

Vitamin B12 in Vegetarian Diets

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Dear Editor,

Vitamin B12 (B12) belongs to a group of water soluble B-vitamins. Methylcobalamin and adenosylcobalamin are two biologically active forms of B12. Hydroxocobalamin, aquacobalamin, nitrocobalamin and cyanocobalamin are metabolized to either of the two active forms once ingested. B12 is essential in synthesis of methionine (methylcobalamin) and in metabolism of lipids, protein and carbohydrates (adenosylcobalamin). Deficiency of B12 is widespread. Vegetarians develop B12 deficiency because of inadequate intake. The deficiency is prevalent regardless of other factors such as demographic characteristics, place of residency, or age. Vegans are at an especially high risk. Deficiency leads to hyperhomocysteinemia. High prevalence of hyperhomocysteinemia has been reported among individuals who adhere to all types of vegetarian diets. Symptoms of B12 deficiency may be mild (e.g. fatigue, difficulty to concentrate) or severe (low bone mineral density (BMD), hearing loss, brain atrophy, and/or hardening of arteries). Intake of eggs, milk and dairy products does not guarantee adequate B12 status. In fact, B12 intake from these products is likely inadequate to maintain sufficient serum B12 concentration. Although fortified foods may help to delay the onset of deficiency, low B12 concentrations and B12 deficiency have been reported in studies with vegetarians who used fortified foods and those who used supplements (perhaps indicating inadequate dose of a supplement). Individuals who adhere to all types of vegetarian diets should be advised to routinely screen for B12 status. The best assessment method of B12 is methylmalonic acid and/or holotranscobalamin II. Vegetarians need to ensure they ingest a reliable B12 source (e.g. B12 supplements). The dose depends large on age and life stage with elderly and pregnant vegan women having the need for the highest supplemental dose.

Vitamin B12 (B12), named cobalamin, belongs to a group of water soluble B-vitamins. Methylcobalamin and ad-

enosylcobalamin are two biologically active forms of B12. Hydroxocobalamin, aquacobalamin, nitrocobalamin and cyanocobalamin are metabolized to either of the two active forms once ingested (1). Methylcobalamin is a cofactor in the conversion of homocysteine to methionine. Adenosylcobalamin is needed for the conversion of Methylmalonyl-CoA to succinyl-CoA. This form is involved in the metabolism of fats, carbohydrates and protein. B12 is essential in DNA synthesis, and thus, in synthesis of all new cells. B12 plays a critical role in erythropoiesis and it is indispensable in synthesis and maintenance of myelin, a coating of the nerve pathways (1).

The process of digestion and absorption of B12 requires adequate synthesis of hydrochloric acid, proteases and an intrinsic factor (IF). B12 is released from dietary proteins by pepsin. Once released, B12 binds with IF forming an IF-B12 (or IF-Cbl) complex and it is absorbed in that form. B12 can also be absorbed via passive diffusion when it is ingested in high doses (e.g. via a supplement) (1).

B12 deficiency is a world-wide problem (2). Among vegetarians B12 deficiency is wide-spread and it is a result of inadequate intake (3). Older vegetarian adults and elderly may be at risk of a deficiency due to inadequate intake and malabsorption. The reported prevalence of deficiency among vegetarians ranged from 11 to 90% and was 62% among pregnant women, between 25 to almost 86% among children, 21 to 41% among adolescents, and 11 to 90% among elderly (3). This range was mainly dependent on deficiency criteria. Although a higher deficiency prevalence was reported among vegans, compared to other vegetarians, a number of studies reported a high deficiency prevalence among lacto- and lacto-ovo-vegetarians, often reaching or exceeded 50% of the samples (3, 4). The deficiency is prevalent regardless of other factors such as demographic characteristics, place of residency, or age. Similarly, high prevalence of hyperhomocysteinemia has been reported among individuals who adhere

to all types of vegetarian diets (3, 4). Although serum homocysteine concentration depends on intake of several nutrients (methionine, folate, B6 and B12), B12 is the determinant factor among vegetarians (4).

