumption group and sociodemographic characteristics	Not stated	Not stated	Not stated
Pregnant and lactat-ing women	Koebnick *et al.* (2002)[39]	Germany	39 healthy pregnant women participated in the study throughout their pregnancies until delivery	To describe ranges of biochemical indices of vitamin B_{12} status and vitamin B_{12} intake in all trimesters of uncomplicated pregnancy	Prospective longitudinal study in which serum vitamin B_{12} and dietary intake data (using 4 d food records) were assessed in weeks 9–12, 20–22 and 36–38. Intake of supplements was recorded	Mean (SD) intake of vitamin B_{12} from the first to the third trimester was 5·6 (2·0) μg/d	Mean (95 % CI) serum B_{12} (pmol/l): 257 (226–292) in 1st month[31], 239 (212–268) in 2nd month[39] and 178 (161–198) in 3rd month[38], with $P < 0{\cdot}0001$ adjusted for maternal age	Not stated	Not stated	The intake of vitamin B_{12} did not correlate with vitamin B_{12} concentrations in blood
	Lindblad *et al.* (2005)[40]	Pakistan	46 women and their IUGR infants as well as 82 pairs with normal birth weight	To investigate whether IUGR was associated with altered maternal and fetal levels of vitamin B_{12}	Prospective observational study. Mothers and fetuses were followed at least 3–4 times since week 12 of pregnancy until the delivery	Not stated	Median (range) serum vitamin B_{12} (pmol/l) in mothers: IUGR 96 (23–266), normal 108 (29–317). Median (range) vitamin B_{12} (pmol/l) in umbilical cord: IUGR 190 (61–913), normal 171 (48–534). Median (range) maternal vitamin B_{12} was 102 (23–317) pmol/l	46 infants were considered IUGR *v.* 82 who had normal birth weight. 21 % of normal birth weight infants' mothers had pre-eclampsia and 26 % of IUGR mothers	*P* values for maternal ($P = 0{\cdot}42$) or umbilical cord ($P = 0{\cdot}24$) vitamin B_{12} levels (pmol/l) in comparison between IUGR and normal birth weight. Birth weight was not significantly different between mothers with pre-eclampsia ($P = 0{\cdot}53$)	Neither maternal nor umbilical cord vitamin B_{12} levels were associated with IUGR. There were no significant differences between IUGR and normal birth weight infants' mothers regarding pre-eclampsia

