Vitamin B-12 and homocysteine status among vegetarians: a global perspective[1–4]

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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).

TABLE 1
Comparison of recommended dietary intake of vitamin B-12 between Europe and the United States[1]

	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.

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

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 ≈10% of total risk. Moderate hyperhomocysteinemia (plasma homocysteine concentrations > 12 μmol/L) is found in 5–10% of the general population but in ≤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 pmol/L; low or marginal: 110–147 pmol/L; deficient: <110 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 μmol/L (normal value: 6–12 μmol/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 ≥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 pmol/L is deficient, 150–250 pmol/L is marginal, >250 pmol/L is adequate, and >16 μmol/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 pmol/L). In the same study, 86% of the vegans, 61% of the vegetarians, and 5% of the omnivores displayed MMA concentrations >271 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 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 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
	pmol/L	*μmol/L*
Majchrzak et al, 2006, Austria (10)		
Vegetarians ($n = 36$)[1]	238.5 ± 99.1	14.0 ± 5.4
Vegans ($n = 42$)[1]	203.2 ± 101.5	16.5 ± 8.2
Omnivores ($n = 40$)[1]	251.5 ± 83.0	12.2 ± 5.6
Koebnick et al, 2005, Germany (11)		
Vegetarians ($n = 38$)[2]	143.2 (121.2–175.9)	17.1 (13.1–20.2)
Vegans ($n = 39$)[2]	126.2 (87.8–182.3)	18.5 (13.5–28.9)
Omnivores ($n = 109$) [2]	174.5 (142.2–249.8)	14.7 (11.9–18.3)
Herrmann et al, 2005, Germany (13)		
Vegetarians ($n = 66$)[3]	192 (127–450)	10.6 (6.4–27.7)
Vegans ($n = 29$)[3]	148 (99–314)	12.8 (5.9–57.1)
Omnivores ($n = 79$)[3]	287 (190–471)	8.8 (5.5–16.1)
Waldmann et al, 2004, Germany (12)		
Moderate vegans ($n = 45$)[3]	185 (97.6–689)	12.3 (4.6–23.6)
Strict vegans ($n = 86$)[3]	122 (71.2–276)	13.4 (6.0–82.5)
Huang et al, 2003, Taiwan (14)		
Vegetarians ($n = 37$)[4]	191.8 (164.0, 220.0)	13.2 (10.6, 15.7)
Omnivores ($n = 32$)[4]	310.9 (278.2, 343.6)	9.8 (9.1, 10.6)
Su et al, 2005, Taiwan (15)		
Vegetarians ($n = 57$)[1]	265.2 ± 179.3	11.0 ± 3.3
Omnivores ($n = 61$)[1]	380.3 ± 199.4	9.0 ± 2.1
Hung et al, 2002, Taiwan (16)		
Vegetarians ($n = 45$)[1]	207.7 ± 127.1	11.20 ± 4.27
Omnivores ($n = 45$)[1]	403.5 ± 138.9	8.64 ± 2.06
Bissoli et al, 2002, Italy (17)		
Vegetarians ($n = 14$)[1]	163.8 ± 57.1	17.4 ± 11.1
Vegans ($n = 31$)[1]	155 ± 73.6	26.9 ± 24.1
Kažimírová et al, 2006, Slovak Republic (18)		
Vegetarians ($n = 24$)[5]	209.79 ± 27.52	Not reported
Omnivores ($n = 24$)[5]	229.33 ± 15.01	Not reported
Koebnick et al, 2004, Germany (19)		
Vegetarian ($n = 60$)[6,7]	159.6 (127–176)	6.7 (5.7–7.5)
Omnivores ($n = 108$)[6,7]	218.6 (169–249)	6.2 (5.7–6.7)
Ambroszkiewicz et al, 2006, Poland (20)		
Vegetarians ($n = 32$)[1,8]	404.9 ± 106.6	6.1 ± 1.2
Karabudak et al, 2008, Turkey (21)		
Vegetarian ($n = 26$)[1]	200.5 ± 137.3	12.6 ± 5.97
Omnivores ($n = 26$)[1]	269.1 ± 234.2	10.8 ± 3.72
Haddad et al, 1999, United States (22)		
Vegans ($n = 25$)[1]	312 ± 125	7.9 ± 1.5
Omnivores ($n = 20$)[1]	313 ± 99	8.0 ± 1.9
Hokin et al, 1999, Australia (23)		
Vegetarians ($n = 245$)[7]	199 (58–538)	Not reported
Omnivores ($n = 53$)[7]	292 (134–721)	Not reported
Leung et al, 2001, China (24)		
Vegetarians ($n = 51$)[7,9]	389 (313–437)	Not reported
Refsum et al, 2001, India (26)		
Vegetarian ($n = 78$)[3]	124 (66–625)	22 (9.6–48)
Omnivores ($n = 126$)[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 μmol/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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REFERENCES

1. Sabaté J. The contribution of vegetarian diets to health and disease: a paradigm shift? Am J Clin Nutr 2003;78(suppl):502S–7S.
2. Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am J Clin Nutr 2003; 78(suppl):517S–20S.
3. Johanna J W. Lampe. Spicing up a vegetarian diet: chemopreventive effects of phytochemicals. Am J Clin Nutr 2003;78(suppl):579S–83S.
4. Koerber K, Männle T, Leitzmann C. Studien mit Vegetariern [Studies with vegetarians]. In: Vollwert-Ernährung [Wholefood nutrition]. Stuttgart, Germany: Haug Verlag, 2004:114–6 (in German).
5. Key TJ, Davey GK, Appleby PN. Health benefits of a vegetarian diet. Proc Nutr Soc 1999;58:271–5.
6. Thorogood M, Mann J, Appleby P, McPherson K. Risk of death from cancer and ischemic heart disease in meat and non-meat eaters. BMJ 1994;308:1667–70.
7. Fraser GE, Lindsted KD, Beeson WL. Effect of risk factor values on lifetime risk of and age at first coronary event. The Adventist Health Study. Am J Epidemiol 1995;142:746–58.
8. Hu FB. Plant-based foods and prevention of cardiovascular disease: an overview. Am J Clin Nutr 2003;78(suppl):544S–51S.
9. Stanger O, Herrmann W, Pietrzik K, et al. Consensus paper on the rational clinical use of homocysteine, folic acid and B-vitamins in cardiovascular and thrombotic diseases: guidelines and recommendations. Clin Chem Lab Med 2004;42:1392–403.
10. Majchrzak D, Singer I, Männer M, et al. B-vitamin status and concentrations of homocysteine in Austrian omnivores, vegetarians and vegans. Ann Nutr Metab 2006;50:485–91.
11. Koebnick C, Garcia AL, Dagnelie PC, et al. Long-term consumption of a raw food diet is associated with favorable serum LDL cholesterol and triglycerides but also with elevated plasma homocysteine and low serum HDL cholesterol in humans. J Nutr 2005;135:2372–8.
12. Waldmann A, Koschizke JW, Leitzmann C, Hahn A. Homocysteine and cobalamin status in German vegans. Public Health Nutr 2004;7:467–72.
13. Herrmann W, Schorr H, Obeid R, Geisel J. Vitamin B-12 status, particularly holotranscobalamin II and methylmalonic acid concentrations, and hyperhomocysteinemia in vegetarians. Am J Clin Nutr 2003;78: 131–6.
14. Huang YC, Chang SJ, Chiu YT, Chang HH, Cheng CH. The status of plasma homocysteine and related B-vitamins in healthy young vegetarians and nonvegetarians. Eur J Nutr 2003;42:84–90.
15. Su TC, Jeng JS, Wang JD, et al. Homocysteine, circulating vascular cell adhesion molecule and carotid atherosclerosis in postmenopausal vegetarian women and omnivores. Atherosclerosis 2006;184:356–62.
16. Hung CJ, Huang PC, Lu SC, et al. Plasma homocysteine levels in Taiwanese vegetarians are higher than those of omnivores. J Nutr 2002; 132:152–8.
17. Bissoli L, di Francesco V, Ballarin A, et al. Effect of vegetarian diet on homocysteine levels. Ann Nutr Metab 2002;46:73–9.
18. Kažimírová A, Barančoková M, Krajčovičová-Kudláčková M, et al. The relationship between micronuclei in human lymphocytes and selected micronutrients in vegetarians and non-vegetarians. Mutat Res 2006;611: 64–70.
19. Koebnick C, Hoffmann I, Dagnelie PC, et al. Long-term ovo-lacto vegetarian diet impairs vitamin B-12 status in pregnant women. J Nutr 2004;134:3319–26.
