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REVIEW

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A systematic review and meta-analysis of functional vitamin B12 status among adult vegans

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Abstract

The dietary intake of vitamin B12 among unsupplemented vegans is notably lower compared to both vegetarians and omnivores. Prolonged low intakes of vitamin B12, such as seen in those adhering to a vegan diet, lead to physiological deficiency of vitamin B12 and an elevated risk of B12-related morbidity. However, while serum B12 serves as a conventional biomarker for assessing B12 status, its utility is limited given its sensitivity and specificity in ascribing physiological deficiency of B12 and the functional vitamin B12 status of those adhering to vegan diets is unclear. We conducted a systematic review and meta-analysis using data based on the full panel of biomarkers of vitamin B12 status to test whether adherence to a vegan diet is associated with an elevated risk of functional vitamin B12 deficiency compared to vegetarian or omnivorous diets. In addition, subgroup analysis was carried out to look at the effect of vitamin B12 supplement use on B12 status among vegans. Our search identified 4002 records, of which 19 studies met the inclusion criteria for the systematic review and 17 studies were taken forward for the meta-analysis. Meta-analysis results revealed significantly lower serum B12, pmol/ (−0.72 [−1.26, −0.18]; $p = 0.01$) and elevated total homocysteine, μmol/L (tHcy) (0.57 [0.26, 0.89]; $p < 0.001$) concentrations, alongside elevated methylmalonic acid, nmol/L (MMA) (0.28 [−0.01, 0.57]; $p = 0.06$) and lower holotranscobalamin, pmol/ (HoloTC) (−0.42 [−0.91, 0.07]; $p = 0.09$) levels among vegan adults compared to omnivores, indicating increased functional B12 deficiency in addition to low vitamin B12 status in vegan adults. There were no differences between vegans and vegetarians in HoloTC (0.04 [−0.28, 0.35]; $p = 0.814$) or MMA (−0.05 [−0.29, 0.20]; $p = 0.708$), but differences were found in serum B12 (−0.25 [−0.40, −0.10]; $p = 0.001$) and for tHcy (0.24 [0.09, 0.39]; $p = 0.002$) concentrations. Subgroup analyses indicated that the use of vitamin B12 supplements among vegans contributes to significant improvements in all biomarker concentrations compared to their unsupplemented counterparts. Our findings underscore the need for improved strategies to redress poor vitamin B12 status with appropriate B12 supplementation use among those adhering to vegetarian and vegan diets.

KEYWORDS
plant-based diets, vegan, vegetarian, vitamin B12

INTRODUCTION

Adoption of plant-based diets is increasing in Western society in large part because it is viewed as a healthier and more sustainable solution (Gibbs & Cappuccio, 2022) to the strain on global supply chains (Cheah et al., 2020). Traditionally, a plant-based diet encompasses a broad spectrum of dietary patterns that accentuate plant-based produce as the primary component and limit or exclude animal products (Kent et al., 2022). Within plant-based diets, there are different positions on animal intake, and some individuals adhering to plant-based diets may choose to limit, rather than entirely exclude, the consumption of red meat, processed meat, white meat and fish. Lacto-ovo vegetarians exclude meat and fish but consume dairy and eggs; lacto-vegetarians exclude meat, fish and eggs but include dairy; and ovo-vegetarians exclude meat, fish and dairy yet include eggs (BDA, 2021). The most stringent position is veganism, which commits to a philosophy rooted in the welfare of animals and excludes the use of all animal products for any purpose.

Within the United Kingdom, approximately 11% of the population follows a plant-based diet, with 3% and 1% identifying as vegetarian and vegan, respectively (Benson et al., 2019). While vegetarian and vegan diets are deemed healthier and rich in certain nutrients, such as fibre and folate, they require careful planning with the inclusion of fortified foods and supplements to ensure a balanced diet (Rocha et al., 2019). In particular, adherence to a strict vegan diet excludes meat, eggs and dairy, increasing the risk of deficiency in zinc, calcium, iodine, selenium and vitamin B12, as these nutrients are primarily found in animal-based foods or have low(er) bioavailability in plant-based foods (Bakaloudi et al., 2021; Neufingerl & Eilander, 2021). Notably, vitamin B12 is only found in animal-based or fortified foods.

Vitamin B12 is a unique micronutrient synthesised exclusively by specific bacteria (Martens et al., 2002). Therefore, plant-based foods inherently lack vitamin B12 unless they have been adequately (bio)fortified or contain bacterial metabolites. In contrast, animal-based foods contain vitamin B12 because animals obtain it through their diet or via symbiotic bacteria in their digestive systems. While some algae or seaweeds contain B12, there is an important question as to whether this is active B12 or just analogues of B12 that are unsuitable for humans (Sayer et al., 2024; Sela et al., 2020). Vitamin B12 is an enzyme cofactor for two enzymes involved in cellular metabolism: methylmalonyl-CoA mutase and methionine synthase (Green et al., 2017). The reaction catalysed by methionine synthase converts homocysteine to methionine, with the concomitant regeneration of tetrahydrofolate from N^5-methyl-tetrahydrofolate. Tetrahydrofolate is required for the synthesis of purines and pyrimidines. Therefore, a deficiency of B12 blocks the regeneration of tetrahydrofolate, which in turn impairs DNA biosynthesis and manifests as megaloblastic anaemia. The reaction of methylmalonyl-CoA mutase converts methylmalonyl-CoA to succinyl-CoA. Succinyl-CoA is a precursor of succinate, a key metabolite in energy production via the Krebs cycle and complex II of the respiratory chain. Thus, B12 deficiency impairs energy production. Severe and prolonged vitamin B12 deficiency can lead to peripheral neuropathy, cognitive impairments and psychological changes if not identified and treated appropriately (Green & Miller, 2022). Women of childbearing age are at higher risk of vitamin B12 deficiency due to increased demands during pregnancy and lactation, which can negatively impact maternal and infant health by raising the risks of low birth weight, preterm birth and neural tube defects (Molloy et al., 2009; Obeid et al., 2017; Rogne et al., 2017).

Plant-based diets (PBDs) comprise a heterogeneous group of diets with varying intakes of animal-based products with correspondingly varying quantities of vitamin B12 (Storz, 2022). In the context of most vegetarian diets, dairy products are typically relied upon as the primary source of B12 intake, although dairy still has a far lower content of vitamin B12 compared with meat. Cow's milk, for example contains a relatively low amount of vitamin B12 (approximately 0.2–0.7 μg/100 g), far lower compared to the substantial B12 content found in cooked beef (2.4 μg/100 g) (Gille & Schmid, 2015). In a study that examined supplement use practice, fewer vegetarians consumed vitamin B12 supplements compared to vegans (51% vs. 90%), with supplemented vegans exhibiting a superior B12 status compared to vegetarians (Storz et al., 2023). From a public health perspective, this underscores the importance of targeting messaging and promoting adequate vitamin B12 intake through fortified foods and supplementation to encompass not just vegans as currently but all individuals following plant-based diets. Among those adhering to PBDs, the early haematological signs of vitamin B12 deficiency may be masked due to the typically high intake of folate in these diets (Cuskelly et al., 2007). In addition, vitamin B12 deficiency can occur without anaemia and the initial presentation of mild(er) and general symptoms such as fatigue, headaches and pins and needles in the hands or feet (Smith et al., 2018).