Table 2 *Continued*

Group	Study	Country	Population (characteristics, *n*)	Objectives	Design	Intake	Status	Health outcome	Results	Conclusion
	Muthayya *et al.* (2006)[42]	India	478 pregnant women were recruited at 12·9±3·3 weeks' gestation	To assess maternal dietary vitamin B_{12} and its biomarkers in apparently healthy pregnant women in order to determine their associations with IUGR	Prospective cohort study. Information on sociodemographic factors at baseline and on maternal anthropometry, dietary intake, clinical status and blood at baseline, second trimester of pregnancy and third trimester of pregnancy were collected	An FFQ for the preceding 3 months of each trimester, validated against 24 h recalls obtained thrice for each trimester, was assessed for each pregnant woman. Mean intakes of vitamin B_{12} were not stated	In a subsample of 185 women, serum vitamin B_{12} medians and IQR for each trimester and for each tertile were obtained	The incidence of IUGR babies was 28·6 % (*n* 108)	AOR = 5·98, 9·28 and 2·81 in trimesters 1 to 3, respectively, for women in the lowest tertile for serum B_{12} concentration during each of the trimesters of pregnancy in relation to risk of IUGR. Coefficients of correlation between vitamin B_{12} intake and status in all three trimesters: trimester 1 (*n* 135, $r = 0·22$, $P = 0·009$); trimester 2 (*n* 140, $r = 0·21$, $P = 0·013$); trimester 3 (*n* 147, $r = 0·20$, $P = 0·017$)	Women in the lowest tertile for serum B_{12} concentration during each of the trimesters of pregnancy had significantly higher risk of IUGR
	Morkbak *et al.* (2007)[41]	Denmark	Apparently healthy lactating mothers (*n* 89) including 23 supplemented with vitamin B_{12}, 41 partly supplemented and 25 not supplemented	To examine longitudinal changes in serum cobalamins during lactation and to investigate the influence of vitamin B_{12} supplementation on these parameters	A 9-month follow-up study for three different statuses of supplementation pregnant groups. Blood samples collected week 3 (baseline) and months 4 and 9 postpartum were analysed for cobalamins	Median (range) of B_{12} supplementation (μg/d): 1 (0–13·5) at baseline (*n* 89); 1 (0–13·5) at 4 months (*n* 87); 0 (0–18·0) at 9 months (*n* 86)	Median (range) cobalamins (pmol/l): 322 (129–1039) at baseline (*n* 89); 317 (114–1247) at 4 months (*n* 87); 315 (145–1193) at 9 months (*n* 86). Median (range) HoloTC (pmol/l): 85 (30–1068) at basleine (*n* 89); 87 (20–1020) at 4 months (*n* 87); 76 (30–972) at 9 months (*n* 86)	Not stated	*P* values for differences in cobalamin status between baseline and 4 months and between 4 months and 9 months were not statistically significant; $P = 0·02$ between baseline and 4 months and $P = 0·01$ between 4 and 9 months for HoloTC	The levels of serum cobalamins and HoloTC after 9 months showed no statistical differences between the groups supplemented or unsupplemented at any of three visits
	Baker *et al.* (2009)[38]	UK	500 pregnant adolescents (14–18 years) were recruited from 2 inner-city populations at gestational age ≤20 weeks	To assess vitamin B_{12} intake and its biomarkers in pregnant women and to determine associations with infant growth	Prospective study. Three non-consecutive 24 h recalls were conducted during the third trimester. Supplement use was also recorded	Mean (SD) intake of vitamin B_{12} of 290 mothers was 5·31 (4·96) μg/d; median (IQR) was 4·31 (2·97–6·11) μg/d	In 290 mothers, mean (95 % CI) serum vitamin B_{12} (pmol/l) of SGA infants (*n* 45) was 188 (166–212) and 175 (167–184) for non-SGA (*n* 245)	17·6 % were SGA (*n* 478)	Ratios of geometric means between SGA and non-SGA births were 1·07 (0·94–1·22) with $P = 0·276$ by simple regression analysis and 1·10 (0·98–1·23) with $P = 0·092$ by multiple regression analysis	Serum vitamin B_{12} in mothers was not associated with SGA infants

Table 2 *Continued*

Group	Study	Country	Population (characteristics, *n*)	Objectives	Design	Intake	Status	Health outcome	Results	Conclusion
	Takimoto *et al.* (2011)[43]	Japan	33 healthy pregnant women at the third trimester, 14 FA users (33·1 (SD 5) years) and 19 non-FA users (32·7 (SD 4·1) years), were recruited in obstetric department in central Tokyo	To describe biochemical indices of maternal vitamin B_{12} status and fetal growth	Prospective study	Not applicable	Mean (SD) maternal vitamin B_{12} (pg/ml) in the third trimester of FA users was 193·8 (68·8) and in non-users was 218·6 (62·2). Mean (SD) maternal vitamin B_{12} (pg/ml) at 1 month after birth of FA users was 355·8 (143·7) and in non-users was 391·2 (141·8)	Mean (SD) gestational length (weeks) was 39·3 (1·2) in FA users and 40·2 (1·1) in non-users. In girls, mean (SD) head circumference (cm) was 32·0 (1·5) in FA users and 33·9 (1·9) in non-users. In boys, mean (SD) birth weight (g) was 2908 (305) in FA users and 3195 (260) in non-users	Statistically significant differences were found between infants from FA users and non-users regarding gestational length (total group), head circumference (only girls) and weight (only boys). Blood vitamin B_{12} was not associated with infants' anthropometric characteristics	Maternal vitamin B_{12} status was not associated with gestational weight, weight, length or head circumference of infants at delivery or 1 month after delivery

IUGR, intra-uterine growth retardation; FA, folic acid supplement intake; IM, intramuscular(ly); SES, socio-economic status; RCT, randomized controlled trial; HoloTC, holotranscobalamin; MMA, methylmalonic acid; IQR, interquartile range; SEM, standard error of measurement; SGA, small-for-gestational age; AOR, adjusted odds ratio.

vitamin B_{12} and MMA were also measured; however, no association with them was found.

