

Vitamin B-12 and homocysteine status among vegetarians: a global perspective¹⁻⁴

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ABSTRACT

Evidence exists that well-planned vegetarian diets provide numerous health benefits and are appropriate for all stages of the life cycle. It is also known that animal foods provide micronutrients that are nonexistent or available only in limited amounts in plant foods. Restriction or exclusion of all animal foods may therefore result in low intake of certain micronutrients such as vitamin B-12, thereby affecting vitamin B-12 status and elevating plasma homocysteine concentrations. Overall, the studies we reviewed showed reduced mean vitamin B-12 status and elevated mean homocysteine concentrations in vegetarians, particularly among vegans. Low vitamin B-12 intake may lead to decreased bioavailability and functional deficiency of cobalamin. Although early noticeable symptoms of vitamin B-12 deficiency are nonspecific (unusual fatigue, digestion problems, frequent upper respiratory infections), the best-known clinical manifestations of cobalamin malabsorption are hematologic (pernicious anemia) and neurologic symptoms. Hyperhomocysteinemia is associated with an increased risk of atherosclerosis and cardiovascular disease. Given these health concerns, vegetarians, particularly vegans, must be advised to carefully plan their diets, to monitor their plasma vitamin B-12 on a regular basis to facilitate early detection of low cobalamin status, and to use vitamin B-12-fortified foods or take vitamin B-12 supplements if necessary. *Am J Clin Nutr* 2009;89 (suppl):1693S-8S.

INTRODUCTION

Vegetarian diets are most frequently subclassified into lacto-vegetarian, ovovegetarian, lactoovovegetarian (depending whether the diet includes only dairy products, eggs, or both, respectively), and vegan diets (avoiding all animal products). Numerous studies emphasize the considerable benefits of appropriately planned vegetarian diets (1-5) and indicate that plant-based diets are associated with a reduced risk of coronary heart disease, several types of cancer, and some other chronic degenerative diseases (5-9). However, studies show that vegetarians, and in particular vegans, have lower serum vitamin B-12 and higher homocysteine concentrations than nonvegetarians, suggesting potential shortfall in nutrients when animal foods are completely avoided (10-27). The position statement on vegetarian diets points out that well-planned vegan and vegetarian diets are appropriate for all stages of the life cycle and offer a number of nutritional benefits, but it also notes that the use of fortified foods or supplements can be helpful in meeting dietary recommendations, especially in diets that completely exclude animal products (28).

In this review, we briefly discuss the metabolism, biochemical assessment of status, and dietary intake of vitamin B-12 and point to the vitamin B-12 status of vegetarians, including vegans, in different geographical regions of the world.

VITAMIN B-12: METABOLISM AND DIETARY INTAKE

Vitamin B-12 is a relatively large and complex vitamin. It is an essential micronutrient that plays a fundamental role in cell division and in one-carbon metabolism (29-31). Vitamin B-12 absorption occurs by both an active and a passive mechanism. Vitamin B-12 in food is protein bound and liberated from food protein by an active mechanism in the stomach where it binds to a salivary R-binder (family of haptocorrins). It is released again in the upper small intestine and attaches to the intrinsic factor (IF). The vitamin B-12-IF complex proceeds to the lower end of the small intestine, where it is absorbed by specific ileal receptors. The 2 main vitamin B-12 transport proteins in human plasma are haptocorrin and transcobalamin. The passive mechanism of vitamin B-12 absorption occurs equally across the absorptive surface of the gastrointestinal tract. It is an inefficient process, and only 1-2% of an oral dose can be absorbed this way. An important component of cobalamin absorption and conservation is the partial reabsorption of biliary vitamin B-12 by the enterohepatic circulation (30).

Vitamin B-12 participates as cofactor in 2 important intracellular metabolic reactions. The first of these is the mitochondrial reaction in which the enzyme methylmalonyl-CoA mutase requires cobalamin in the form of 5'-deoxyadenosylcobalamin (converting methylmalonyl-CoA to succinyl-CoA) and the second is the cytosolic reaction that requires cobalamin in the form of methylcobalamin for folate-dependent methylation of the sulfur amino acid homocysteine to form methionine, catalyzed by the enzyme methionine synthase. Methionine metabolism is regulated by vitamin B-12, folate, and vitamin B-6. The methionine synthase reaction is also necessary for normal DNA synthesis (29-31).

