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Systematic Review

Plasma total homocysteine status of vegetarians compared with omnivores: a systematic review and meta-analysis

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Abstract

There is strong evidence indicating that elevated plasma total homocysteine (tHcy) levels are a major independent biomarker and/or a contributor to chronic conditions, such as CVD. A deficiency of vitamin B_{12} can elevate homocysteine. Vegetarians are a group of the population who are potentially at greater risk of vitamin B_{12} deficiency than omnivores. This is the first systematic review and meta-analysis to appraise a range of studies that compared the homocysteine and vitamin B_{12} levels of vegetarians and omnivores. The search methods employed identified 443 entries, from which, by screening using set inclusion and exclusion criteria, six eligible cohort case studies and eleven cross-sectional studies from 1999 to 2010 were revealed, which compared concentrations of plasma tHcy and serum vitamin B_{12} of omnivores, lactovegetarians or lacto-ovovegetarians and vegans. Of the identified seventeen studies (3230 participants), only two studies reported that vegan concentrations of plasma tHcy and serum vitamin B_{12} did not differ from omnivores. The present study confirmed that an inverse relationship exists between plasma tHcy and serum vitamin B_{12}, from which it can be concluded that the usual dietary source of vitamin B_{12} is animal products and those who choose to omit or restrict these products are destined to become vitamin B_{12} deficient. At present, the available supplement, which is usually used for fortification of food, is the unreliable cyanocobalamin. A well-designed study is needed to investigate a reliable and suitable supplement to normalise the elevated plasma tHcy of a high majority of vegetarians. This would fill the gaps in the present nutritional scientific knowledge.

Key words: Hyperhomocysteinaemia: Vitamin B_{12}: Vegetarians: Omnivores

There are approximately four million vegetarians within the UK population[1]. In addition 5 % of British adults are practising semi-vegetarians, whose diet contains a greatly reduced intake of products of animal origin[2]. Worldwide, there are 75 million vegetarians by choice and 1450 million by necessity[3]. This agrees with the Foods Standards Agency[4] approximation of 25 % of the world's population consuming a largely vegetarian diet. The most commonly known vegetarians are vegan, lactovegetarian (LV) and lacto-ovovegetarian (LOV).

Hyperhomocysteinaemia (>15 μmol/l, as defined by Ravaglia *et al.*[5]) has been shown to be linked with chronic conditions, among which is CVD[6,7]. Other studies have shown that CHD is linked to homocysteine concentrations, with a substantial risk occurring at >10 μmol/l plasma total homocysteine (tHcy)[8,9]. Furthermore, each 5 μmol/l increase in plasma tHcy is associated with an approximate 20 % increased risk of CHD events[6,7], irrespective of the diet. The present review sets out to determine the homocysteine and vitamin B_{12} status of vegetarians compared with omnivores, as they may be a group of the population who may have the potential to be at greater risk than omnivores to these homocysteine-related diseases. This is due to the lack of intake of animal produce, the only natural abundant source of vitamin B_{12}, whose deficiency can raise homocysteine levels[10,11]. Vitamin B_{12} is required in the important remethylation pathway, where homocysteine is remethylated to methionine in a reaction catalysed by the enzyme methionine synthase and the cofactor vitamin B_{12}[12], but only in its

Abbreviations: LV, lactovegetarian; LOV, lacto-ovovegetarian; tHcy, total homocysteine; THF, 5-methyl tetrahydrofolate.

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methylcobalamin form[13]. Homocysteine acquires a methyl group from 5-methyl tetrahydrofolate (THF), which is catalysed from 5,10-methyleneTHF by the enzyme methylenetetrahydrofolate reductase and folic acid/folate from the diet, which enters the remethylation pathway as THF, via dihydrofolate acid, which has been reduced to THF by the enzyme dihydrofolate reductase. The exact pathomechanisms of cobalamin deficiency that cause the typical clinical symptoms of vitamin B_{12} deficiency, especially the neurological symptoms, in human subjects have not been fully clarified. The methyl folate trap hypothesis[14–16] has been widely accepted over decades, despite the difficulty in testing the theory in any meaningful way. The methyl trap hypothesis proposes that due to a vitamin B_{12} deficiency, folate can be trapped as methylfolate, which is metabolically inactive. This is due to the fact that vitamin B_{12} is required for the transfer of the methyl group from 5-methylTHF to form THF, so that it can return to the tetrahydrofolate pool for conversion to 5,10-methyleneTHF. As the transfer of the methyl group of 5-methylTHF to homocysteine is impaired in vitamin B_{12} deficiency, it results in a rise in homocysteine levels[17,18].