A number of case reports among vegetarian, especially vegan, adults have been published. Deficiency symptoms experienced by these individuals were many and included hematological, neurological, dermatological, gastrointestinal and psychiatric abnormalities (5). Symptom manifestations may include synthesis of large, immature, oblong-shaped erythrocytes (megaloblasts), weakness, fatigue, lightheadedness, tingling and burning sensation, numbness, muscle imbalance, skin hyperpigmentation, glossy and "beefy" color of the tongue, forgetfulness, pancytopenia, and myelin deterioration in both central and peripheral nervous systems (5).

Although some researchers believe that biochemical deficiency of B12 exists without accompanied symptoms, B12 deficiency is associated with symptoms that are not easily detected, such as low bone mineral density (BMD), hearing loss, brain atrophy, and/or hardening of arteries (6). Findings from a meta-analysis showed that B12 and homocysteine status were associated with BMD and postmenopausal osteoporosis (7). Also, findings reported by Kwok et al. showed that supplementation with 500 µg/day of B12 for 12 weeks by asymptomatic vegetarians not only statistically significantly increased serum B12 (mean baseline serum B12 = 134.0 pmol/L vs 379.6 pmol/L, $P = 0.0001$) and reduced Hcy (mean baseline Hcy = 16.7 µmol/L vs 11.3 µmol/L, $P = 0.01$) concentrations but also enhanced flow-mediated dilation of the brachial artery and improved intima-media thickness of the carotid artery (8). It is also important to realize that myelin deterioration that may lead to neurological manifestations may occur among "asymptomatic" individuals with low B12 and, that these problems may occur before hematological changes arise. Consequently, "asymptomatic" individuals may develop long-term complications prior to diagnosis of B12 deficiency.

Especially worrisome is a high number of published case studies with B12 deficiency among infants and children born to vegan women (although reports are less frequent, children born to lacto-ovo-vegetarian women have developed similar problems) (9). Long-term complications, related especially to neurological manifestations that include often severe developmental delays, are not uncommon. B12 deficiency has been associated with some forms of birth defects (e.g. neural tube defects) (10).

B12 recommendations vary from 1.9 µg/day to 3.0 µg/day (11-13). Although the recommendation for B12 for an adult, as issued by the Institute of Medicine, is 2.4 µg/day, results of several new studies indicated that this recommendation may have been underestimated and that a higher daily intake may be needed to avoid B12 depletion (14-19). This is especially true for elderly individuals who may need several times to one or two hundred times higher dose (20).

B12 is not found in foods of plant origin even though very small amounts of B12 may be found in some food items made exclusively from plant foods (due to either contamination during processing, the addition of small amounts of animal-derived ingredients or fortification). However, the amount of B12 found in these foods, except for foods that have been fortified, is negligible (1).

Milk, dairy products and eggs contains between 0.3 to 1.4 µg/100g of B12. Boiling and/or pasteurization destroys 5 to 50% of B12, depending on the duration of cooking. Bioavailability of B12 from various preparations of eggs (e.g., scrambled, boiled) ranges from less than 4% to just over 9% (21).

Fermented soy products, such as tempeh and other plant foods, do not contain biologically active forms of B12. B12 synthesis by bacteria in the small intestine is negligible. Research findings regarding algae, such as spirulina, nori and kombu are contradictory (1). Some researchers reported relatively high content of active B12 forms while others found exclusively or almost exclusively inactive B12 analogues, which actually may interfere with metabolism of the active B12 compounds (22).

Although several assessment techniques of B12 status exist, there is no "gold standard" for evaluation of B12 deficiency. B12 status can be determined using serum or plasma B12, holotranscobalamin II (holoTCII), serum or urinary methylmalonic acid (MMA), serum homocysteine, and mean corpuscular volume (MCV). HoloTCII and MMA are the most accurate, whereas serum or plasma B12 and especially MCV are less reliable (1).