There are several ways to measure vitamin B12 status, the most common being through serum vitamin B12 (SB12) concentrations (NICE, 2015). Values below 148 pmol/L indicate a strong deficiency, although this marker has low specificity and sensitivity (Smith et al., 2018). Other biomarkers provide a more robust and functional indication of vitamin B12 status. These include holotranscobalamin (HoloTC), methylmalonic acid (MMA) and total homocysteine (tHcy), which are used to provide a functional and more complete cellular/sub-cellular report of vitamin B12 status and to detect deficiency accurately (Hannibal et al., 2016). Elevated serum/plasma levels of MMA and tHcy suggest impaired B12-dependent enzymatic reactions, indicating a functional deficiency even when serum B12 levels might appear normal. Additionally, low serum/plasma

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levels of HoloTC signify a deficiency. Serum B12 and HoloTC are considered static biomarkers of vitamin B12 status because they measure the levels of vitamin B12 directly present in the blood, reflecting immediate and current B12 availability. In contrast, MMA and tHcy are considered functional biomarkers because they indicate the metabolic activity of the two vitamin B12-dependent enzymes, thereby reporting on the physiological functionality of vitamin B12 in the body.

Results from previous studies have consistently shown that the intake of vitamin B12 is typically lower among individuals adhering to plant-based diets compared to those who consume omnivorous diets (Neufingerl & Eilander, 2021; Obersby et al., 2013). A major shortcoming of these studies has been that they have principally focused on the assessment of SB12 levels or one other biomarker only. Therefore, a comprehensive analysis based on all four biomarkers, including the functional biomarkers MMA and tHcy, for a holistic biochemical assessment among vegans has not been undertaken.

To address this gap, we conducted a systematic review and meta-analysis investigating the association of the full panel of static and functional biomarkers of vitamin B12 status, including SB12, HoloTC, MMA and tHcy, among vegan adults in comparison to individuals adhering to vegetarian or omnivore diets. Additionally, our study aimed to test whether the use of vitamin B12-containing supplements is associated with a reversal of the effect of low dietary vitamin B12 through its impact on concentrations of functional indicators of vitamin B12 status among vegans.

METHODS

This systematic review and meta-analysis was conducted using the PRISMA checklist guidelines (Page et al., 2021) and registered with Prospero: CRD42023455642.

Data sources and search strategy

Population, Intervention, Comparison, Outcome (PICOs) was employed as the foundational framework for constructing the systematic review (Table S1a).

The search strategy of selected studies was described using the PRISMA flow diagram (Page et al., 2021). A literature search using three databases: PubMed, Scopus and Web of Science for publications, was conducted in February 2024. Searches were limited to English language and human trials. The search strategy to identify all studies examining parameters of vitamin B_{12} status in vegans was developed with terms relating to veganism, vitamin B12 status and biomarkers, as documented in Table S1b. In addition, a hand search was performed to identify relevant articles by checking the reference list of previously published systematic reviews and studies with similar topics.

Selection criteria

The eligibility criteria were created based on our research aim and objectives to select studies during the screening selection process. We aimed to include interventional and observational studies that compared vitamin B12 status in vegans with omnivores and/or vegetarians with at least one indicator of B12 status (SB12, HoloTC, MMA and tHcy). We excluded studies published only as abstracts or without original data (e.g. conference posters). The inclusion criteria involved studies on vegan adults, a suitable comparison of either omnivores or vegetarians, at least one biomarker of vitamin B12 status, duration of vegan diet and healthy adult participants (age 18–64 years) of European or Western European ancestry. We excluded reviews, studies on animal models or participants on prescribed medication known to influence B12 status (e.g. Metformin, Proton pump inhibitors and Histamine H2 Blockers).

Outcomes

The primary outcomes were a decrease in concentrations of SB12 and HoloTC and an increase in concentrations of MMA and tHcy in vegans than in vegetarians or omnivores. In addition to the primary outcomes, we conducted subgroup analyses on the effects of vitamin B12 in individuals using or consuming B12 supplements.

Study selection process

Entries from the three databases were inputted into Zotero software (Zotero, 2022), and any duplicate entries were eliminated. Two reviewers (AN and KA) independently screened the remaining articles using the Rayyan software (Rayyan, 2022). First, the titles and abstracts of the entries were screened, and then the full text was examined for the remaining articles. Articles were included or excluded based on the a priori specified eligibility criteria. Any discrepancies between reviewers were resolved through discussion.

Data collection

For each article, data on variables pertaining to author, year of publication, study design, participant characteristics, inclusion and exclusion criteria, supplement use, duration on a vegan diet and static/functional biomarker concentrations were extracted. Results from the systematic review were taken forward to meta-analysis if the appropriate data was available.

Assessment of bias of included studies

The Joanna Briggs Institute (JBI) critical appraisal tool checklists (https://jbi.global/critical-appraisal-tools) were used to assess the quality of each included study (Moola et al., 2020). The selection of checklists was decided by the specific study design criteria (intervention, case-control and cross-sectional). Two reviewers (AN and KA) separately completed the quality assessment; any discrepancies were resolved through discussion. The recommended approach for authors using the JBI tool is to classify a study's quality as poor, moderate or good (Moola et al., 2020). We categorised the studies' risk of bias based on the number of 'no' domains in the risk of bias assessment. We categorised the risk of bias in the studies based on the presence of 'no' domains in the risk of bias assessment. Given the varying number of domains across different study types, we adjusted our classification criteria. Studies with no 'no' domains were classified as 'good' quality. Studies with one or more 'no' domains were classified as 'poor' with a high risk of bias. However, studies were classified as 'moderate' quality if they had no 'no' domains but had two or fewer 'unsure' domains.

Meta-analysis

The standardised mean difference (SMD) was used as the effect measure in the meta-analyses for the continuous outcomes. The SMD provides a standardised measure of effect size that allows for direct comparison of vitamin B12 status across different studies.

Statistical analysis

All analyses were conducted using the statistical software Jamovi (Jamovi, 2022). The mean value and standard deviation (SD) of the four biomarker concentrations in the vegan and control groups (omnivore and vegetarian) were used for the meta-analysis. For uniformity, all biomarker measurements were converted to consistent units: serum B12 (pmol/L), HoloTC (pmol/L), MMA (nmol/L) and tHcy (μmol/L).

To deal with studies that only provided the median and upper/lower percentiles, the authors were contacted and asked to provide the mean and SD. If this was not possible or we did not receive a response, median values were assumed to be approximately equal to the mean, as previously implemented by other meta-analyses (Obersby et al., 2013). The upper and lower percentiles were converted to the SD using the following formula (Krzywinski & Altman, 2014):

$$\mathrm{SD} = \frac{\text{high percentile} - \text{lower percentile}}{2.7}$$

This step was undertaken to ensure that as much data as possible was included in the meta-analysis. The JAMOVI software application MAJOR (1.2.3) (Viechtbauer, 2010) was used for the SMD analysis for each biomarker using the SMD as the outcome measure, applying a random-effects model ($p < 0.05$). Reported results are based on tested differences between concentrations for each biomarker in the vegan participants and controls, including omnivores and vegetarians (vegan vs. omnivore and vegan vs. vegetarian). The means and SD from all studies were aggregated to calculate an overall mean and SD for each biomarker and dietary group.