The RDA for vitamin B_{12} is $2{\cdot}5\,\mu g$[19], of which the body stores considerable amounts (several mg) in the liver. The body recycles approximately 75 % of vitamin B_{12} it uses; serum vitamin B_{12} starts to decline and plasma tHcy rises when the absorption of the ingested vitamin B_{12} input is less than that dissipated by the body[20]. Thus, a delay of 5–10 years may separate the beginning of a vegetarian diet and the onset of deficiency symptoms that usually occur when serum vitamin B_{12} is reduced to below 150 pmol/l, which marks the onset of pernicious anaemia[21].

It is also noted that cell-surface receptors located in the ileum require free Ca to enable the vitamin B_{12} absorption – intrinsic factor complex to aid absorption of vitamin B_{12}[22–24].

Lack of Ca in the vegetarian diet could, therefore, inhibit vitamin B_{12} absorption[22–24]. Prolonged Fe deficiency damages the gastric mucosa and promotes atrophic gastritis and gastric atrophy, including loss of gastric acid and intrinsic factor secretion and, therefore, diminished vitamin B_{12} absorption. As vegetarians have reduced Fe intake[25–27], this would cause vitamin B_{12} deficiency[28]. Furthermore, vitamin B_{12} is excreted in the presence of high levels of soluble fibre (such as pectin), probably via an effect on the enterohepatic cycle of vitamin B_{12}, a common feature of vegetarian diets[29]. Furthermore, vegetarian diets contain high levels of n-6 PUFA, whilst they are low in n-3 PUFA. This imbalance, together with inherent low vitamin B_{12} levels and consequential high concentrations of plasma tHcy, can be shown to have a thrombotic tendency that raises the risk of developing CVD[30].

Hypothesis and objective

The hypothesis is that there is a correlation between levels of plasma tHcy and the intake of dietary animal produce, the only natural abundant source of vitamin B_{12}.

The main objective of the present systematic review and meta-analysis is, therefore, to assess the plasma tHcy and serum vitamin B_{12} status of LV–LOV and vegans, as compared with omnivores, from a wide range of cohort and cross-sectional published studies that have met the set criteria.

Materials and methods

Electronic searches

The search engines selected were PubMed, as it contains entries from MEDLINE, EMBASE, JAMA, BMJ, Cochrane Databases and Lancet, together with Science Direct, ACP Journal Club, CCTR, AMED, Highwire Press and EBSCO host databases. A search for systematic reviews, meta-analyses, cohort case studies, cross-sectional studies and randomised controlled trials was carried out using the search terms 'Hyperhomocysteinemia'; 'Vitamin B_{12}'; 'Omnivores and vegetarians'; and 'Supplementation with vitamin B_{12} to normalise homocysteine in vegetarians'; this revealed 443 entries for studies undertaken during the period from January 1999 to June 2011.

Participants

In the studies examined, omnivores were defined as individuals who consumed both plant and animal products. LV were defined as individuals who did not consume animal produce, but consumed dairy products. LOV had the same diet as LV, but they consumed eggs too. Vegans were defined as individuals who abstained from all types of animal products and semi-vegetarians were defined as individuals who occasionally included animal products in their diet.

Inclusion and exclusion criteria

The flow chart in Fig. 1 outlines the initial inclusion and exclusion criteria employed in the selection of six cohort case studies and eleven cross-sectional studies; this was followed by these studies being finally assessed by one author and checked by another for methodological validity employing standardised data extraction tools from JBI000308[31] with any disagreements being resolved through discussion with a third reviewer. All initially screened studies met these requirements and are included in the present systematic review and meta-analysis, and summarised in Table 1.

Statistical analyses

Data from the selected seventeen studies in the vast majority of cases have been calculated as mean values for each diet group. In the case of the small number of cases that employed median values, it has been assumed in the calculations that these are approximately equal to the mean value, and that, in the case of two studies, the small number of vegans has been included in the LV–LOV group. As the number (n) is <30 for the group of the studies, comparison between groups has been undertaken by Student's two-tailed unpaired test to determine the significant difference between the values of plasma tHcy and serum vitamin B_{12} for LV–LOV and vegans against omnivores[32]. Table 2 summarises the calculated values.

