Dietary source of vitamin B₁₂ intake and vitamin B₁₂ status in female elderly Koreans aged 85 and older living in rural area

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Abstract
Recently, we found and analyzed vitamin B₁₂ in some Korean traditional plant foods which had not reported, yet. This study was to investigate vitamin B₁₂ intake and its dietary sources and the vitamin B₁₂ status in the very old elderly Koreans. We measured serum vitamin B₁₂ level and estimated the amounts of vitamin B₁₂ intake from different dietary sources in female elderly Koreans aged 85 and over who had consumed a relatively low animal traditional diet for the whole life. The average age of the subjects (n = 127) was 98.0 years (85-108 years). The assessment on energy and nutrient intake involved a one-day 24-hour recall, and serum vitamin B₁₂ concentration was measured by radioimmunoassay. Overall diet pattern was not different between the 85-99 yr-old group and centenarians, except centenarians were taking more dairy product. The average ratio of plant food to animal food consumption was 87.5:12.5 in weight. The average vitamin B₁₂ intake of our subjects was 3.2 µg/day; and 52.7% of subjects consumed under estimated average requirement, 2.0 µg/day. On dietary source, 67.3% of dietary vitamin B₁₂ was from meat, eggs and fishes and 30.6% was from plant foods, such as soybean-fermented foods, seaweeds, and kimchi. The average serum vitamin B₁₂ concentration was 450.5 pg/mL, and low serum vitamin B₁₂ (<200 pg/mL) was found in 9.6% of subjects. Dietary vitamin B₁₂ intake was significantly lower in subjects with low serum vitamin B₁₂ (0.79 µg/day) than those with normal serum vitamin B₁₂ (3.47 µg/day). There were no significant difference in vitamin B₁₂ intake and its dietary sources and serum vitamin B₁₂ level between the 85-99 yr-old group and centenarians. In conclusion, several plant-origin foods including seaweed, soybean-fermented foods, and kimchi, may contribute significantly to good vitamin B₁₂ status in very old elderly Koreans.

Key Words: Female elderly, dietary source of vitamin B₁₂, serum vitamin B₁₂ level

Introduction
The prevalence of vitamin B₁₂ deficiency was underestimated in the past for several reasons, including the erroneous belief that deficiency is unlikely except in strict vegetarians or patients with pernicious anemia, and that it usually takes ~20 y for stores of the vitamin to become depleted [1].
Numerous reports have indicated the increasing prevalence of low vitamin B₁₂ levels among different segments of the general population [2-5]. Poor vitamin B₁₂ status is associated with neurological problems [6,7], hematological disorders [6,8], and other health-related conditions, including poor cognition and Alzheimer’s disease [9,10], depression [11], hearing loss [12], cancer [13], and poor bone health [14,15]. Recently, the rapid aging of many populations has increased attention to vitamin B₁₂.
It is known that more vegetarians or elderly people suffer from vitamin B₁₂ deficiency compared to omnivores or younger adults. Because natural sources of vitamin B₁₂ in human diets have known to be restricted to animal-origin food, it has been believed that those people with low animal food diets are more susceptible to cobalamin deficiency [16]. However, vitamin B₁₂ also exists in soybean-fermented foods, seaweeds, and tea leaves [17-19]. Recently, we reported that doenjang, a Korean soybean-fermented paste, contained 0.04-1.85 µg cobalamin/100 g, and that dried laver contained 66.76 µg/100 g (laver is normally consumed in dried form); these figures are surprisingly high compared to those of 0.9-1.2 µg/100 g for eggs [20], and also 1.33 µg per one sheet of dried laver (2 g) was higher than 0.45-0.6 µg per one egg (50 g) when calculated B₁₂ content per one serving size.
In terms of the relationship between vitamin B₁₂ and aging, it has been reported that atrophic gastritis increases with aging, thereby inducing decreased production of the gastric acid and digestive enzymes that are needed to cleave protein-bound
vitamin B₁₂ from the natural chemical form of vitamin B₁₂ found in foods. Therefore, Americans aged over 50 years have been advised to consume vitamin B₁₂ in the crystalline form [6].