Statistical analysis of heterogeneity

Heterogeneity was calculated using the I^2 value, which describes the degree of variance between the studies attributed to heterogeneity. This was defined as non-important <25%, moderate 25%–50%, substantial 51%–75% and considerable >75% for I^2 (Higgins et al., 2003). To investigate the influence of the degree of heterogeneity on the results, subgroup sensitivity analysis was performed for each biomarker concentration for vegan participants versus vegetarian and omnivore groups and vegans who supplemented compared to those that did not supplement with vitamin B12. We used Tau^2 to estimate the variability of the effect sizes of individual studies after accounting for the within-study sampling variability. It represents the variability in the effects of individual studies that are not explained by chance or random error.

Funnel plots were used to detect publication bias by applying the rank correlation test and Egger's regression test, using the standard error of the observed outcomes as a predictor to check for asymmetry (Egger et al., 1997). Furthermore, the studentised residuals test assessed for outliers, identifying influential data points by measuring the standardised residuals against a threshold value, aiding in the detection of observations that significantly impact the model's assumptions and results.

RESULTS

Selection process

We identified 4002 records using three electronic databases (Figure 1). After excluding duplicate citations, we screened the titles and abstracts of 3169 publications. Of these records, 3099 were excluded as irrelevant,

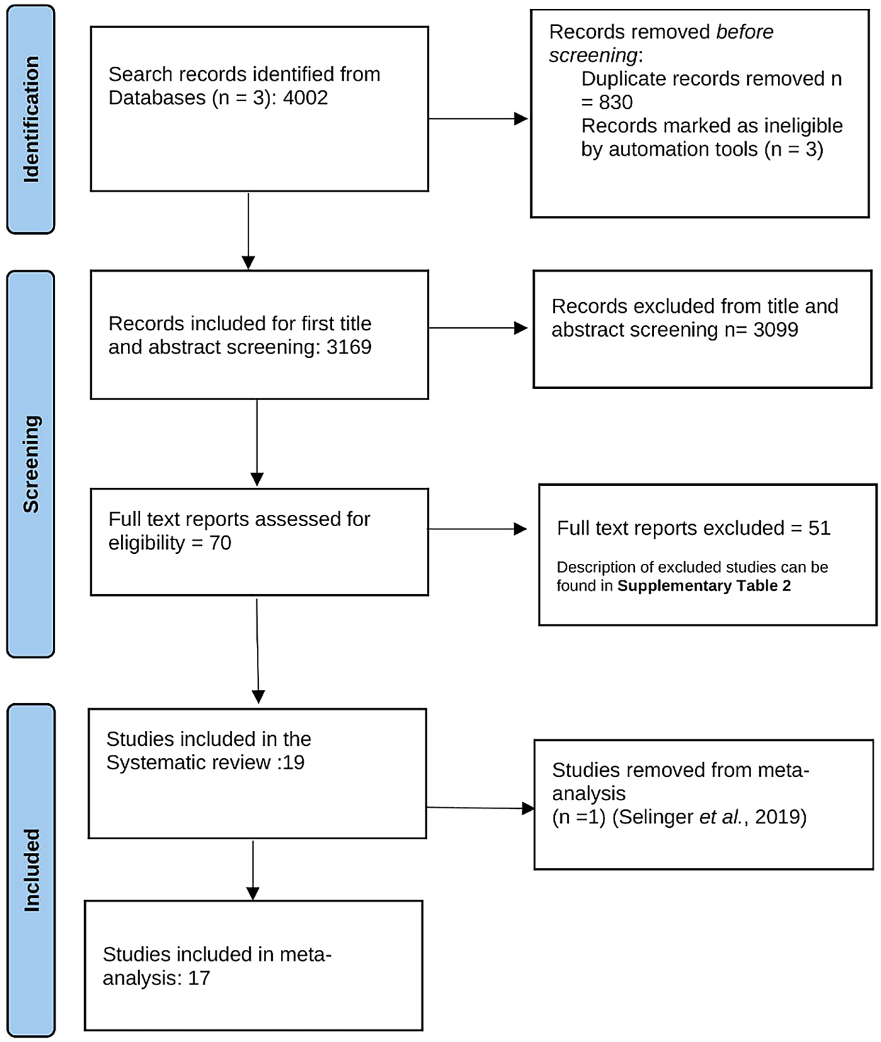

FIGURE 1 Prisma flow diagram of the systematic review and meta-analysis. Prisma flow diagram of the step-by-step process of obtaining the studies used for the systematic review and meta-analysis. An initial 1894 articles were retrieved from three databases: Web of Science, Pubmed and Scopus. Duplicates were removed using the Zotero software. The remaining articles were screened by AN and KA, and discrepancies were resolved through discussion. A total of 17 studies were used in the meta-analysis.

and a total of 70 papers were assessed in full text. Table S2 presents the excluded studies and reasons for exclusion. After full texts were screened, 19 papers were included in the systematic review. We attempted to reach out to authors in cases where we deemed additional information might be necessary. The majority of the contacted authors either did not respond to our request or indicated they lacked access to the data. Only one study, conducted by Storz et al. (2023), responded and provided the requested data, which was used within the analysis.

Overall, 19 studies were included in the systematic review, of which data from 17 studies were taken forward and used within the primary meta-analysis and 4 in the subgroup meta-analysis. (Bissoli et al., 2002; Dawczynski et al., 2022; Elorinne et al., 2016; Gallego-Narbon et al., 2019; Gilsing et al., 2010; Haddad et al., 2020; Henjum et al., 2023; Herrmann et al., 2003, 2001; Krajcovicová-Kudlácková et al., 2000; Lederer et al., 2019; Majchrzak et al., 2006; Mann et al., 1999; Nebl et al., 2019; Selinger et al., 2019; Siebert et al., 2017; Storz et al., 2023; Waldmann et al., 2004; Weikert et al., 2020). The manuscript by Siebert et al. (2017) was excluded from the primary meta-analysis as an appropriate comparison group was not specified (lacto-ovo vegetarian or omnivore); the study was included in the subgroup analysis for vegans who supplemented and did not supplement with vitamin B12. Finally, the manuscript by Selinger et al. (2019) was removed as only the mean, minimum and maximum biomarker values were provided, and the mean and standard deviation could not be calculated.

Study characteristics

Table 1 provides an overview of the characteristics of 19 studies included in a systematic review and studies in the meta-analysis. Data from a total of $n = 930$ vegan, $n = 1019$ vegetarian and $n = 1166$ omnivore participants were included. The mean age and standard deviations varied across the studies, reflecting the diverse age ranges of the participants. The duration of a vegan diet ranged from a few weeks to several years. All studies were published between 1999 and 2022. Out of the 19 studies included, two were intervention

TABLE 1 Characteristics for 19 studies included in the systematic review and meta-analysis. The total number of individuals within each dietary group included 930 vegans, 1166 omnivores and 1019 vegetarians. Two of the studies were intervention trials (RCT).