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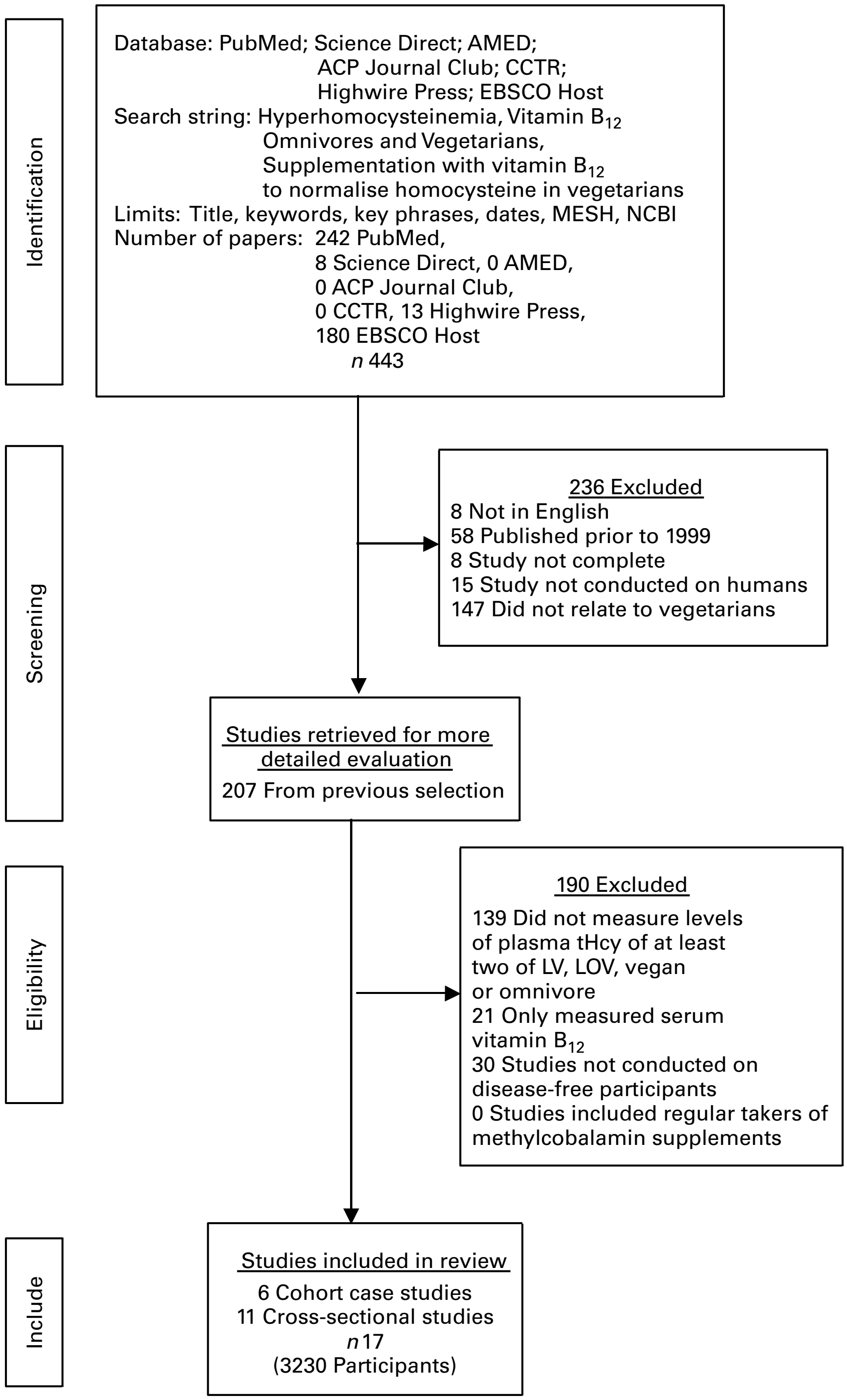

Fig. 1. Flow chart. Initial inclusion and exclusion criteria for selected studies for plasma total homocysteine (tHcy) and serum vitamin B_{12} status of omnivores, lactovegetarians (LV) or lacto-ovovegetarians (LOV) and vegans.

Results

Of a total of 443 entries, the search revealed six cohort case studies and eleven cross-sectional studies, as summarised in Table 1.