Serum B12 assessment is among the most often used. Serum B12 indicative of a deficiency is a matter of debate. While most researchers and clinicians use relatively low concentration (e.g. < 150 pmol/L), others used higher values (e.g. 221 pmol/L). Even higher serum vitamin B12 concentrations were associated with hyperhomocysteinemia and elevated methylmalonic acid (MMA). If disease prevention is used as a criterion of B12 status, serum B12 concentration > 330 pmol/L would normalize homocysteine (< 10 µmol/L) and decrease risk of cardiovascular disease (23). Serum B12 concentration ≥ 400 pmol/L would be most protective against brain atrophy and cognitive decline (6).

Normal urine MMA value is between 0.58 to 3.56 µmol/mmol creatinine (1). Concentration > 270 nmol/L has been used most often as a deficiency criterion for serum MMA (24). HoloTCII < 35 pmol/L is often used to indicate a deficiency. However, higher values have been shown to be more protective of brain atrophy and cognitive decline (25). Homocysteine is the less specific but also useful marker. Homocysteine concentration is also affected by renal function and the use of certain medications including methotrexate and theophylline. The reference value for homocysteine is another matter of debate. Concentration of ≥ 12, and even ≥ 10 µmol/L, indicates hyperhomocysteinemia. MCV > 96 fl indicates megaloblastic anemia (1). In evaluation of B12 status, it is recommended to use

two of the above mentioned assessment techniques simultaneously (23).

Although B12 is believed to be non-toxic, in case of a very high serum B12 concentrations (most likely > 1,000 pmol/L) acne-like dermatological symptoms may occur among some individuals (26). Some reports have showed an association between serum B12 and haptocorrin concentrations and cancer (e.g. prostate cancer). It is believed that this association is due to reverse causation or that it may be coincidental rather than causative (27).

Vegetarian diets may be recommended as a therapy for diabetes patients (28). Patients who use metformin are at risk of B12 deficiency since this drug interferes with B12 absorption (29). Vegetarians who had a procedure that required general anesthesia are at risk of B12 deficiency since nitrous oxide, a common anesthetic agent, inactivates B12 via an oxidation of cobalt (30).

Vegetarians need to ensure they ingest a reliable B12 source. Intake of eggs, milk and dairy products does not guarantee adequate B12 status. In fact, B12 intake from these products is likely inadequate to maintain sufficient serum B12 concentration (21, 27, 31, 32). This would be especially true for elderly vegetarians (20). Although fortified foods may help to delay the onset of deficiency, low B12 concentrations and B12 deficiency have been reported in studies with vegetarians who used fortified foods and those who used supplements (perhaps indicating inadequate dose of a supplement) (3). Because food fortification practices change and B12 content of eggs and dairy products is variable, the most reliable way for vegetarians to ensure adequate B12 status is to use vitamin B12 supplements (1-4). B12 deficiency has been reported among non-vegetarians ingesting B12 supplements in doses lower than 75 µg/day (20). A supplemental dose depends mainly on age. It should be determined on an individual bases according to biochemical B12 screening results. All vegetarians should be regularly screened for B12 status.

References

1. Pawlak R, James PS, Raj S, Cullum-Dugan D, Lucus D. Understanding vitamin B12. *Am J Lifestyle Med.* 2013;7(1):60-5.
2. Allen LH. How common is vitamin B-12 deficiency? *Am J Clin Nutr.* 2009;89(2):693S-6S. doi: 10.3945/ajcn.2008.26947A. [PubMed: 19116323]
3. Pawlak R, Parrott SJ, Raj S, Cullum-Dugan D, Lucus D. How prevalent is vitamin B12 deficiency among vegetarians? *Nutr Rev.* 2013;71(2):110-7. doi: 10.1111/nure.12001. [PubMed: 23356638]
4. Pawlak R. Is vitamin B12 deficiency a risk factor for cardiovascular disease in vegetarians? *Am J Prev Med.* 2015;48(6):e11-26. doi: 10.1016/j.amepre.2015.02.009. [PubMed: 25998928]
5. Rusher DR, Pawlak R. A Review of 89 Published Case Studies of Vitamin B12 Deficiency. *J Hum Nutr Food Sci.* 2013;1(2):1008.
6. Smith AD, Refsum H. Do we need to reconsider the desirable blood level of vitamin B12? *J Intern Med.* 2012;271(2):179-82. doi: 10.1111/j.1365-2796.2011.02485.x. [PubMed: 22092891]
7. Zhang H, Tao X, Wu J. Association of homocysteine, vitamin B12, and folate with bone mineral density in postmenopausal women: a meta-analysis. *Arch Gynecol Obstet.* 2014;289(5):1003-9. doi: 10.1007/s00404-013-3075-6. [PubMed: 24193243]
8. Kwok T, Chook P, Qiao M, Tam L, Poon YK, Ahuja AT, et al. Vita-