			Number of participants *n*			Mean age	
Study	**Study design**	**Country**	**VG**	**OMV**	**LOV**	**VG**	**OMV**
Mann 1999	Cross-sectional	Australia	18	18	43	33 (±7.7)	34.2 (±9.4)
Krajovica 2000	Case–control	Slovakia	32	59	62	41.5 (1.8)	
Hermann 2001	Case–control	Germany	7	44	34	22	23
Bissoli 2002	Cross-sectional	Italy	31	29	14	45.8 (15.8)	43.8 (17.3)
Herrmann 2003	Case–control	Germany and the Netherlands	29	79	66		
Waldman 2004	Cross-sectional	Germany	86		45	43.8 ± 15.6	
Majchrzak 2006	Cross-sectional	Austria	42	40	36	30.7 ± 9.9	38.4 ± 14.8
Gilsing 2010	Cross-sectional	UK	232	226	231	27.5 (11)	30.5 (10.5)
Elorrine 2016	Case–control	Finland	22	21		33	35
Siebert 2017	RCT	Spain	76			29.4 (±7.1)	
Gallego- Nabron 2019	Cross-sectional	Spain	54		49		
Lederer 2019	RCT	Germany	26	27		33.2	29.9
Nebl 2019	Cross-sectional	Germany	28	27	26	27.5 ± 4.24	27.4 ± 4.0
Selinger 2019	Case–control	Prague	151	84		32.8 (±7.4)	32 (+/−7)
Haddad 2020	Cross-sectional	USA	76	488	221	64 ± 12.4	56.9 ± 12.8
Weikert 2020	Cross-sectional	Germany	36	36		37.5 (32.5–44.0)	38.5 (32.46)
Dawczynski 2022	Cross-sectional	Germany	58	65	65	25 (19–56)	33 (18–61)
Henjum 2022	Cross-sectional	Norway	115		90	30.4 (9.1)	
Storz 2023	Cross-sectional	Germany	38	39	37	27.5 (11)	30.5 (10.5)

Abbreviations: HoloTC, holotranscobalamin; LOV, lacto-ovo vegetarian; MMA, methylmalonic acid; OMV, omnivore; tHcy, total homocysteine; VG, vegan.

trials (RCT), while the remaining 17 were observational studies (cross-sectional and case-control). Most of the studies were conducted in Europe, with six studies from Germany (the most represented country), one study from the United States (Haddad et al., 2020) and another from Australia (Mann et al., 1999). A total of 4 studies were included in the subgroup analysis, which compared vegan B12 supplement users with non-users (Gallego-Narbon et al., 2019; Herrmann et al., 2003; Nebl et al., 2019; Siebert et al., 2017). The total sample size included in the sub-group analysis was $n = 123$ supplement users and $n = 64$ non-supplement users.

		Sex (female %)					
LOV	Total	VG	OMV	LOV	Biomarkers	Duration on a vegan diet	Quality of evidence
34.9±9		0	0	0	Serum B12, tHcy	Not stated	Poor
		69			Serum B12, tHcy	Not stated	Poor
22		71			Serum B12, MMA tHcy	Minimum 1 year	Poor
48.5 (14.5)		39	38	57	Serum B12, tHcy	Minimum 5 years	Moderate
	37	55			Serum B12, HoloTC, MMA, tHcy	Minimum 1 year	Poor
44.6±15		59		64	Serum B12, tHcy	Minimum 1 year	Poor
34.2±13.6		50	72	72	Serum B12, tHcy	Among vegetarians and vegans were 67% who had been following diet at least 5 years, 25%–31% 1–5 years and 8%–2% 1 year.	Moderate
27 (9)		0	0	0	Serum B12	38% of vegans on diet <7 years	Good
		73	52		Serum B12, tHcy	The average duration of a vegan diet = is 8.6 years.	Poor
		68			Serum B12, HoloTC, MMA, tHcy	Not stated	Poor
	30.3 (7.7)	78			Serum B12, MMA, THcy	Not stated	Poor
		69	56		Serum B12, HoloTC, MMA, tHcy	4 weeks	Poor
27.6±4.31		64	59	62	Serum B12, HoloTC, MMA, tHcy	0.5-1 year-21% 1-2 years- 14% 2-3 years- 25% >3 years- 39%	Moderate
		48	46		Serum B12, HoloTC, tHcy	Minimum 3 years	Poor
60.2±13.9		72	67	62	Serum B12, HoloTC, MMA, tHcy	Not stated	Moderate
		50	50		Serum B12, HoloTC, MMA, tHcy	The average duration of a vegan diet = 4.8 (3.1–8.7) years	Good
28 (18–65)		69	62	72	Serum B12, HoloTC, MMA, tHcy	Minimum 1 year	Moderate
29.6 (9.5)		64		82	Serum B12, MMA, tHcy	Average duration = 4.7 years for vegans	Moderate
27 (9)		11	60	68	Serum B12, HoloTC, MMA, tHcy	Longer than 24 months	Good

Table S3 presents a detailed overview of the data provided for vitamin B12 supplementation and duration of diets for each study included.

Serum vitamin B12 and tHcy were the most common biomarkers used to assess vitamin B12 status in vegan participants. Seven studies measured all four biomarkers (Dawczynski et al., 2022; Haddad et al., 2020; Herrmann et al., 2003; Lederer et al., 2019; Nebl et al., 2019; Storz et al., 2023; Weikert et al., 2020). Table 2 presents the overall mean (SD) value for the four biomarkers, categorised by biomarker and diet.

TABLE 2 Overall mean (SD) values for each biomarker in each of the dietary groups.

Diet group	Serum B12 (pmol/L)			HoloTC (pmol/L)			MMA (nmol/L)			tHcy (μmol/L)		
	N[a]	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
Vegan	17	249.4	87.8	7	62.9	33.9	9	**269**	263	15	**13.4**	7.8
Vegetarian	14	252	85.7	5	60.6	36.6	7	213.7	173.2	12	12.5	5.2
Omnivore	13	384.9	109.6	7	75.5	28.6	7	180.5	89.0	11	9.7	3.8
Vegan supplement users[b]	4	276.9	154.1	3	**43**	30.47	4	**340.1**	429.2	2	11.4	5.2
Vegan non-supplement users	4	**183.9**	85.7	3	**26**	26	4	**484.5**	678.9	3	**14.4**	9.8

Note: The data presents the number of studies included for each biomarker to produce a combined mean (SD) concentration. The data show that in general, the vegan group has the lowest serum B12 and holotranscobalamin (HoloTC) concentration and the highest methylmalonic acid (MMA) and total homocysteine (tHcy) compared to vegetarian and omnivorous groups. The vegetarian group has slightly lower Holo-TC concentrations than the vegan group; however, both are in the range of normal B12 status. Vegan supplement users had higher vitamin B12 and HoloTC concentrations and lower MMA and tHcy concentrations than the non-supplement users, based on 4 studies. Values in bold highlight values outside the normal range relating to the primary outcomes for low vitamin B12 status defined as: Serum B12 (<180 pmol/L), HoloTC (<50 pmol/L), tHcy (≥13 μmol/L, and MMA) (>260 nmol/L) in vegan participants.

[a]Number of studies included.

[b]Includes both vitamin B12 supplements or multivitamins containing vitamin B12.

Risk of bias and quality of evidence

Overall, 16 of the 19 studies included in the systematic review had a moderate to poor risk of bias. The inclusion and exclusion criteria were appropriately defined in 85% of the studies. The primary category in which studies were flagged with a 'No' was related to performance and detection bias, as these studies did not adequately consider or address potential confounding variables. The classification of risk of bias for each study, depending on their study design, can be found in Table 1. A more comprehensive view detailing the risk of bias in the included studies can be found in Table S4.

Primary outcomes

The meta-analysis revealed standardised mean differences in SB12 levels among the different dietary groups. Vegans exhibited lower levels compared to both omnivores (−0.72 [−1.26, −0.18], $p=0.01$; 13 studies, $I^2=93\%$ considerable heterogeneity) and vegetarians (−0.25 [−0.40, −0.10], $p=0.001$; 14 studies, $I^2=31.8\%$ moderate heterogeneity) in the SMD analysis after excluding outliers (Table 3 and Figure 2). Table S5 presents the mean/median, standard deviation and inter-quartile ranges for each vitamin B12 biomarker for 13 studies included in the meta-analysis. Table S6 presents the raw data for the subgroup analysis for vegan supplement users and non-supplement users. To further explore publication bias, Figure S1 presents funnel plots for each biomarker with outliers for SB12 removed, while Figure S2 depicts funnel plots and Forrest plots, including outliers for SB12. Detailed results for the SMD, including outliers for Serum B12, are provided in Table S7.