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Vitamin B-12 is the only vitamin that is synthesized exclusively by microorganisms. Dietary sources are primarily of animal origin, including meats, dairy products, and eggs (28). It is not supplied by plant foods unless they have been exposed to specific bacterial action (29, 30). Vegans may be able to ingest small amounts of cobalamin with the use of special microbially fermented products, but the bioavailability of vitamin B-12 in these products has yet to be clarified. There is no evidence to suggest that vitamin B-12-producing bacteria in the human large bowel can contribute substantially to the vitamin B-12 needs of the individual. Humans in rural areas may obtain some vitamin B-12 through ingestion of cobalamin contained in (feces) bacteria-contaminated plant foods (29, 32). Thus, based on our current knowledge and understanding, vegans and to a lesser degree other vegetarians are advised to use vitamin B-12-fortified foods or supplements. The current dietary recommendation for vitamin B-12 for some European countries (31, 33) and the United States (34) is presented in **Table 1**.

VITAMIN B-12 DEFICIENCY AND ASSESSMENT OF STATUS

The 2 main causes of cobalamin deficiency are inadequate dietary intake (eg, lack of foods of animal origin because of poverty, vegan diet, or both) and malabsorption (eg, because of IF deficiency or functional abnormality, chronic gastritis, atrophic gastritis, total or partial gastrectomy, ileal resection, Crohn disease, severe pancreatitis, tropical and nontropical sprue, HIV, use of several prescription drugs, and chronic alcohol abuse). Malabsorption is by far the most common cause of clinically

evident vitamin B-12 deficiency. The best-known clinical manifestation of cobalamin malabsorption is pernicious anemia. This is frequently caused by autoimmune destruction of gastric parietal cells but also by stomach surgery, intestinal disorders or infections, strict vegetarian diets, or simply inadequate diets (30, 32, 35, 36). Vitamin B-12 deficiency may result in defective DNA synthesis, homocysteine accumulation, and impaired regeneration of methionine (30, 36–38). Genetic factors can affect vitamin B-12 assimilation, transport, and metabolism (congenital IF deficiency or functional abnormality; congenital transcobalamin deficiency; congenital haptocorrin deficiency; cobalamin mutations that result in hyperhomocysteinemia, methylmalonic academia, or both). Severe but rare disorders involving gene deletion or mutation generally result in serious complications during infancy or childhood, whereas milder but more prevalent conditions can arise at any age as a result of polymorphisms of genes involved in vitamin B-12 pathways (39, 40).

The most prevalent single nucleotide polymorphism (SNP) is at the base position 776 (776C→G) in transcobalamin. Apotranscobalamin and holotranscobalamin (holoTC) were found to be lower and serum methylmalonic acid higher in individuals homozygous for the 776G allele than in persons homozygous for the 776C allele. In most observations total cobalamin and homocysteine were not significantly different among the genotypes. Clinical significance of the 776C→G polymorphism seems to be associated with spontaneous abortion and cleft lip or palate as well as Alzheimer disease. The 2756A→G polymorphism in methionine synthase and the 66AG→ polymorphism in methionine synthase reductase are also associated with various birth defects (39–41).

Early noticeable symptoms of vitamin B-12 deficiency are nonspecific and include unusual fatigue, appetite loss, digestion problems and nausea, anxiety, mild depression, numbness and tingling in the hands and feet, frequent upper respiratory infections, and impaired memory. Clinical symptoms can be divided into 3 main categories: hematologic symptoms (megaloblastic anemia, pernicious anemia), neurologic symptoms (myelosis funicularis), and gastrointestinal symptoms (Möller-Hunter glossitis) (30).