Table 2 demonstrates that the primary outcome of the meta-analysis is that an inverse relationship exists between plasma tHcy and serum vitamin B_{12} for all three diets, indicating that vegans have the highest mean plasma tHcy value of 16·41 (SD 4·80) μmol/l as well as the lowest mean serum vitamin B_{12} value of 172 (SD 59) pmol/l.

LV–LOV exhibited a mean plasma tHcy value of 13·91 (SD 3·15) μmol/l and a mean serum vitamin B_{12} value of 209 (SD 47) pmol/l. Omnivores recorded a mean plasma tHcy value of 11·03 (SD 2·89) μmol/l and a mean serum vitamin B_{12} value of 303 (SD 72) pmol/l. Fig. 2 indicates the

Table 1. Details of the selected studies of plasma total homocysteine (tHcy) and serum vitamin B_{12} status among omnivores, lactovegetarians or lacto-ovovegetarians and vegans (1999–2010)
(Mean values and standard deviations; medians, 5th–95th percentiles and 25th–75th percentiles; geometric mean values and 95 % confidence intervals)

				Plasma tHcy (μmol/l)						Serum vitamin B_{12} (pmol/l)					
Study, date of publication and sex	Volunteers (*n*)	Average age (years)	Duration of being vegetarian (years)	Mean	SD	Median	5th–95th percentiles	25th–75th percentiles	95 % CI	Mean	SD	Median	5th–95th percentile	25th–75th percentiles	95 % CI
Haddad *et al.* (1999)[34]															
Omnivores*	20	33·5		8·0	1·9					313	99				
Vegans*	25	36·0	> 1	7·9	1·5					312	125				
Mann *et al.* (1999)[39]															
Omnivores†	18	34·2		11·0	2·5					403	169				
Lactovegetarians or lacto-ovovegetarians†	43	34·9	Not stated	15·8	9·3					211	98				
Vegans†	18	33·0	Not stated	19·2	10·7					145	68				
Krajcovicova-Kudlackova *et al.* (2000)[40]															
Omnivores‡	59	40·9		10·2	0·3					345	8·2				
Lactovegetarians or lacto-ovovegetarians‡	62	35·1	8·5 Mean	13·2	0·3					215	5·1				
Vegans‡	32	41·5	8·5 Mean	15·8	0·9					140	4·9				
Herrmann *et al.* (2001)[41]															
Omnivores‡	44	23·0				9·8	5·9–16·7					276	172–406		
Lactovegetarians or lacto-ovovegetarians‡	34	22·0	> 1			11·0	5·7–20·8					253	153–376		
Vegans‡	7	22·0	> 1			15·2	9·3–18·5					217	153–438		
Refsum *et al.* (2001)[45]															
Omnivores*	126	41·0				19·4	9·7–45·7					161	62–492		
Lactovegetarians or lacto-ovovegetarians*	78	41·0	Not stated			22·0	9·6–48					124	66–625		
Hung *et al.* (2002)[50]															
Omnivores§	45	38·0		8·6	2·0					404	139				
Lactovegetarians or lacto-ovovegetarians§	45	38·0	> 2	11·2	4·3					208	127				
Cappuccio *et al.* (2002)[33]															
Omnivores†	583	50·7		11·2‖					11, 11·5				Not applicable		
Lactovegetarians or lacto-ovovegetarians†	46	50·7	Not stated	15·1‖					14, 16·4				Not applicable		
Omnivores§	669	50·7		8·9‖					8·7, 9·1				Not applicable		
Lactovegetarians or lacto-ovovegetarians†	92	50·7	Not stated	11·5‖					10·8, 12				Not applicable		
Bissoli *et al.* (2002)[35]															
Lactovegetarians or lacto-ovovegetarians*	14	48·5	> 5	17·4	11·1					164	57				
Vegans	31	45·8	> 5	26·9	24·1					155	74				
Huang *et al.* (2003)[43]															
Omnivores‡	32	22·9		9·8‖					9·1, 10·6	311‖					278, 343
Lactovegetarians or lacto-ovovegetarians‡‖	37	28·9	> 1	13·2‖					10·6, 15·7	192‖					164, 220
Herrmann *et al.* (2003)[36]															
Omnivores*	79	46·0				8·8	5·5–16·1					287	190–471		
Lactovegetarians or lacto-ovovegetarians*	53	40·0	> 1			10·9	6·8–28·2					179	124–330		
Vegans*	12	39·0	> 1			14·3	6·5–52·1					126	92–267		