The meta-analysis showed no significant differences in HoloTC concentrations between vegans and omnivores (−0.42[−0.91, 0.07], $p=0.093$; 7 studies, $I^2=89.7\%$ considerable heterogeneity), and with no differences observed between vegans and vegetarians (0.04[−0.28, 0.35], $p=0.814$; 5 studies, $I^2=68.8\%$ substantial heterogeneity) (Table 3 and Figure 3).

For MMA, there was a moderate difference between vegans and omnivores (0.28 [−0.01, 0.57], $p=0.06$, 7 studies, $I^2=70.7\%$, indicating substantial heterogeneity), which was not significant. No difference was observed between vegans and vegetarians (−0.05 [−0.29, 0.20], $p=0.71$; 7 studies, $I^2=66.05\%$, indicating substantial heterogeneity) (Table 3).

The meta-analysis also identified differences in tHcy levels between vegans and omnivores (0.57 [0.26, 0.89], $p<0.001$; 11 studies, $I^2=81.74\%$, indicating considerable heterogeneity) and between vegans and vegetarians (0.24 [0.09, 0.39], $p=0.002$; $I^2=41.78\%$, indicating moderate heterogeneity). Forrest plots for MMA and tHcy can be found in Figure S3.

Subgroup sensitivity analysis for supplementation with B12 in vegans

Finally, we carried out a subgroup analysis using data from vegan participants to compare vitamin B12 status between vegans who were using vitamin B12-containing supplements against those who did not use any form of vitamin B12 (studies $n=4$). We noted a difference and improvement between vegans that use B12 supplemented versus unsupplemented vegans for each biomarker (Table 3) with generally non-important heterogeneity ($I^2=0\%$–16%). The data for tHcy exhibited moderate heterogeneity but was not significant ($I^2=42\%$, $p=0.187$). For each biomarker, examination of the studentised residuals showed no indication of outliers or overly influential studies. Funnel plots (Figure S1 i–l) and Forest plots (Figure S3 e–h) for all the data concerning the supplemented versus non-supplemented vegan participants can be found in the supplementary material.

TABLE 3 Overall effect and heterogeneity of the results with outliers removed. A mean difference analysis for each biomarker was carried out using the SMD (standard mean difference) as the outcome measure. The degree of heterogeneity was estimated using I^2 statistic.

	Overall effect			Heterogeneity			
Variables	**SMD [95%, CI]**	***Z***	***p***	I^2	**Tau²**	**Std. err.**	***p***
Vegan versus omnivore							
Serum vitamin B12[a,b]	−0.72 [−1.26, −0.18]	−2.60	**0.01**	93%	0.76	0.37	**<0.001**
HoloTC	−0.42 [−0.91, 0.07]	−1.68	0.09	89.7%	0.384	0.25	**<0.001**
MMA	0.28 [−0.01, 0.57]	1.90	0.06	70.7%	0.113	0.09	**0.003**
HCY	0.57 [0.26, 0.89]	3.57	**<0.001**	81.74%	0.238	0.13	**<0.001**
Vegan versus vegetarian							
Serum vitamin B12[a,b,c]	−0.25 [−0.40, −0.10]	−3.27	**0.001**	31.8%	0.02	0.03	0.16
HoloTC[c]	0.04 [−0.28, 0.35]	0.236	0.81	68.8%	0.09	0.09	**0.03**
MMA	−0.05 [−0.29, 0.20]	−0.38	0.71	66.05%	0.07	0.06	**0.01**
HCY	0.24 [0.09, 0.39]	3.09	**0.002**	41.78%	0.03	0.03	**0.05**
Supplemented vegan versus non supplemented vegan							
Serum vitamin B12	0.73 [0.39, 1.09]	4.16	**0.001**	16%	0.021	0.104	0.38
HoloTC	0.49 [0.13, 0.85]	2.64	**0.01**	0	0	0.112	0.61
MMA	−0.33 [−0.64, −0.03]	−2.14	**0.03**	0%	0	0.081	0.75
tHcy	−0.41 [−0.87, 0.05]	−1.76	0.08	42%	0.069	0164	0.18

Note: Significant *p*-values are identified in bold. Results were considered significant with a $p<0.05$. Degree of heterogeneity based on the I^2 statistics was classified as (i) non important if <25%, (ii) moderate if 25–50, (iii) substantial if 51–75 and, (iv) considerable if >75%.

Abbreviations: HCY, homocysteine; Hcy, total homocysteine; HoloTC, holotranscobalamin; MMA, methylmalonic acid.

[a]Study Krajovika removed as outlier.

[b]Study Gisling removed as outlier.

[c]Study Storz removed as outlier.

DISCUSSION

The present systematic review and meta-analysis set out to test the association of the full panel of static and functional biomarkers of vitamin B12 status, including SB12, HoloTC, MMA and tHcy, among vegan adults in comparison to individuals adhering to vegetarian or omnivore diets. Our results show that individuals adhering to a vegan diet exhibit lower vitamin B12 status compared to vegetarians and omnivores, as indicated by both static (SB12) and functional (tHcy) indicators of vitamin B12 status. Furthermore, our results show that a regimen of vitamin B12 supplements is a simple and effective way to mitigate the risk of vitamin B12 deficiency among those adhering to a vegan diet. To the best of our knowledge, this study is the first systematic review and meta-analysis to develop a consensus on vitamin B12 status among vegans using the full cassette of functional and static biomarkers of vitamin B12 status.

The complex relationship between biomarkers of vitamin B12 status in different diets

The relationship between the four biomarkers of B12 status is complex. For the vast majority of healthy subjects, nutritional insufficiency and deficiency of B12 are characterised by reduced SB12 and HoloTC, and increased plasma tHcy and MMA. However, exceptions to this exist and there are examples where certain individuals or groups of the population exhibit values in one marker contrary to the above norm. Previous studies in omnivore populations demonstrated that both Hcy and MMA can be markedly elevated even at SB12 values above the traditional cut-off value of 148–200 pmol/L (Carmel, 2011; Refsum et al., 2006; Selhub et al., 2008). Typical symptoms of low vitamin B12 status have also been observed in individuals exhibiting elevated MMA and/or tHcy accompanied by normal SB12 concentrations (David Smith & Refsum, 2012). The Hordaland study conducted in Norway analysed the relationship between SB12 and the functional biomarkers MMA and tHcy, identifying cut-off values of 334 and 393 pmol/L for SB12, dependent on elevated MMA and tHcy, respectively (Vogiatzoglou et al., 2009). A study by Naik et al. (2018) examining a cohort of 119 healthy vegetarian Indians showed that 50% of participants classified as B12-deficient based on SB12 cut-offs, 50%–70% presented with low plasma HoloTC, and 70%–90% had elevated plasma Hcy. Strikingly all participants in the study were clinically asymptomatic for B12 deficiency. It is thus conceivable that adjusted