In the RCT by Gewa *et al.*[36], the targeted population group was children and the studied relationship was I-H. The authors discovered that children with a daily high intake of vitamin B_{12} gained a significant 0·24 more points in the Digit Span-forward test (as part of the entire cognitive test) than others with a low intake level, considering intakes of vitamin B_{12} predictors of the Digit Span-forward test.

Pregnant and lactating women

Regarding the pregnant and lactating women group, six prospective observational studies were included[38–43]. Four of them studied the relationship between status and health outcomes in the fetus (intra-uterine growth retardation (IUGR), small for gestational age (SGA) and growth in general)[38,40,42,43]. In Lindblad *et al.*'s study[40], the results suggested that in infants with normal birth weight, cord blood levels of vitamin B_{12} were correlated with maternal levels of serum vitamin B_{12}. However these correlations were weaker when infants had IUGR. In the study by Baker *et al.*[38], serum vitamin B_{12} levels in mothers were not associated with the risk of SGA infants. However, in Muthayya *et al.*'s study[42] women in the lowest tertile for serum vitamin B_{12} concentration during each of the trimesters of pregnancy had significantly higher risk of delivering IUGR infants. In this last study, a correlation between vitamin B_{12} intake and status was also reported in all three trimesters. In Takimoto *et al.*'s study[43], maternal vitamin B_{12} status, assessed in the third trimester of the pregnancy, was not associated with gestational weight, weight, length or head circumference of infants at delivery or at 1 month after delivery. Two studies[39,41] described longitudinal changes in vitamin B_{12} biomarkers through pregnancy, I-S being the main relationship examined. In one longitudinal study[39], vitamin B_{12} intake in pregnant women was not associated with serum vitamin B_{12}. In the study by Morkbak *et al.*[41], which is the only selected study on pregnant women, in spite of there being three different supplementation groups, as there were no significant differences between them, results were presented for all three groups together. No change was observed in serum vitamin B_{12} throughout the study period, whereas a significant decrease was observed for HoloTC from baseline to the 9th month.

The observed I-S and S-H relationships were not consistent and further conclusions cannot be extracted.

Quality of included studies

Table 3 summarizes the method used to assess the quality of the included studies. Only three studies had a high risk of bias[29,33,36]. Five studies had a moderate risk of bias[28,31,34,35,38] and eight studies reflect low risk of bias[30,32,37,39–43]. The most repeated reason for risk of bias across the studies was an inadequate explanation about the drop-outs and an inadequate assessment of exposure (method to assess vitamin B_{12} intakes).

Table 3 Assessment of methodological quality of included randomized controlled trials, longitudinal and cross-sectional studies