Table 1. *Continued*

				Plasma tHcy (μmol/l)						Serum vitamin B_{12} (pmol/l)					
Study, date of publication and sex	Volunteers (*n*)	Average age (years)	Duration of being vegetarian (years)	Mean	SD	Median	5th–95th percentiles	25th–75th percentiles	95 % CI	Mean	SD	Median	5th–95th percentile	25th–75th percentiles	95 % CI
Waldmann *et al.* (2003)[46]															
Lactovegetarians or lacto-ovovegetarians*	45	44·6	>1			12·3	4·6–23·6					185	97·6–689		
Vegans*	86	43·8	>1			13·4	6·0–82·5					122	71·2–276		
Koebnick *et al.* (2005)[37]															
Omnivores*	109	44·5				14·7		12–18·3				175		142–250	
Lactovegetarians or lacto-ovovegetarians*	38	44·5	>1			17·1		13–20·2				143		121–176	
Vegans*	39	44·5	>1			18·5		13·5–29				126		88–182	
Su *et al.* (2006)[47]															
Omnivores§	61	57·7		9·0	2·1					380	199				
Lactovegetarians or lacto-ovovegetarians§	57	59·2	>5	11·0	3·3					265	179				
Majchrzak *et al.* (2006)[42]															
Omnivores*	40	38·4		12·2	5·6					252	83				
Lactovegetarians or lacto-ovovegetarians*	36	34·2	<1 to >5							239	99				
Vegans*	42	30·7	<1 to >5	14·0	5·4					203	102				
Karabudak *et al.* (2008)[48]				16·5	8·2										
Omnivores§	26	27·4		10·8	3·7					269	235				
Lactovegetarians or lacto-ovovegetarians§	26	29·0	10·5 ± 6·7							201	137				
Yen *et al.* (2010)[38]				12·6	6·0										
Omnivores*	28	35·9		9·6	2·2					359	138				
Lactovegetarians or lacto-ovovegetarians*¶	21	34·8	>0·5							307	267				
Krivosikova *et al.* (2010)[49]				10·4	5·7										
Omnivores§	131	40·8		12·5	4·5					306	137				
Lactovegetarians or lacto-ovovegetarians§	141	41·9	Not stated	16·5	5·6					247	161				

* Mixed population of adult volunteers.
† Male adult volunteers only.
‡ Sex of volunteers not stated.
§ Female adult volunteers only.
|| Geometric mean.
¶ Combined measured levels of plasma tHcy and serum vitamin B_{12} of lactovegetarians or lacto-ovovegetarians and vegans.

Table 2. Plasma total homocysteine (tHcy) and serum vitamin B_{12} levels of lactovegetarians or lacto-ovo vegetarians and vegans compared with omnivores from the selected seventeen studies shown in Table 1 (study by Cappuccio *et al.*[33] omitted)

(Mean values and standard deviations)

	Plasma tHcy (μmol/l)				Serum vitamin B_{12} (pmol/l)			
Diet	Mean	SD	*n*	*P*	Mean	SD	*n*	*P*
Omnivores	11·03	2·89	14		303	72	14	
Lactovegetarians or lacto-ovovegetarians	13·91	3·15	15	<0·025	209	47	15	<0·005
Vegans	16·41	4·80	9	<0·005	172	59	9	<0·005

relationship between plasma tHcy and serum vitamin B_{12} for the three diets.

Statistical heterogeneity

The null hypothesis states that the mean values of plasma tHcy and serum vitamin B_{12} for omnivores, LV–LOV and vegans are homogeneous. Table 3 reports the results of an ANOVA, which demonstrates that the null hypothesis can be rejected.