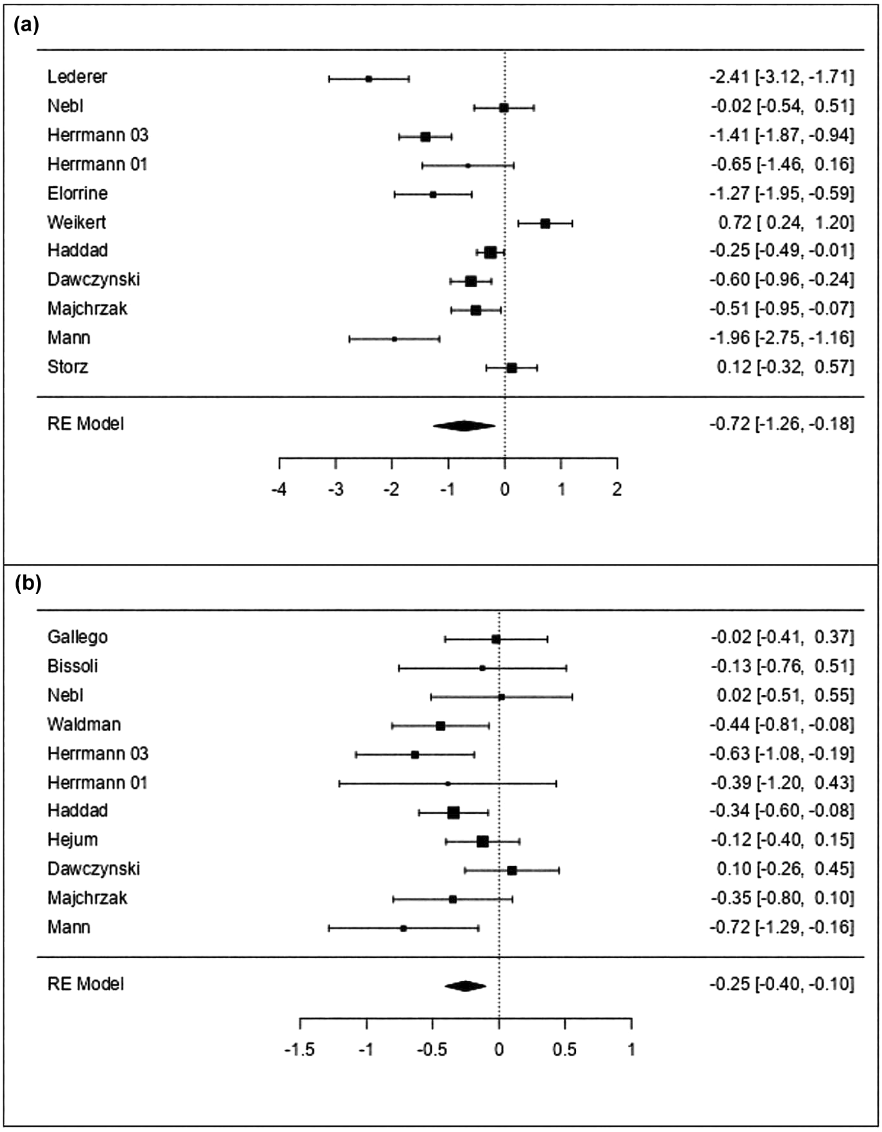

FIGURE 2 Forest plots illustrating the standardised mean difference effect of serum B12 (SB12) status using the random-effects model with outliers removed. Subfigures 2a and 2b show the standardised mean difference for vegans and omnivores ($p=0.01$, $I^2=93\%$) and between vegans and vegetarians ($p=0.001$, $I^2=31.8\%$). (a) Comparison of SB12 status between vegan (left) and omnivorous (right) participants. (b) Comparison of SB12 status between vegan (left) and vegetarian (right) participants.

reference ranges for B12 biomarkers may be necessary to capture true-to-health B12 status in individuals with different dietary patterns (Hannibal, 2018; Naik et al., 2018). However, the current review is limited to Caucasian populations, and caution should be exercised when extrapolating these findings to Indian populations, where biomarkers of vitamin B12 status may be influenced by long-term vegetarianism or genetic differences. The complex relationship of B12 biomarkers demands even more careful assessment in women who adhere to plant-based diets and are pregnant, lactating or more generally, of child-bearing age (David Smith & Refsum, 2012; Devalia et al., 2014; Smith et al., 2018). Vitamin B12 deficiency during pregnancy can negatively impact maternal and infant health by raising, for example the risk of low birth weight, preterm birth and neural tube defects (Molloy et al., 2009; Obeid et al., 2017; Rogne et al., 2017).

Our biomarker findings align with anticipated trends for different diets. The mean SB12 concentrations among meat eaters, vegans and vegetarians were 384.9, 249.4 and 252 pmol/L, respectively. These outcomes are consistent with those reported in a previous systematic review by Neufingerl and Eilander (2021), which investigated the nutritional status of plant-based eaters compared to meat eaters and similarly found higher mean SB12 levels among meat eaters (309 pmol/L) in contrast to vegetarians (220 pmol/L) and vegans (226 pmol/L).

HoloTC, or active B12, is often put forth as the most stable indicator of vitamin B12 status, especially sensitive to early stages of vitamin B12 deficiency and throughout pregnancy (Heil et al., 2012). This holds true in light of the current findings; non-supplemented vegans exhibit the lowest HoloTC values, which highlights a low to negligible vitamin B12 intake. This concept supports the notion that HoloTC serves as a sensitive

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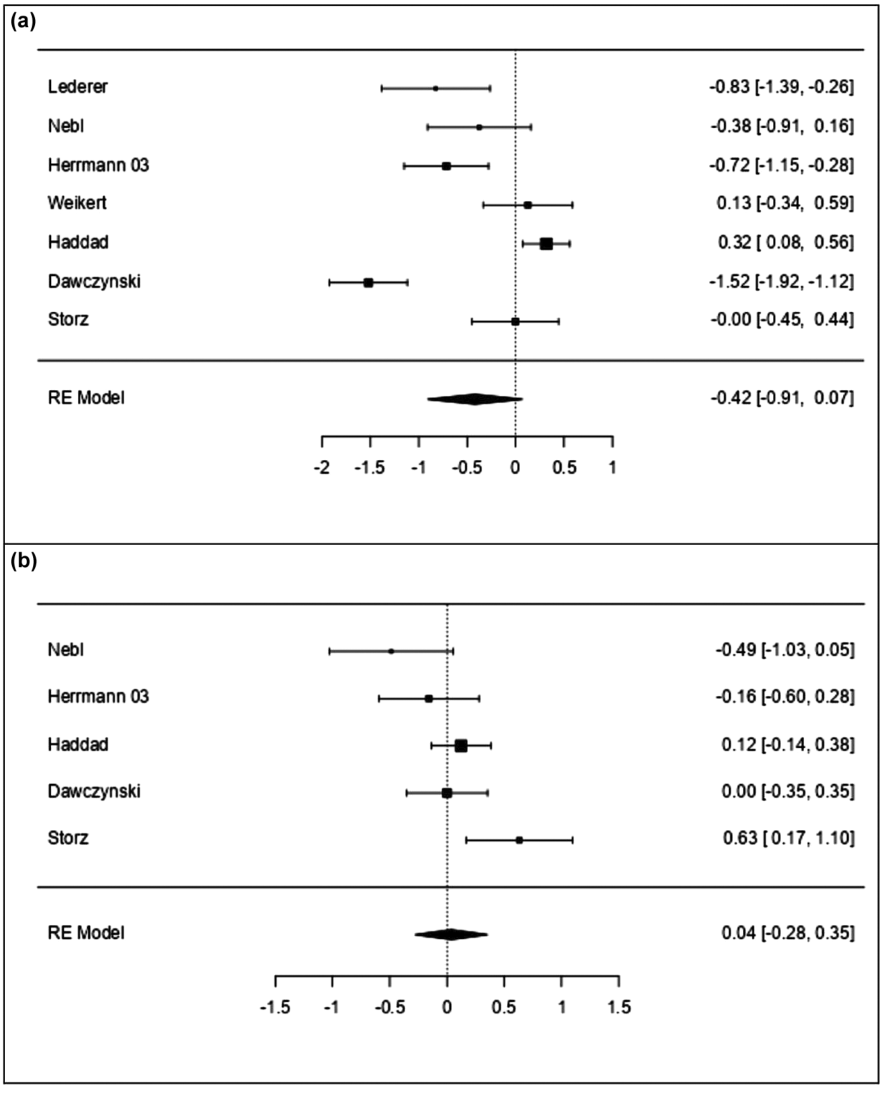