20. Ambroszkiewicz J, Klemarczyk W, Chełchowska M, Gajewska J, Laskowska-Klita T. Serum homocysteine, folate, vitamin B12 and total antioxidant status in vegetarian children. Adv Med Sci 2006;51:265–8.
21. Karabudak E, Kiziltan G, Cigerim N. A comparison of some of the cardiovascular risk factors in vegetarian and omnivorous Turkish females. J Hum Nutr Diet 2008;21:13–22.
22. Haddad EH, Berk LS, Kettering JD, Hubbard RW, Peters WR. Dietary intake and biochemical, hematologic, and immune status of vegans compared with nonvegetarians. Am J Clin Nutr 1999;70(suppl):586S–93S.
23. Hokin BD, Butler T. Cyanocobalamin (vitamin B-12) status in the Seventh-day Adventist ministers in Australia. Am J Clin Nutr 1999; 70(suppl):576S–8S.
24. Leung SSF, Lee RHY, Sung RYT, et al. Growth and nutrition of Chinese vegetarian children in Hong Kong. J Paediatr Child Health 2001;37: 247–53.
25. Yajnik CS, Deshpande SS, Lubree HG, et al. Vitamin B12 deficiency and hyperhomocysteinemia in rural and urban Indians. J Assoc Physicians India 2006;54:775–82.
26. Refsum H, Yajnik CS, Gadkari M, et al. Hyperhomocysteinemia and elevated methylmalonic acid indicate a high prevalence of cobalamin deficiency in Asian Indians. Am J Clin Nutr 2001;74:233–41.
27. Dhonukshe-Rutten RA, van Dusseldorp M, Schneede J, de Groot LC, van Staveren WA. Low bone mineral density and bone mineral content are associated with low cobalamin status in adolescents. Eur J Nutr 2005;44:341–7.
28. Position of the American Dietetic Association and Dieticians of Canada. Vegetarian diets. J Am Diet Assoc 2003;103:748–65.
29. Elmadfa I, Leitzmann C. Vitamine, verschiedene [Vitamins, miscellaneous diets].. In: Ernährung des Menschen [Human nutrition]. Eugen, Germany: Ulmer GmbH & Co, 2004:366–74, 380–97, 615–9 (in German).
30. Green R, Miller JW. Vitamin B_{12}. In: Zempleni J, Rucker RB, McCormick DB, Suttie JW, eds. Handbook of vitamins. Baton Raton, FL: CRC Press, 2007:413–57.
31. Deutsche Gesellschaft für Ernährung. Österreichische Gesellschaft für Ernährung, Schweizerische Gesellschaft für Ernährungsforschung, Schweizerische Vereinigung für Ernährung. Wasserlösliche vitamine

[German, Austrian and Swiss Societies of Nutrition. Watersoluble vitamins]. In: D-A-CH-Referenzwerte für die Nährstoffzufuhr [D-A-CH-reference values for nutrient and energy intakes]. Frankfurt, Germany: Umschau Braus GmbH, 2000:113–23, 131–5 (in German).
32. Elmadfa I, Fritzsche D. Allgemeines über Vitamine [Vitamins]. In: Die große GU Vitamin und Mineralstofftabelle [The large GU vitamin- and mineral- and trace elements-table]. Munich, Germany: Gräfe und Unzer Verlag, 2004:24–6 (in German).
33. Scientific Committee for Food. Directorate-General, Industry. Vitamin B_{12}. In: Reports of the Scientific Committee for Food. Thirty-first series. Nutrient and energy intakes for the European Community. Commission of the European Communities. Luxembourg: Office for Official Publication of the European Communities, 1992:107–16.
34. National Academy of Sciences, Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for vitamins, minerals, and macronutrients. Washington, DC: US Government Printing Office, 2002.
35. Antony AC. Vegetarianism and vitamin B-12 (cobalamin) deficiency. Am J Clin Nutr 2003;78:3–6.
36. Stabler SP. Vitamin B-12. In: Bowman BA, Russell RM, eds. Present knowledge in nutrition. Washington, DC: International Life Sciences Institute, 2001:230–40.
37. Geisel J, Schorr H, Bodis M, et al. The vegetarian lifestyle and DNA methylation. Clin Chem Lab Med 2005;43:1164–9.
38. Friso S, Choi SW. Trans-HHS Workshop: diet, DNA methylation processes and health gene-nutrient interactions and DNA methylation. J Nutr 2002;132:2382S–7S.