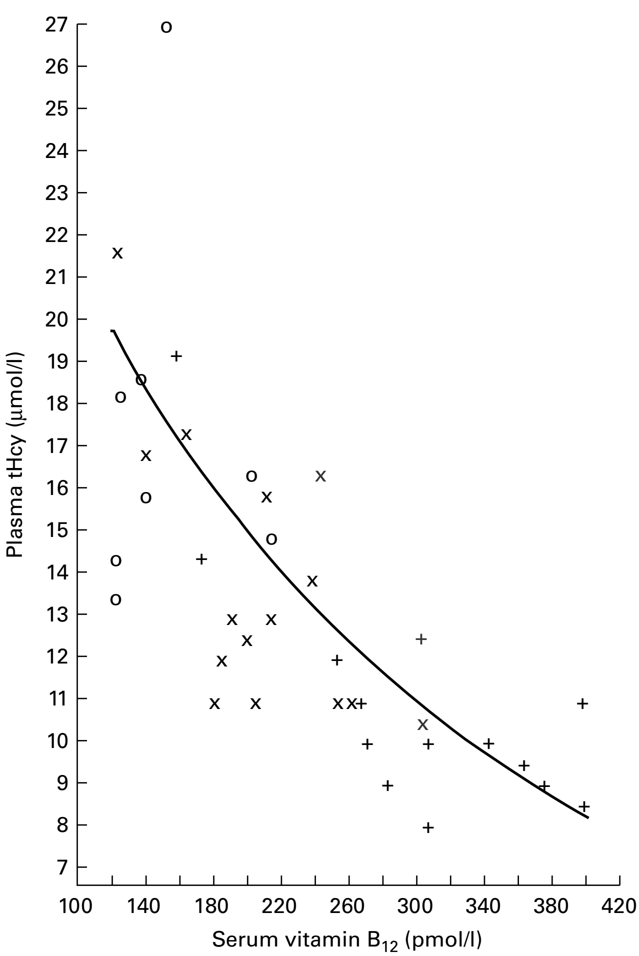

Fig. 2. Correlation between plasma total homocysteine (tHcy) and serum vitamin B_{12} for omnivores, lactovegetarians (LV) or lacto-ovovegetarians (LOV) and vegans, with median values approximated to be equal to mean values of five studies[36,37,41,45,46] and combined measured levels of plasma tHcy and serum B_{12} of LV or LOV and vegans of two studies[38,43] from 1999 to 2010 (study by Cappuccio *et al.*[33] excluded) taken from Table 1. $Y = 24{\cdot}57\,e^{-0{\cdot}003x}$ (R^2 0·598). +, Omnivores; x, LV–LOV; ○, vegans.

Discussion

A total of fifteen of the seventeen selected studies that met the inclusion and exclusion criteria show a good agreement that serum vitamin B_{12} and plasma tHcy exhibit an inverse relationship. The study by Cappuccio *et al.*[33] did not monitor serum vitamin B_{12} levels. The study by Haddad *et al.*[34] concluded that, statistically, vegans had similar plasma tHcy to omnivores (i.e. 8·0 against 7·9 μmol/l, respectively) and serum vitamin B_{12} levels (i.e. 313 against 312 pmol/l, respectively). In this case, it was noted that 36 % of the participating vegans were users of vitamin B_{12} supplements, although the type, dosage and frequency of usage were not reported. Nevertheless, this could confound the statistics, which have given a result that is incompatible with the other fifteen studies. Furthermore, a small proportion of LV–LOV and vegans were found to be consuming vitamin B_{12} supplements and/or consuming vitamin B_{12}-fortified foods in the studies conducted by Bissoli *et al.*[35], Herrmann *et al.*[36] and Koebnick *et al.*[37]. None of these studies recorded details regarding type, dosage and frequency of consumption. The conclusion reached by the respective researchers was that fortification does not lower plasma tHcy or increase serum vitamin B_{12} levels significantly. The remaining studies stated that no vitamin B_{12} supplements had been used by the participants. Yen *et al.*[38] concluded that vegetarian parents and their preschool children had lower vitamin B_{12} intake than omnivorous parents and their preschool children, but had similar vitamin B_{12} and homocysteine concentrations. As far as the adults are concerned, these results are incompatible with the observations of six studies that compared LV–LOV, vegan and omnivores' serum vitamin B_{12} and plasma tHcy levels[36,37,39–42]. Huang *et al.*[43] concluded that vegetarians have lower vitamin B_{12} status than omnivores, leading to raised plasma tHcy, and that vitamin B_6 and folate have little effect on plasma homocysteine concentration when individuals have adequate vitamin B_6 and folate status. Kluijtmans *et al.*[10] and Selhub[11] demonstrated that hyperhomocysteinaemia can be caused by a deficiency of folate. They also demonstrated that, normally, homocysteine elevation is much less affected in cases of vitamin B_6 deficiency. However, Majchrzak *et al.*[42] have shown that in vegetarian diets and, particularly, in vegan diets, which contain relatively high levels of folate, folate deficiency is unlikely to occur, whereas omnivore diets are more predisposed to this. An exception to this is possibly seen in India, where traditionally folate deficiency has been linked to poverty, which may cause problems, with 33 % of the population being vegetarians by necessity[44]. There is strong