FIGURE 3 Forest plots illustrating the standardised mean difference effect of holotranscobalamin (HoloTC) status using the random-effects model with outliers removed. Subfigures 3a and 3b show the standardised mean difference between the vegans and omnivores ($p=0.093$, $I^2=89.7\%$) and between vegans and vegetarians ($p=0.814$, $I^2=68.8\%$). (a) Comparison of HoloTC status between vegan (left) and omnivorous (right) participants. (b) Comparison of HoloTC status between vegan (left) and vegetarian (right) participants.

biomarker for identifying suboptimal vitamin B12 status in individuals on plant-based diets (Herbert, 1994; Lederer et al., 2019).

Vegans demonstrated the highest mean level of tHcy (13.4 µmol/L), followed by vegetarians (12.5 µmol/L) and omnivores (9.7 µmol/L) (Table 2). These results agree with a previous systematic review by Obersby et al. (2013) that reported that vegans had the highest mean tHcy of 16.41 µmol/L, followed by vegetarians with 13.91 µmol/L, and omnivores with 11.03 µmol/L. In healthy adults, the reference range for plasma tHcy is 5–13 µM. tHcy >13 µM in folate-fortified populations and tHcy>15 µM in non-folate-fortified populations are classified as hyperhomocysteinaemia (Refsum et al., 2004). tHcy is not a specific biomarker for vitamin B12 as it responds also to folate status, and additionally, age, sex and renal function could all affect tHcy levels independently of vitamin B12 status (Rehman et al., 2020). Because healthy vegetarians and vegans generally have an adequate intake of folate (Neufingerl & Eilander, 2021), the elevated Hcy is attributable to low B12 intake.

An MMA >260 nmol/L is generally considered an indication of B12 insufficiency/deficiency in adults (Clarke et al., 2003; Hannibal et al., 2016). The reported mean values of MMA in our study suggest that vegans, irrespective of whether they are using vitamin B12 supplements, exhibit a clearly elevated MMA profile and approaching B12 deficiency (340 and 485 nmol/L, respectively); MMA was within the normal range in both vegetarians and omnivores. Often, MMA is used as a specific marker of vitamin B12 status; however, it should be stated that MMA levels are also influenced by renal function and, to a lesser degree, degradation of odd-chain fatty acids, cholesterol and some amino acids (Bråtveit et al., 2024; Herrmann et al., 2001). Nevertheless, the elevation of MMA in vegans in combination with other biomarkers indicates suboptimal B12 status in this group, which, if left unaddressed, could progress to vitamin B12 deficiency.

Finally, our analysis of the SMD for all biomarkers across diets confirmed that the omnivore group exhibited the highest vitamin B12 status marker levels compared to both vegetarian and vegan groups. Differences in B12 status between vegetarians and vegans were well captured in SB12 and Hcy but not in HoloTC and MMA. Interestingly, SB12, HoloTC and MMA describe distinct B12 statuses between supplemented and unsupplemented vegans, but this was not true for Hcy.

Effect of cobalamin supplements on biomarkers of vitamin B12 status

Vegans who use vitamin B12-containing supplements exhibit more adequate biomarker values compared to their unsupplemented counterparts. Notably, the substantial heterogeneity observed across the three dietary groups was markedly reduced for each biomarker when comparing vegan vitamin B12 supplement users to non-users. Although this analysis was limited to only 4 studies as opposed to 19, which would in itself limit the degree of heterogeneity being introduced, our results do suggest that supplementation status can and does play a role in explaining the differences in biomarker levels and underscore the importance of accounting for supplement use in studies assessing nutritional status across different dietary practices. This reduction in heterogeneity strengthens the argument that vitamin B12 supplementation is an important factor in mitigating the risks associated with a vegan diet and highlights the need for more detailed data collection on supplementation habits in future research.

The prevalence of vitamin B12 supplement use is generally greater among vegans compared with vegetarians. Previous reports show that up to 71% of vegans versus 41% of vegetarians use vitamin B12-containing supplements (Henjum et al., 2023; Schupbach et al., 2017). In a study that examined supplement use practices among individuals adhering to long-term plant-based diets, fewer vegetarians consumed vitamin B12 supplements compared to vegans (51% vs. 90%) (Storz et al., 2023). Vegans who used B12-containing supplements exhibited mean SB12 concentrations comparable to those of vegetarians; however, the amount of B12 taken in supplemental form by vegans included in this analysis may have been insufficient to support an optimal vitamin B12 status based on all four biomarkers. Here, it is important to emphasise that there are numerous brands and various formulations of vitamin B12 supplements available in the market and that the 'quality' of most remains an open question. A number of vitamin B12 supplements incorporate cyanobacteria that often contain pseudo-vitamin B12 or B12 analogues (Watanabe, 2007). These forms of vitamin B12 provide no benefits as they are biologically/functionally inert in humans. While no chemical form of vitamin B12 is superior for the supplementation of genetically healthy individuals (Obeid et al., 2015; Paul & Brady, 2017), the market offers a variety of forms that include methylcobalamin, adenosylcobalamin, hydroxocobalamin and cyanocobalamin, either individually or in combination. A point of concern is the lack of guidelines on what would be an adequate supplement quantity to support metabolism in diets devoid of B12. An expert consensus report indicated that 4–20 μg of B12/day is adequate to prevent B12 deficiency in healthy individuals (Niklewicz et al., 2023). A recent study based on long-term omnivores, vegetarians and vegans calculated that a total intake (dietary + supplemental) of 250 μg B12/day for 1 year among healthy vegans was sufficient to support a B12 status comparable to that of healthy omnivores (Storz et al., 2023). While satisfactory to support an adequate B12 status, this daily intake is likely excessive. Dose-finding studies are thus required to determine the optimal dose and frequency of B12 supplementation necessary to support an adequate B12 status in vegans.