New and more sensitive screening methods allow earlier and more accurate diagnosis of subclinical vitamin B-12 deficiencies. It is useful to use several techniques to assess cobalamin deficiency because no single assay is completely adequate. The standard screening test is measurement of total plasma cobalamin. Studies indicate that measurement of holoTC, plasma vitamin B-12 bound to transcobalamin, has equal diagnostic accuracy. Measuring both holoTC and total vitamin B-12 provides a better assessment of vitamin B-12 deficiency than does either assay alone (42–44). Additional assessment of specific metabolites (functional variables) such as methylmalonic acid (MMA), total plasma homocysteine (tHcy), cystathionine, and 2-methylcitric acid allows early detection of metabolic disturbances, even before severe changes occur. Elevated serum MMA is considered highly specific to cobalamin metabolism (except modest elevations in the case of chronic renal insufficiency). Plasma homocysteine is not suggested as a single indicator of vitamin B-12 status because conditions such as folate or vitamin B-6 deficiencies can also cause elevated plasma homocysteine concentrations (29, 30, 36). Serum cystathionine concentrations are usually elevated in cobalamin

TABLE 1

Comparison of recommended dietary intake of vitamin B-12 between Europe and the United States¹

| | Vitamin B-12 μg/d |
|--------------------------------------|----------------------|
| Europe | |
| Infants | |
| 0–4 mo | 0.4 |
| 4–12 mo | 0.8 |
| Children | |
| 1–4 y | 1.0 |
| 4–7 y | 1.5 |
| 7–10 y | 1.8 |
| 10–13 y | 2.0 |
| 13–15 y | 3.0 |
| Adolescents and adults (15 to >65 y) | 3.0 |
| Pregnancy | 3.5 |
| Lactation | 4.0 |
| United States | |
| Infants | |
| 0–6 mo | 0.4 |
| 7–12 mo | 0.8 |
| Children | |
| 1–3 y | 0.9 |
| 4–8 y | 1.2 |
| 9–13 y | 1.8 |
| Adolescents and adults (14 to >50 y) | 2.4 |
| Pregnancy | 2.6 |
| Lactation | 2.8 |

¹ European information is adapted from reference 31, and US information is adapted from reference 34.

deficiency but also in folate and B-6 deficiencies. Elevated 2-methylcitric acid concentrations occur in connection with innate errors of mutase and adenosylcobalamin synthesis and severe vitamin B-12 deficiency (30, 42–44). Because cobalamin and folate deficiencies have a similar profile, subclinical deficiency of cobalamin can only be confidently diagnosed when metabolite concentrations return to normal after treatment with cobalamin (35).

HOMOCYSTEINE

Strong evidence gathered over the past decade indicates that even mild hyperhomocysteinemia represents an independent risk factor for atherosclerosis and thromboembolic diseases. As an independent risk factor for cardiovascular disease, hyperhomocysteinemia is thought to be responsible for $\approx 10\%$ of total risk. Moderate hyperhomocysteinemia (plasma homocysteine concentrations $> 12 \mu\text{mol/L}$) is found in 5–10% of the general population but in $\leq 40\%$ of patients with vascular disease (9, 29, 30). Homocysteine is an amino acid, mainly derived from dietary sources of the essential amino acid methionine. It can either be remethylated to methionine (requiring folate, vitamin B-12, vitamin B-2) or converted to cysteine in the transsulfuration pathway (requiring vitamin B-6). The remethylation process requires 2 key enzymes: methionine synthase (using methylcobalamin as coenzyme) and methylenetetrahydrofolate reductase (MTHFR) with 5-methyltetrahydrofolate as the methyl donor. An alternative homocysteine remethylation cycle is catalyzed by betaine (29, 30, 45). Numerous factors may affect homocysteine metabolism. Elevated homocysteine concentrations can be the result of genetic defects or renal failure but are most frequently due to deficiencies of folate, vitamin B-12, vitamin B-6, and vitamin B-2 (29, 45). Several SNPs that affect folate metabolism can also lead to elevated plasma homocysteine concentrations. A common SNP in the *MTHFR* gene (*C677T*) is particularly important and results in a thermolabile phenotype associated with increased homocysteine concentrations and reduced DNA methylation. Vegetarians may have a higher demand for folate to neutralize the genotype effect. Some studies suggest that *MTHFR 677TT* polymorphisms may be associated with moderately reduced colorectal cancer risk (46, 47).