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Table 3. ANOVA table for differences of plasma total homocysteine and serum vitamin B_{12} between omnivores and vegans and omnivores and lactovegetarians or lacto-ovovegetarians*

	Sum of squares	df	Mean square	P	$F_{\alpha, 2, 35}$	Outcome
Plasma total homocysteine						
Between groups	164·124	2	82·062	6·033	3·267	Significant: $P<0·01$
Within groups	476·087	35	13·602			
Total	640·211	37				
Serum vitamin B_{12}						
Between groups	112 389	2	56 195	14·42	3·267	Significant: $P<0·001$
Within groups	136 349	35	3896			
Total	248 738	37				

* Mean values utilised from Table 2.

evidence, with a significance of $P<0·005$ from four studies that, compared with omnivores, a large proportion of vegans develop hyperhomocysteinaemia (>15 μmol/l plasma tHcy) and serum vitamin B_{12} deficiency (≤150 pmol/l)[37,39–41]. Furthermore, a significant proportion of LV–LOV subjects in the study conducted by Koebnick *et al.*[37] showed a hyperhomocysteinaemia condition, with a strong significance of $P<0·001$. A total of ten studies[35,40–43,46–50] reported that vegans and/or LV–LOV were found to have plasma tHcy >10 μmol/l and serum vitamin B_{12} of >150 pmol/l (i.e. not deficient[21]), although vegan and LV–LOV levels of serum vitamin B_{12} were substantially lower than omnivores, with mean values of 172 and 209 against 303 pmol/l, respectively (Table 2). This was generally in accordance with studies conducted by Joosten *et al.*[51] and Herrmann *et al.*[52].

Refsum *et al.*[45] reported that, in India, both omnivores and LV–LOV have high plasma Hcy levels (i.e. 19·4 *v.* 22·0 μmol/l, respectively), indicating hyperhomocysteinaemia together with low serum vitamin B_{12} levels (i.e. 161·0 *v.* 124·0 pmol/l, respectively). It is, however, noted that even the diet of non-vegetarians in India contains only low proportions of animal produce and hence relatively low amounts of vitamin B_{12}[52]. Also, it is noted that a high proportion of the Indian population is expected to have, in addition, folate deficiency. This could be a contributing factor for the high levels of plasma tHcy and low levels of serum vitamin B_{12} in Indian omnivores. Furthermore, most vegetarians and omnivores in India begin consuming essentially a vegetarian diet as infants, which leads to low vitamin B_{12} intake, with the only source of vitamin B_{12} coming from bacterial-contaminated food, for most of their lives[53]. India has large proportions of its population who suffer from malnutrition, tropical sprue and gastrointestinal infections, which often result in malabsorption[54–56]. It would seem reasonable to deduce that the high prevalence of vitamin B_{12} deficiency accompanied by elevated plasma tHcy can only be expected for both omnivores and LV–LOV in India.

The examined studies took steps to eliminate possible well-known confounding factors that may distort the results and were appropriately adjusted for factors such as smoking, age and sex. However, there is a minimal risk of distortion due to inter-assay and inter-population bias and variability in the present study. It can be clearly observed from Table 2 that there is an inverse relationship between plasma tHcy and serum vitamin B_{12}. Moreover, statistical evidence in Table 2 indicates that vegans have the highest mean values of plasma tHcy and the lowest mean levels of serum vitamin B_{12}. LV–LOV show intermediate levels, whereas omnivores exhibit high level of serum vitamin B_{12} and the lowest levels of plasma tHcy. This is compatible with work done by Herbert & Das[21]. Studies undertaken by Gilsing *et al.*[57], who researched British male omnivores, LV–LOV and vegans, found that 226 omnivores had mean serum vitamin B_{12} levels of 281 (95 % CI 270, 292) pmol/l, 231 LV–LOV had mean serum vitamin B_{12} levels of 182 (95 % CI 175, 189) pmol/l and 232 vegans had mean serum vitamin B_{12} levels of 122 (95 % CI 117, 127) pmol/l. Furthermore, work done by Herbert & Das[21], who studied vitamin B_{12} deficiency of LV, LOV, vegans and semi-vegetarians from the American Vegetarian Society, found that 92 % of the vegans, 64 % of the LV, 47 % of the LOV and 20 % of semi-vegetarians had serum vitamin B_{12} levels of ≤150 pmol/l, which indicates vitamin B_{12} deficiency[21].