Vitamin B12 status among vegan women of childbearing age: A neglected area of research

The vitamin B12 status of pregnant and lactating vegan women has not been investigated to date. Our literature analysis identified only one study by Waldmann et al. (2004) that presented data on vitamin B12 status for each sex, including women of childbearing age. The study (Waldmann et al., 2004) found that non-supplemented vegan women were vitamin B12-deficient with an SB12 value of 113 pmol/L and elevated tHcy levels of 17.3 μmol/L, indicating hyperhomocysteinaemia (Son & Lewis, 2022). Analysis of blended data for vegetarians and vegans followed a similar pattern, yet with slightly greater values (SB12 = 151 pmol/L and tHcy = 14.3 μmol/L). Results from newborn screening analysis paired with maternal B12 status examination in Germany revealed that maternal B12 deficiency occurred predominantly due to unknown origin (56%), dietary causes (32%) and organic causes (8%) (Reischl-Hajiabadi et al., 2022). Results from the German study showed that all mothers on a vegan diet and most of those on a vegetarian diet took vitamin supplements during pregnancy, and only 55.8% of mothers with a balanced omnivore diet took folic acid or other vitamins (Reischl-Hajiabadi et al., 2022). The frequency of vitamin supplement use amongst those consuming plant-based diets during pregnancy varies by country. A study in Portugal examining 12 newborns who were exclusively breastfed, asymptomatic for B12 deficiency, yet presenting with elevated Hcy and/or MMA, revealed that a non-supplemented vegetarian diet was the primary cause of maternal B12 deficiency (9 of 12 causes), followed by pernicious anaemia (3 of 12 cases) (Lipari Pinto et al., 2022). A nationwide screening of over 0.5

million newborns in Spain showed that of the total diagnosed newborn B12 deficiencies, maternal vegetarian diets or poor eating accounted for 5% of cases, and pernicious anaemia explained 15% of cases (Martín-Rivada et al., 2022). Importantly, retrospective studies showed that the type of testing performed by newborn screening platforms does not capture B12 deficiencies that manifest clinically in the infant stage, that is beyond the neonatal phase (Ljungblad et al., 2022; Tangeraas et al., 2023). Based on the available literature and according to our findings, we anticipate that vegan and vegetarian mothers and foetuses, particularly those not using an adequate cobalamin supplementation dose/regimen, entering trimester 1 would encounter a much higher risk of functional vitamin B12 deficiency as pregnancy progresses and during lactation. These projections have grave public health implications for this specific but growing stratum of the population. They highlight the importance of closely monitoring B12 status during pregnancy and lactation using a battery of functional vitamin B12 biomarkers. This monitoring should be accompanied by risk-mitigating guidelines and policy recommendations to ensure adequacy of vitamin B12 status at the start and throughout pregnancy and lactation to prevent adverse health outcomes for both the mother and foetus.

Strengths and limitations of our study

In this meta-analysis, we employed study-level data. While we ensured standardisation of our analyses across studies, it is important to note that study-specific factors would invariably introduce heterogeneity into the study. These include reporting and availability of data (some studies failed to provide the necessary mean and standard deviation for the meta-analysis calculation) as well as participant characteristics (demographic, health status, duration of diet and compliance with supplementation), as well as methodological differences, including differences in the assays used.

We acknowledge that the approach of assuming the median values to be approximately equal to the mean has limitations due to the potential influence of outliers and discrepancies between these values. However, efforts were made to minimise these discrepancies by appropriately standardising the values and contacting the authors for more information to address this concern, although many did not respond nor have the available data. Another limitation relates to the results on some of the funnel plots, indicating that the overall effect sizes might have been exaggerated due to publication bias. To mitigate this, a systematic search of the literature was undertaken, using a variety of databases. Despite conducting a comprehensive search, the number of eligible studies included in the systematic review and meta-analysis was relatively small (19 for the systematic review and 17 for the meta-analysis). This limited the pool of studies and restricted the generalisability of the findings.

Furthermore, no older adults were included in the analysis, and the studies were limited to Caucasian populations. Additionally, the duration of adherence to a vegan diet was not a criterion for inclusion or exclusion. These factors further limit the generalisability and applicability of our findings across different populations and dietary patterns.

Strengths of this meta-analysis include a comprehensive search strategy conducted through a search of multiple databases (PubMed, Web of Science and Scopus) to identify relevant studies. This ensured that as many relevant studies were included as possible, minimising the risk of missing essential data. Additionally, the comprehensive inclusion and exclusion criteria used to screen the articles helped to ensure that only studies meeting specific criteria were included, enhancing the quality and relevance of the data included in your analysis. The results of the meta-analysis were consistent with previous literature and showed significant trends towards lower SB12 concentrations and higher MMA and tHcy levels among unsupplemented vegan participants compared to omnivores. This consistency strengthens the validity of the results and supports the conclusion that there is a significant decrease in functional vitamin B12 status among unsupplemented vegans compared to omnivores. Finally, this is the first study to specifically highlight vitamin B12 status differences among vegans, vegetarians and omnivores using functional as well as static biomarkers. An important limitation highlighted by our study is that among studies examining vitamin B12 status in adult individuals adhering to plant-based diets, none have investigated B12 status among vegan pregnant and lactating women. Nevertheless, based on knowledge of the time course of vitamin B12 status in omnivore women during pregnancy and lactation, we estimate that women of childbearing age adhering to vegan and vegetarian diets are at elevated risk of developing B12 deficiency, which can increase the risk of B12 deficiency in the child.

Future work and recommendations

As stated above, we would advise those who follow a vegan and vegetarian diet to ensure they follow an appropriate vitamin B12 supplementation regimen/dose and opt to select plant-based alternative foods adequately fortified with vitamin B12. As mentioned previously by Niklewicz et al. (2023), the use of GMC-certified supplements is highly recommended as they will contain formulations and dosing that are reliable. To ensure optimal health and prevent long-term complications associated with vitamin B12 deficiency or insufficiency, individuals following plant-based diets should be vigilant about their B12 status. Regular monitoring of vitamin B12 status using a variety of biomarkers, including MMA, HoloTC, tHcy and SB12, may be necessary to identify early signs of functional impairment and enable appropriate interventions, including vitamin B12 supplementation or dietary modifications.

Public health efforts should aim to raise awareness about the risks and benefits of both vegan and vegetarian diets, emphasising the importance of maintaining a balanced dietary intake that can include the consumption of adequately fortified products and/or an appropriate regimen of supplement use. This is particularly crucial for women of childbearing age adhering to a vegan or vegetarian diet, including those who are planning pregnancies or are lactating, as they are at a generally higher risk of vitamin and mineral inadequacies and deficiencies, including but not limited to vitamin B12. Additionally, healthcare providers should be aware of the potential for vitamin B12 deficiency in vegans and vegetarians and consider screening for it, even in the absence of typical haematological markers. Early detection and intervention can help prevent or manage the health consequences associated with vitamin B12 deficiency.

CONCLUSIONS

In summary, this meta-analysis, utilising functional and static biomarkers, further confirms previous findings that vegan adults present lower vitamin B12 status and increased functional B12 deficiency than non-vegans. The analysis also demonstrates that vitamin B12 supplementation is associated with improved vitamin B12 status among vegans. Moreover, vegans and vegetarians are susceptible to vitamin B12 insufficiency, which may not be evident solely through serum vitamin B12 measurements. Therefore, the inclusion of both functional and static biomarkers is crucial for the accurate assessment of B12 status. Randomised controlled studies are necessary to outline a supplementation regimen aimed at sustaining B12 adequacy in these populations. In consideration of the findings, we provide clear advice for all vegans to ensure adequate vitamin B12 exposure either through the use of GMC vitamin B12-containing supplements in combination with a diet that contains adequate vitamin B12-fortified foods to sustain both planetary and human health.

AUTHOR CONTRIBUTIONS

A. N: Methodology, Formal analysis, Writing of original Draft. L. H: Writing, Review and Editing. M. J. W: Review and Editing. K. R. A: Conceptualisation, Methodology, Writing of original Draft, Supervision. All authors provided critical input and feedback, which contributed to the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

On request, the extracted data used for analysis can be made available.

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SUPPORTING INFORMATION

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