VITAMIN B-12 STATUS AND PLASMA HOMOCYSTEINE AMONG VEGETARIANS: A GLOBAL PERSPECTIVE

Some of the studies that measured serum vitamin B-12 and plasma tHcy in vegetarians from different geographical areas in the world are described in **Table 2**. Although the interconnection between vitamin B-12 and homocysteine is not completely understood, increasing evidence shows that low serum cobalamin results in elevated serum tHcy in children, adults, and the elderly (48–52). One of the limitations of this review is the lack of consistency in the assessment of vitamin B-12 status and plasma tHcy concentration among studies, making it difficult to make meaningful comparisons.

We assessed plasma vitamin B-12 in 42 vegans, 36 vegetarians, and 40 omnivores in Austria (10). On the basis of the criteria (normal: $>147 \text{ pmol/L}$; low or marginal: $110\text{--}147 \text{ pmol/L}$; deficient: $<110 \text{ pmol/L}$) established by Sauberlich (53), the mean plasma vitamin B-12 concentrations were within the normal

range in all 3 groups we studied; however, the mean value in the vegan group was significantly lower than in the omnivore group ($P < 0.01$) with 2.4% of vegans identified as deficient. More than 66% of vegans, 52% of vegetarians, and 45% of omnivores had homocysteine concentrations between 12 and $30 \mu\text{mol/L}$ (normal value: $6\text{--}12 \mu\text{mol/L}$).

Several studies have looked at the vitamin B-12 status of vegetarians in Germany (11–13, 19). Koebnick et al (11) studied participants from raw food communities who adhered to a diet in which $\geq 70\%$ of their total intake was raw food for a period of 24 mo. Most consumed a mixed diet, including raw meat and fish. On the basis of the criteria they used (vitamin B-12 $< 150 \text{ pmol/L}$ is deficient, $150\text{--}250 \text{ pmol/L}$ is marginal, $>250 \text{ pmol/L}$ is adequate, and $>16 \mu\text{mol/L}$ is elevated homocysteine), only 21% of the participants had an adequate vitamin B-12 status and 51% had elevated tHcy. In another study by Herrmann et al (13), 90% of the vegans, 73% of the vegetarians, but only 11% of the omnivores displayed low holoTC concentrations ($<35 \text{ pmol/L}$). In the same study, 86% of the vegans, 61% of the vegetarians, and 5% of the omnivores displayed MMA concentrations $>271 \text{ mmol/L}$, indicating greater risk of suboptimal vitamin B-12 status among vegetarians than among omnivores. Among vegetarians, vegans or strict vegetarians tended to have the lowest serum vitamin B-12 concentrations (13). In Germany, a study of pregnant women also showed an increased risk in vitamin B-12 deficiency among lactoovovegetarians and low meat eaters than for omnivores eating a typical Western diet (19).

Studies from Taiwan (14–16), Italy (17), Turkey (21), the Slovak Republic (18), the United States (22), and Australia (23) all report lower mean serum concentrations of vitamin B-12 among vegetarians than among omnivores. Even when the mean serum concentration of vitamin B-12 is within the normal range, a significantly higher percentage of vegetarians have $<200 \text{ pmol}$ serum vitamin B-12/L, indicating a deficient state, than do the percentage of omnivores in the same cohorts. Also in many of these studies elevated serum tHcy was documented in a higher percentage of vegetarians compared with omnivore controls. In a study in Chinese vegetarian children (24) the prevalence of vitamin B-12 deficiency was not high based on serum values; however, as others have shown, often times other indicators of vitamin B-12 status may be compromised (22), making it necessary to measure more than one marker of vitamin B-12 status. It is also likely that the frequent intake of eggs, sprouted legumes, and fermented soybean products in this cohort may have provided adequate vitamin B-12 in the diets of the Chinese vegetarian children (24).

Several researchers stress the increased risk of cobalamin deficiency in children and adolescents born to either vegetarian mothers or nonvegetarian mothers on a poor diet. Ambroszkiewicz et al (20) reported adequate mean serum concentrations of vitamin B-12 and tHcy in vegetarian children but below the recommended range in the vegan children. Two other studies showed low cobalamin status in pregnant women and low bone mineral density in adolescents with low cobalamin status who were fed a macrobiotic diet until the age of 6 y (MMA and tHcy were elevated) (11, 27). The findings of Ueland and Monsen (52) that infants ($<1 \text{ y}$) born to mothers with an adequate nutrition had moderately elevated tHcy and low serum cobalamin cause some concern. These findings raise the question of whether cobalamin deficiency may also be widespread and undetected in infants born to nonvegetarian mothers.