- min B-12 supplementation improves arterial function in vegetarians with subnormal vitamin B-12 status. *J Nutr Health Aging.* 2012;16(6):569-73. [PubMed: 22659999]
9. Dror DK, Allen LH. Effect of vitamin B12 deficiency on neurodevelopment in infants: current knowledge and possible mechanisms. *Nutr Rev.* 2008;66(5):250-5. doi: 10.1111/j.1753-4887.2008.00031.x. [PubMed: 18454811]
10. Gu Q, Li Y, Cui ZL, Luo XP. Homocysteine, folate, vitamin B12 and B6 in mothers of children with neural tube defects in Xinjiang, China. *Acta Paediatr.* 2012;101(11):e486-90. doi: 10.1111/j.1651-2227.2012.02795.x. [PubMed: 22860981]
11. Institute of Medicine of the National Academies. Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. Report on the scientific evaluation of dietary reference intake and its panel on folate, other B vitamins and choline and subcommittee on upper reference levels of nutrients Food and Nutrition Board Institute on Medicine. Washington; 1998.
12. World Health Organization. Requirements of vitamin A, iron, folate and vitamin B12. Report of a Joint FAO/WHO Expert Consultation. Rome: Food and Agriculture Organization of the United Nations. Geneva; 1988.
13. National Research Council. Recommended Dietary Allowances. Washington; 1989.
14. Bor MV, von Castel-Roberts KM, Kauwell GP, Stabler SP, Allen RH, Maneval DR, et al. Daily intake of 4 to 7 microg dietary vitamin B-12 is associated with steady concentrations of vitamin B-12-related biomarkers in a healthy young population. *Am J Clin Nutr.* 2010;91(3):571-7. doi: 10.3945/ajcn.2009.28082. [PubMed: 20071646]
15. Bor MV, Lydeking-Olsen E, Moller J, Nexø E. A daily intake of approximately 6 microg vitamin B-12 appears to saturate all the vitamin B-12-related variables in Danish postmenopausal women. *Am J Clin Nutr.* 2006;83(1):52-8. [PubMed: 16400049]
16. Tucker KL, Rich S, Rosenberg I, Jacques P, Dallal G, Wilson PW, et al. Plasma vitamin B-12 concentrations relate to intake source in the Framingham Offspring study. *Am J Clin Nutr.* 2000;71(2):514-22. [PubMed: 10648266]
17. Kwan LL, Bermudez OI, Tucker KL. Low vitamin B-12 intake and status are more prevalent in Hispanic older adults of Caribbean origin than in neighborhood-matched non-Hispanic whites. *J Nutr.* 2002;132(7):2059-64. [PubMed: 12097693]
18. Doets EL, In 't Veld PH, Szczecinska A, Dhonukshe-Rutten RA, Cave-laars AE, van 't Veer P, et al. Systematic review on daily vitamin B12 losses and bioavailability for deriving recommendations on vitamin B12 intake with the factorial approach. *Ann Nutr Metab.* 2013;62(4):311-22. doi: 10.1159/000346968. [PubMed: 23796635]
19. Koebnick C, Hoffmann I, Dagnelie PC, Heins UA, Wickramasinghe SN, Ratnayaka ID, et al. Long-term ovo-lacto vegetarian diet impairs vitamin B-12 status in pregnant women. *J Nutr.* 2004;134(12):3319-26. [PubMed: 15570032]
20. Evatt ML, Terry PD, Ziegler TR, Oakley GP. Association between vitamin B12-containing supplement consumption and prevalence of biochemically defined B12 deficiency in adults in NHANES III (third national health and nutrition examination survey). *Public Health Nutr.* 2010;13(1):25-31. doi: 10.1017/S136898009990279. [PubMed: 19519972]
21. Watanabe F. Vitamin B12 sources and bioavailability. *Exp Biol Med (Maywood).* 2007;232(10):1266-74. doi: 10.3181/0703-MR-67. [PubMed: 17959839]
22. Herbert V. Vitamin B-12: plant sources, requirements, and assay. *Am J Clin Nutr.* 1988;48(3 Suppl):852-8. [PubMed: 3046314]
23. Obersby D, Chappell DC, Dunnett A, Tsiami AA. Plasma total homocysteine status of vegetarians compared with omnivores: a systematic review and meta-analysis. *Br J Nutr.* 2013;109(5):785-94. doi: 10.1017/S000711451200520X. [PubMed: 23298782]
24. Carmel R. Biomarkers of cobalamin (vitamin B-12) status in the epidemiologic setting: a critical overview of context, applications, and performance characteristics of cobalamin, methylmalonic acid, and holotranscobalamin II. *Am J Clin Nutr.* 2011;94(1):348S-58S. doi: 10.3945/ajcn.111.013441. [PubMed: 21593511]