In the research carried out by Ueland *et al.*[6], Humphrey *et al.*[7], Malinow *et al.*[8] and Ubbink[9], it was demonstrated that a substantial risk of developing CHD exists at a plasma tHcy level of >10 μmol/l and that, furthermore, each 5 μmol/l increase in plasma tHcy is associated with an approximately 20 % increase risk of CHD events. This, together with the fact that the present study indicates that there is an inverse relationship between plasma tHcy and serum vitamin B_{12}, is not unreasonable to deduce that these danger levels will be breached by some vegetarians well before they reach the deficiency level of serum vitamin B_{12} (≤150 pmol/l) and symptoms of pernicious anaemia usually occur[21]. Levels at which this could occur apply to all vegetarian groups, with exception of Haddad *et al.*[34], as can be observed in Table 1. Meta-analyses conducted by the Homocysteine Studies Collaboration[58] and Wald *et al.*[59] have demonstrated that lowering homocysteine concentrations by 3 μmol/l substantially reduces the risk of CVD. Moreover, Ward *et al.*[60] showed that there is a benefit to health in reducing the risk of primary CVD by lowering homocysteine levels. In contrast, The Heart Outcome Prevention Evaluation (HOPE 2) Investigation[61] found that supplements combining vitamin B_{12} and folic acid did not reduce the risk of major secondary cardiovascular events in patients with vascular disease.

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A further finding is that the mean overall homocysteine level of vegans shown in Table 2 of 16·41 (SD 4·80) μmol/l ($P<0·005$) and mean serum vitamin B_{12} of 172 (SD 59) pmol/l ($P<0·005$) indicates that most vegans can be classified as being likely to suffer from hyperhomocysteinaemia due to a deficiency of vitamin B_{12} that will increase their risk of developing CVD. Moreover, LV–LOV with a mean overall homocysteine level of 13·91 (SD 3·15) μmol/l ($P<0·025$) and mean serum vitamin B_{12} of 209 (SD 47) pmol/l ($P<0·005$) also have an increased risk of developing CVD. Furthermore, omnivores from the results recorded in the present review (mean plasma tHcy 11·03 (SD 2·89) μmol/l) can be considered generally to have a borderline increased risk of developing homocysteine-related CVD, probably due to inadequate status of folate[42]. Statistical tests (independent samples *t* tests and ANOVA) showed a significant difference in mean levels of tHcy and serum vitamin B_{12} between omnivores, LV–LOV and vegans. Whilst the diets of some vegetarians are aimed at the well-documented benefits of promoting health, due to the restriction or absence of food from animal origin, this as far as CVD is concerned is probably due to reduced saturated fat, lower total serum cholesterol levels, lower prevalence of obesity and slightly lower blood pressure, as compared with omnivores. However, this may not negate the risk of vegetarians with elevated plasma tHcy being susceptible to homocysteine-related CVD, as indicated by Ueland *et al.*[6], Humphrey *et al.*[7], Malinow *et al.*[8] and Ubbink[9]. The present review reveals that there is only poor evidence available of vegetarians consuming vitamin B_{12} supplements and/or vitamin B_{12}-fortified food and beverages. However, supplements, fortified food and beverages normally contain the less efficient cyanocobalamin form of vitamin B_{12}, which when it enters the bloodstream must be converted to methylcobalamin[62], the only form of vitamin B_{12} that has a methyl donor that is required to neutralise homocysteine[63]. It takes 4–9 weeks for this conversion to take place[64], assuming there are no disruptions by genetic factors, age-related problems and metabolic obstacles that may be present. Furthermore, research suggests that vitamin B_{12} that is not dissolved in the mouth will not (up to 88 %) be absorbed[65], due to the lack of R-binder mostly obtained from saliva, which is required to start the absorption process. The aforementioned study indicates that supplementation with cyanocobalamin can be poorly absorbed, which will have little or no effect on raising vitamin B_{12} levels.

A well-designed study is needed to investigate supplementary methylcobalamin by, for example, a 1 mg lozenge dissolved in the mouth (that can bypass the above potential problems), and takes advantage of absorption by mediated intrinsic factor, non-intrinsic-mediated diffusion and sublingual intake and on its affects on elevated homocysteine levels of vegetarians, who may have a resultant susceptibility to hyperhomocysteinaemia-related diseases. This would fill gaps in present nutritional scientific knowledge.

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