Study	Sequence generation adequate	Allocation concealment adequate	Blinding adequate	Drop-outs adequate and outcome data complete	Funder adequate	Lack of other potential threats to validity	Confounders	Assessment of exposure adequate	Overall risk of bias
Dagnelie and van Staveren (1994)[32]	–	–	–	Unclear	Yes	Yes	Yes	Yes	Low
Worthington-White *et al.* (1994)[29]	Unclear	Yes	Yes	Unclear	Yes	Unclear	–	–	High
Jones *et al.* (2007)[30]	–	–	–	–	Yes	Yes	Yes	Yes	Low
Bjorke-Monsen *et al.* (2008)[28]	Yes	Yes	Yes	No	Yes	Yes	–	–	Moderate
Hay *et al.* (2008)[31]	–	–	–	Unclear	Yes	Yes	No	Yes	Moderate
Papoutsakis *et al.* (2006)[33]	–	–	–	–	No	Yes	Yes	No	High
Gewa *et al.* (2009)[36]	Unclear	Unclear	No	Unclear	Yes	–	–	–	High
Hay *et al.* (2011)[37]	–	–	–	–	Yes	Yes	Yes	Yes	Low
Steluti *et al.* (2011)[34]	–	–	–	–	Yes	Yes	Yes	No	Moderate
Yeung *et al.* (2011)[35]	–	–	–	–	Yes	Yes	Yes	No	Moderate
Koebnick *et al.* (2002)[39]	–	–	–	Unclear	Yes	Yes	Yes	Yes	Low
Lindblad *et al.* (2005)[40]	–	–	–	Yes	Yes	Yes	Yes	Yes	Low
Muthayya *et al.* (2006)[42]	–	–	–	Yes	Yes	Yes	Yes	Yes	Low
Morkbak *et al.* (2007)[41]	–	–	–	Unclear	Yes	Yes	Yes	Yes	Low
Baker *et al.* (2009)[38]	–	–	–	Unclear	Yes	No	Yes	Yes	Moderate
Takimoto *et al.* (2011)[43]	–	–	–	Yes	Yes	Yes	Yes	Yes	Low

Discussion

From 5815 identified papers, only sixteen were suitable to be included in the review according to EURRECA's eligibility criteria. From these, five papers focused only on descriptions of vitamin B_{12} intakes and biomarkers without any stated association. Because of the small number of eligible papers included in the review, only a few main conclusions can be drawn for the specific population groups studied.

Infants

In this population group, vitamin B_{12} (ingested or injected) was significantly and positively associated with vitamin B_{12} biomarkers. Serum vitamin B_{12} was investigated in all four studies. The evidence, however, was not sufficient for HoloTC (only one study[31]) or MMA (two studies[28,31], while in one study[31], associations were not found for MMA).

In this population group, the two included interventions were performed through injection of vitamin B_{12}. Although injection could be a more reliable method of intervention, in general oral administration is better tolerated in the absence of neurological problems[44,45]. Moreover, it should be noted that exposure to vitamin B_{12} via oral supplements or intramuscular injections is very different as e.g. bioavailability issues are different.

Children and adolescents

Among the four cross-sectional studies[33–35,37] included in this population group, the only available finding was the positive association between children's intake of vitamin B_{12} and serum HoloTC in one of them. In that study, serum vitamin B_{12} and MMA were also investigated without any obtained association. The other three cross-sectional studies did not look for any association.

In the RCT of Gewa *et al.*[36], it was demonstrated that higher vitamin B_{12} intakes are associated with higher scores in one part of a cognitive test. However, only one study represents very limited data from which to extract a clear conclusion and in this respect, drawing conclusions may not be justified.

Pregnant and lactating women

Regarding the S-H relationships searched for in this group, as well as for I-S ones, no conclusions can be drawn due to the discrepancies in the results. One study[42] showed an association between status and fetal growth and three[38,40,43] showed no association. On the other hand, due to the heterogeneity shown in results regarding I-S relationships in all five included studies, it is possible to conclude that intake of vitamin B_{12} in pregnant and lactating women is not related to vitamin B_{12} concentration in their blood. This fact can be derived from the vitamin B_{12} gradient in the placenta, between the fetus and the mother. During pregnancy, vitamin B_{12} had been noted to decrease in mothers but not its transport molecules. Such an observation of the placenta facilitating the transport of a critical nutrient (as occurs with vitamin B_{12}) for fetal growth and development when the mother is deficient is another revelation of how important the placenta is in maintaining the development of the fetus[46,47]. In the other four studies[38–41] there were no significant or relevant associations present.