TABLE 2

Vitamin B-12 and homocysteine status among vegetarians, vegans, and omnivores: summary of studies from different countries

| Study (reference) | Plasma vitamin B-12 | Plasma homocysteine |
|---|----------------------|---------------------|
| | <i>pmol/L</i> | <i>μmol/L</i> |
| Majchrzak et al, 2006, Austria (10) | | |
| Vegetarians (<i>n</i> = 36) ¹ | 238.5 ± 99.1 | 14.0 ± 5.4 |
| Vegans (<i>n</i> = 42) ¹ | 203.2 ± 101.5 | 16.5 ± 8.2 |
| Omnivores (<i>n</i> = 40) ¹ | 251.5 ± 83.0 | 12.2 ± 5.6 |
| Koebnick et al, 2005, Germany (11) | | |
| Vegetarians (<i>n</i> = 38) ² | 143.2 (121.2–175.9) | 17.1 (13.1–20.2) |
| Vegans (<i>n</i> = 39) ² | 126.2 (87.8–182.3) | 18.5 (13.5–28.9) |
| Omnivores (<i>n</i> = 109) ² | 174.5 (142.2–249.8) | 14.7 (11.9–18.3) |
| Herrmann et al, 2005, Germany (13) | | |
| Vegetarians (<i>n</i> = 66) ³ | 192 (127–450) | 10.6 (6.4–27.7) |
| Vegans (<i>n</i> = 29) ³ | 148 (99–314) | 12.8 (5.9–57.1) |
| Omnivores (<i>n</i> = 79) ³ | 287 (190–471) | 8.8 (5.5–16.1) |
| Waldmann et al, 2004, Germany (12) | | |
| Moderate vegans (<i>n</i> = 45) ³ | 185 (97.6–689) | 12.3 (4.6–23.6) |
| Strict vegans (<i>n</i> = 86) ³ | 122 (71.2–276) | 13.4 (6.0–82.5) |
| Huang et al, 2003, Taiwan (14) | | |
| Vegetarians (<i>n</i> = 37) ⁴ | 191.8 (164.0, 220.0) | 13.2 (10.6, 15.7) |
| Omnivores (<i>n</i> = 32) ⁴ | 310.9 (278.2, 343.6) | 9.8 (9.1, 10.6) |
| Su et al, 2005, Taiwan (15) | | |
| Vegetarians (<i>n</i> = 57) ¹ | 265.2 ± 179.3 | 11.0 ± 3.3 |
| Omnivores (<i>n</i> = 61) ¹ | 380.3 ± 199.4 | 9.0 ± 2.1 |
| Hung et al, 2002, Taiwan (16) | | |
| Vegetarians (<i>n</i> = 45) ¹ | 207.7 ± 127.1 | 11.20 ± 4.27 |
| Omnivores (<i>n</i> = 45) ¹ | 403.5 ± 138.9 | 8.64 ± 2.06 |
| Bissoli et al, 2002, Italy (17) | | |
| Vegetarians (<i>n</i> = 14) ¹ | 163.8 ± 57.1 | 17.4 ± 11.1 |
| Vegans (<i>n</i> = 31) ¹ | 155 ± 73.6 | 26.9 ± 24.1 |
| Kažimírová et al, 2006, Slovak Republic (18) | | |
| Vegetarians (<i>n</i> = 24) ⁵ | 209.79 ± 27.52 | Not reported |
| Omnivores (<i>n</i> = 24) ⁵ | 229.33 ± 15.01 | Not reported |
| Koebnick et al, 2004, Germany (19) | | |
| Vegetarian (<i>n</i> = 60) ^{6,7} | 159.6 (127–176) | 6.7 (5.7–7.5) |
| Omnivores (<i>n</i> = 108) ^{6,7} | 218.6 (169–249) | 6.2 (5.7–6.7) |
| Ambroszkiewicz et al, 2006, Poland (20) | | |
| Vegetarians (<i>n</i> = 32) ^{1,8} | 404.9 ± 106.6 | 6.1 ± 1.2 |
| Karabudak et al, 2008, Turkey (21) | | |
| Vegetarian (<i>n</i> = 26) ¹ | 200.5 ± 137.3 | 12.6 ± 5.97 |
| Omnivores (<i>n</i> = 26) ¹ | 269.1 ± 234.2 | 10.8 ± 3.72 |
| Haddad et al, 1999, United States (22) | | |
| Vegans (<i>n</i> = 25) ¹ | 312 ± 125 | 7.9 ± 1.5 |
| Omnivores (<i>n</i> = 20) ¹ | 313 ± 99 | 8.0 ± 1.9 |
| Hokin et al, 1999, Australia (23) | | |
| Vegetarians (<i>n</i> = 245) ⁷ | 199 (58–538) | Not reported |
| Omnivores (<i>n</i> = 53) ⁷ | 292 (134–721) | Not reported |
| Leung et al, 2001, China (24) | | |
| Vegetarians (<i>n</i> = 51) ^{7,9} | 389 (313–437) | Not reported |
| Refsum et al, 2001, India (26) | | |
| Vegetarian (<i>n</i> = 78) ³ | 124 (66–625) | 22 (9.6–48) |
| Omnivores (<i>n</i> = 126) ³ | 161 (62–492) | 19.4 (9.7–45.7) |