39. von Castel-Dunwoody KM, Kauwell GP, Shelnutt KP, et al. Transcobalamin 776C→G polymorphism negatively affects vitamin B-12 metabolism. Am J Clin Nutr 2005;81:1436–44.
40. Aléssio AC, Höehr NF, Siqueira LH, Bydlowski SP, Annichino-Bizzacchi JM. Polymorphism C776G in the transcobalamin II gene and homocysteine, folate and vitamin B_{12} concentrations. Association with MTHFR C677T and A1298C and MTRR A66G polymorphism in healthy children. Thromb Res 2007;119:571–7.
41. Herrmann W, Obeid R, Schorr H, Zarzour W, Geisel J. Homocysteine, methylenetetrahydrofolate reductase C677T polymorphism and the B-vitamins: a facet of nature-nurture interplay. Clin Chem Lab Med 2003;41:547–53.
42. Miller J, Garrod MJ, Rockwood AL, et al. Measurement of total vitamin B12 and holotranscobalamin, singly and in combination, in screening for metabolic Vitamin B12 deficiency. Clin Chem 2006;52:278–85.
43. Herrmann W, Obeid R, Schorr H, Geisel J. The usefulness of holotranscobalamin in predicting vitamin B12 status in different clinical settings. Curr Drug Metab 2005;6:47–53.
44. Herrmann W, Obeid R, Schorr H, Geisel J. Functional vitamin B12 deficiency and determination of holotranscobalamin in populations at risk. Clin Chem Lab Med 2003;41:1478–88.
45. Bailey LB. Folic acid. In: Zempleni J, Rucker RB, McCormick DB, Suttie JW, eds. Handbook of vitamins. Boca Raton, FL: CRC Press, 2007:386–412.
46. Sharp L, Little J. Polymorphisms in genes involved in folate metabolism and colorectalneoplasia: a HuGE review. Am J Epidemiol 2004;159:423–43.
47. Sharp L, Little J, Schofield AC, et al. Folate and breast cancer: the role of polymorphisms in methylenetetrahydrofolate reductase (MTHFR). Cancer Lett 2002;181:65–71.
48. Rogers LM, Boy E, Miller JW, Green R, Sabel JC, Allen LH. High prevalence of cobalamin deficiency in Guatemalan schoolchildren: associations with low plasma holotranscobalamin II and elevated serum methylmalonic acid and plasma homocysteine concentrations. Am J Clin Nutr 2003;77:433–40.
49. Kim MK, Ordovas JM, Selhub J, Campos H. B vitamins and plasma homocysteine concentrations in an urban and rural area of Costa Rica. J Am Coll Nutr 2003;22:224–31.
50. Gonzalez-Gross M, Sola R, Albers U, et al. B-vitamins and homocysteine in Spanish institutionalized elderly. Int J Vitam Nutr Res 2007; 77:22–33.
51. Taneja S, Bhandari N, Strand TA, et al. Cobalamin and folate status in infants and young children in a low-to-middle income community in India. Am J Clin Nutr 2007;86:1302–9.
52. Ueland PM, Monsen AL. Hyperhomocysteinemia and B-vitamin deficiencies in infants and children. Clin Chem Lab Med 2003;41:1418–26.
53. Sauberlich HE. Laboratory test assessment of nutritional status. 2nd ed. Boca Raton, FL: CRC Press, 2000.
54. Stabler SP, Allen RH. Vitamin B12 deficiency as a worldwide problem. Annu Rev Nutr 2004;24:299–326.
55. Rajaram S, Sabaté J. Preface. Am J Clin Nutr 2009;89(suppl):1541S–2S.
56. Jacobs DR Jr, Gross MD, Tapsell LC. Food synergy: an operational concept for understanding nutrition. Am J Clin Nutr 2009;89(suppl): 1543S–8S.
57. Jacobs DR Jr, Haddad EH, Lanou AJ, Messina MJ. Food, plant food, and vegetarian diets in the US dietary guidelines: conclusions of an expert panel. Am J Clin Nutr 2009;89(suppl):1549S–52S.
58. Lampe JW. Interindividual differences in response to plant-based diets: implications for cancer risk. Am J Clin Nutr 2009;89(suppl):1553S–7S.
59. Simon JA, Chen Y-H, Bent S. The relation of α-linolenic acid to the risk of prostate cancer: a systematic review and meta-analysis. Am J Clin Nutr 2009;89(suppl):1558S–64S.