Use of biomarkers in studies

One of the currently open questions regarding vitamin B_{12} is to determine the best biomarker to assess its status. In the present review, data were insufficient to draw conclusions about the effectiveness of serum HoloTC or MMA as a biomarker of vitamin B_{12} status (only one study showed a positive association between vitamin B_{12} intakes and HoloTC in children[37]). However, MMA and HoloTC are more sensitive markers for vitamin B_{12} deficiency than plasma vitamin B_{12}[48] by reflecting sudden changes in vitamin B_{12} homeostasis, whereas plasma vitamin B_{12} seems to reflect the accumulation of vitamin B_{12}[49]. On the other hand, they are extremely variable in these periods of life, making difficult their interpretation[31]. Moreover, due to the ability of serum/plasma vitamin B_{12} to describe the status of vitamin B_{12} through time, without being influenced by punctual intake, serum/plasma vitamin B_{12} is the most common biomarker to assess vitamin B_{12} status.

Cognitive function

One of the constraints to the lack of data in the research on vitamin B_{12} intake and cognitive function is that even detailed examinations are not sufficiently accurate to detect developmental delays in young infants. However, reports on short- and long-term neurological effects related to vitamin B_{12} deficiency in young infants demonstrate the importance of an adequate vitamin B_{12} status during the first months of life[28]. Vitamin B_{12} is also suggested to be related with neurocognitive function in school-aged children[50]. In the present systematic review, two papers on this topic suggested this association (one in infants, the other in children). However, in the infants study[32], the differences in scores in psychomotor tests were associated with type of diet (macrobiotic or omnivorous) and not with intake of vitamin B_{12} (however, the authors found significant differences in vitamin B_{12} intakes between diet groups).

Megaloblastic anaemia

Although being selected as a relevant health outcome for infants and children and adolescents, no paper on megaloblastic anaemia was finally included. However, some bibliography has reported megaloblastic anaemia as a typical symptom of vitamin B_{12} deficiency, usually as a consequence of previous maternal vitamin B_{12} deficiency[51]. Absence of included studies investigating this outcome suggests the low quality of reporting of the available studies, which were mostly old case reports.

No studies were found for megaloblastic anaemia in pregnant and lactating women. The explanation for no revealed hits could be that the literature about megaloblastic anaemia in this vulnerable group is linked mostly to intake and status of folate rather than the intake and status of vitamin B_{12}[52].

Growth

Of four papers focusing on fetal/infant growth (SGA, IUGR or general growth) in pregnant and lactating women, as only one has shown a positive association, no clear conclusion can be extracted in this regard.

Fetal malformations

The literature reveals that neural tube defects are the most common fetal malformation linked to deficiency of vitamin B_{12} in mothers[53]. However, due to the strict inclusion criteria of the present systematic review, no studies on this topic were included.

Maternal pre-eclampsia

This health outcome was mentioned in only one of the longitudinal studies in the pregnant and lactating women group. However, there was no significant difference in vitamin B_{12} status among mothers who suffered pre-eclampsia compared with mothers without pre-eclampsia[40]. In another similar study, no significant differences were observed in both maternal and fetal serum vitamin B_{12} between a severe pre-eclampsia group *v.* mild pre-eclampsia and control groups[54].

Conclusions

The current systematic review emphasizes a number of knowledge gaps in the field of vitamin B_{12} research for young populations and pregnant and lactating women, derived from the scarcity and the low quality of available studies.

One of the reasons for this scarce literature on vitamin B_{12} in young population groups could be that mild vitamin B_{12} deficiency is more prevalent among elderly people in association with a number of chronic diseases[55].

There is also evidence that vitamin B_{12} deficiency is uncommon in young populations, unless they belong to a vegan community, or live in a developing area, or have a congenital malabsorption syndrome[56]. However, the prevalence in younger groups may be higher than formerly recognized[57].

RCT with enough power and varying doses of dietary intakes and duration of supplementation are required in order to establish vitamin B_{12} recommendations for young populations. Further studies to correlate serum/plasma vitamin B_{12}, MMA and HoloTC and also to explore vitamin B_{12} adequacy in young age groups are needed.

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