¹ All values are means ± SDs.² All values are medians; 25th to 75th percentiles in parentheses.³ All values are medians, 5th to 95th percentiles in parentheses.⁴ All values are means; 95% CIs in parentheses.⁵ All values are means ± SEMs.⁶ Subjects were women at various stages of pregnancy.⁷ All values are means; range in parentheses.⁸ Subjects were children, including 5 vegans (values originally in pg/ml: 548.6 ± 144.4).⁹ Subjects were children aged 4–14 y.

Although most of the studies mentioned identify lower vitamin B-12 and higher homocysteine concentrations in vegetarians in comparison with nonvegetarians, studies from India show high prevalence of vitamin B-12 deficiency and hyperhomocysteinemia in vegetarians as well as in omnivores. Yajnik et al (25) recruited 441 middle-aged men, vegetarians and nonvegetarians from rural areas (41% lactovegetarians), 142 slum dwellers (11% lactovegetarians), and 150 from urban areas (44% lactovegetarians). Sixty-seven percent of all recruited men had low cobalamin concentration (<150 pmol/L), and 58% had hyperhomocysteinemia (>15 μ mol/L). Eighty-one percent of the urban middle-class men had low cobalamin concentrations, and 79% of them had hyperhomocysteinemia. Similar findings were reported by Refsum et al (26), indicating that, perhaps more than diet, genetic mechanisms may be contributing to the prevalence of inadequate vitamin B-12 status among Asian Indians. However, vegetarians still had a 4.4 times higher risk of low cobalamin concentrations and a 3.0 times higher risk of hyperhomocysteinemia than did those who ate nonvegetarian food frequently in this population (25).

Findings from the literature confirm that vegetarians, especially vegans, have lower cobalamin status and a higher homocysteine concentration than omnivores. Although these studies point to the potential inadequacy of vegan and vegetarian diets for cobalamin status (54), one cannot ignore the overall benefits of plant-based diets to health. Also of note is that vitamin B-12 deficiency and hyperhomocysteinemia are not always unique to vegetarians, although it is generally more difficult for vegetarians and especially vegans to meet vitamin B-12 requirements than it is for omnivores (except those with poor-quality diet because of poverty). Given the likelihood that low cobalamin status is widespread, vegetarians and vegans must be advised to not only carefully plan their diets but also to use vitamin B-12-fortified foods (or supplements) if necessary. They should be encouraged to monitor their vitamin B-12 status on a regular basis to facilitate early detection of low cobalamin status and timely treatment before clinical manifestations can develop. These recommendations also apply to high-risk omnivore groups. (Other articles in this supplement to the Journal include references 55–81.)

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