25. Hooshmand B, Solomon A, Kareholt I, Rusanen M, Hanninen T, Leiviska J, et al. Associations between serum homocysteine, holotranscobalamin, folate and cognition in the elderly: a longitudinal study. *J Intern Med*. 2012;**271**(2):204-12. doi: 10.1111/j.1365-2796.2011.02484.x. [PubMed: 22077644]
26. Arora MK, Seth S, Dayal S. Homocysteine, folic acid and vitamin B12 levels in females with severe acne vulgaris. *Clin Chem Lab Med*. 2012;**50**(11):2061-3. doi: 10.1515/cclm-2012-0228. [PubMed: 23093336]
27. Collin SM, Metcalfe C, Refsum H, Lewis SJ, Zuccolo L, Smith GD, et al. Circulating folate, vitamin B12, homocysteine, vitamin B12 transport proteins, and risk of prostate cancer: a case-control study, systematic review, and meta-analysis. *Cancer Epidemiol Biomarkers Prev*. 2010;**19**(6):1632-42. doi: 10.1158/1055-9965.EPI-10-0180. [PubMed: 20501771]
28. Cullum-Dugan D, Lucas D, Pawlak R. *Academy of Nutrition and Dietetics. Vegetarian Toolkit*. 2015. Available from: <http://www.eatrightstore.org/product/F7AE3CE6-D2D1-4715-80BC-615040832949>.
29. Niafar M, Hai F, Porhomayon J, Nader ND. The role of metformin on vitamin B12 deficiency: a meta-analysis review. *Intern Emerg Med*. 2015;**10**(1):93-102. doi: 10.1007/s11739-014-1157-5. [PubMed: 25502588]
30. Kiasari AZ, Firouzian A, Baradari AG, Nia HS, Kiasari SH. The Effect of Vitamin B12 Infusion on Prevention of Nitrous Oxide-induced Homocysteine Increase: A Double-blind Randomized Controlled Trial. *Oman Med J*. 2014;**29**(3):194-7. doi: 10.5001/omj.2014.48. [PubMed: 24936269]
31. Gilsing AM, Crowe FL, Lloyd-Wright Z, Sanders TA, Appleby PN, Allen NE, et al. Serum concentrations of vitamin B12 and folate in British male omnivores, vegetarians and vegans: results from a cross-sectional analysis of the EPIC-Oxford cohort study. *Eur J Clin Nutr*. 2010;**64**(9):933-9. doi: 10.1038/ejcn.2010.142. [PubMed: 20648045]
32. Elmadfa I, Singer I. Vitamin B-12 and homocysteine status among vegetarians: a global perspective. *Am J Clin Nutr*. 2009;**89**(5):1693S-8S. doi: 10.3945/ajcn.2009.26736Y. [PubMed: 19357223]