60. Pierce JP, Natarajan L, Caan BJ, et al. Dietary change and reduced breast cancer events among women without hot flashes after treatment of early-stage breast cancer: subgroup analysis of the Women's Healthy Eating and Living Study. Am J Clin Nutr 2009;89(suppl):1565S–71S.
61. Newby PK. Plant foods and plant-based diets: protective against childhood obesity? Am J Clin Nutr 2009;89(suppl):1572S–87S.
62. Barnard ND, Cohen J, Jenkins DJA, et al. A low-fat vegan diet and a conventional diabetes diet in the treatment of type 2 diabetes: a randomized, controlled, 74-wk clinical trial. Am J Clin Nutr 2009;89(suppl): 1588S–96S.
63. Mangat I. Do vegetarians have to eat fish for optimal cardiovascular protection? Am J Clin Nutr 2009;89(suppl):1597S–601S.
64. Willis LM, Shukitt-Hale B, Joseph JA. Modulation of cognition and behavior in aged animals: role for antioxidant- and essential fatty acid–rich plant foods. Am J Clin Nutr 2009;89(suppl):1602S–6S.
65. Fraser GE. Vegetarian diets: what do we know of their effects on common chronic diseases? Am J Clin Nutr 2009;89(suppl):1607S–12S.
66. Key TJ, Appleby PN, Spencer EA, Travis RC, Roddam AW, Allen NE. Cancer incidence in vegetarians: results from the European Prospective Investigation into Cancer and Nutrition (EPIC-Oxford). Am J Clin Nutr 2009;89(suppl):1620S–6S.
67. Key TJ, Appleby PN, Spencer EA, Travis RC, Roddam AW, Allen NE. Mortality in British vegetarians: results from the European Prospective Investigation into Cancer and Nutrition (EPIC-Oxford). Am J Clin Nutr 2009;89(suppl):1613S–7S.
68. Craig WJ. Health effects of vegan diets. Am J Clin Nutr 2009;89(suppl): 1627S–33S.
69. Weaver CM. Should dairy be recommended as part of a healthy vegetarian diet? Point. Am J Clin Nutr 2009;89(suppl):1634S–7S.
70. Lanou AJ. Should dairy be recommended as part of a healthy vegetarian diet? Counterpoint. Am J Clin Nutr 2009;89(suppl):1638S–42S.
71. Sabaté J, Ang Y. Nuts and health outcomes: new epidemiologic evidence. Am J Clin Nutr 2009;89(suppl):1643S–8S.
72. Ros E. Nuts and novel biomarkers of cardiovascular disease. Am J Clin Nutr 2009;89(suppl):1649S–56S.
73. Rajaram S, Haddad EH, Mejia A, Sabaté J. Walnuts and fatty fish influence different serum lipid fractions in normal to mildly hyperlipidemic individuals: a randomized controlled study. Am J Clin Nutr 2009;89(suppl):1657S–63S.
74. Lampe JW. Is equol the key to the efficacy of soy foods? Am J Clin Nutr 2009;89(suppl):1664S–7S.
75. Badger TM, Gilchrist JM, Pivik RT, et al. The health implications of soy infant formula. Am J Clin Nutr 2009;89(suppl):1668S–72S.
76. Messina M, Wu AH. Perspectives on the soy–breast cancer relation. Am J Clin Nutr 2009;89(suppl):1673S–9S.
77. Lönnerdal B. Soybean ferritin: implications for iron status of vegetarians. Am J Clin Nutr 2009;89(suppl):1680S–5S.
78. Chan J, Jaceldo-Siegl K, Fraser GE. Serum 25-hydroxyvitamin D status of vegetarians, partial vegetarians, and nonvegetarians: the Adventist Health Study-2. Am J Clin Nutr 2009;89(suppl):1686S–92S.
79. Marlow HJ, Hayes WK, Soret S, Carter RL, Schwab ER, Sabaté J. Diet and the environment: does what you eat matter? Am J Clin Nutr 2009; 89(suppl):1699S–703S.
80. Carlsson-Kanyama A, González AD. Potential contributions of food consumption patterns to climate change. Am J Clin Nutr 2009;89(suppl): 1704S–9S.
81. Eshel G, Martin PA. Geophysics and nutritional science: toward a novel, unified paradigm. Am J Clin Nutr 2009;89(suppl):1710S–6S.