Historically, Koreans have consumed a plant-based diet including steamed rice, staple food. Even today, the elderly, especially those living in rural areas, adhere to this traditional diet, even though young people have moved toward Western dietary patterns. There has been concern that vitamin B₁₂ status among the elderly is poor. However, data are not available on vitamin B₁₂ status and intake among elderly Koreans, and only a few reports have been published regarding vitamin B₁₂ intake or serum vitamin B₁₂ level among young adults [21,22] and pregnant women [23]. Moreover, the vitamin B₁₂ intake in extant reports are considered to represent underestimates because the Korean national database on vitamin B₁₂ content in foods does not contain data on plant-origin foods. Recently, we analyzed some plant-origin foods widely consumed in Korea [20], and updated this database accordingly.

In this study, we first assessed the vitamin B₁₂ status and intake pattern among very old elderly female Koreans living in rural areas by measuring serum vitamin B₁₂ level and estimating daily vitamin B₁₂ intake. Especially, we were interested in how much some unique plant-origin foods consumed by Koreans, such as soybean-fermented foods, seaweeds and kimchi, contribute to their vitamin B₁₂ intake.

Subjects and Methods

Subjects

Females aged 85 years and older were recruited in rural areas in Korea during summer of 2003 to 2005. Candidates were randomly selected based on birth records, and those living in facilities were excluded. Our research team including dietician, nurse, medical doctor and students visited each home after getting the consent. We kept the ethical and standard of institutional review board of author’s institute and the Helsinki Declaration during this study. For recruiting subjects, we explained about the purpose and process of this study to Government officer and village leaders, and also explained and took the consent from every candidate or her/his family member. Finally, 127 subjects, including 70 centenarians, participated in our study. The age of the subjects ranged from 85 to 108 years, and the average age was 98.0 ± 5.7 years. The age distribution of subjects is shown in Table 1.

Table 1. Age distribution of subjects

| Age (yr) | Total (n = 127) | 85-99 yr (n = 57) | ≥ 100 yr (n = 70) |
|----------|----------------|------------------|-----------------|
| 98.0 ± 5.7 | 92.5 ± 4.2 | 102.2 ± 1.9 |

1) Mean ± SD
2) Range

Food and nutrient intake including vitamin B₁₂

Well-trained interviewer wrote down one-day diet history of each subject by 24-h recall and weighing method with the help of her/his family members. The intake of dairy product, meat and eggs, fish and shellfish, cereals, potatoes and starch, sweets, legumes and tofu, vegetables and seaweeds, fruits, and soybean-fermented foods, as well as total food intake were calculated. The energy and nutrient intake of subjects were calculated using a computer software package, CAN-Pro 2.0, developed by the Korean Nutrition Society. In particular, we calculated daily vitamin B₁₂ intake and the proportions of vitamin B₁₂ from plant-origin foods such as soybean-fermented foods, seaweeds, kimchi and etc.

Blood test

Blood samples were drawn and delivered the same day to the laboratory in a cold iced box. Serum vitamin B₁₂ level was assessed by radioimmunoassay using a SimulTRAC-SNB Radioassay kit (ICN, New York, USA).

Statistical analysis

Analyses were performed using SAS version 9.2 software (SAS Institute, Inc., Cary, NC). All data were expressed as means and standard deviations, frequencies, or percentages. Statistically significant differences between groups were determined by t-test or Chi-square test.

Results

Food intake

The food intake pattern is shown in Table 2. The average total food intake of subjects was 761.1 g/day. Meals were comprised primarily of plant foods (87.5% of total) such as cereals, legumes and their products, vegetables, fruits, and so on. The average intake of cereals was 232.6 g/day, and almost of this consumption derived from rice, a staple food for Koreans. The subjects consumed 26.3 g/day of legumes, nuts, and tofu, a representative soybean product consumed in Korea. They consumed 216.1 g/day of vegetables and seaweeds, including 82.2 g/day of kimchi, the most popular vegetable-fermented food in Korea, and large portion of vegetable intake was derived from various blanched vegetables (namul in Korean). They also consumed 23.1 g/day of soybean-fermented foods, such as doenjang, chungkookjang, gochujang, and ganjiang. The average fruit intake was 71.0 g/day. On the other hand, subjects consumed 95.5 g/day of animal foods (12.5% of total), including 52.7 g of meat, poultry, and eggs; 30.9 g of fish and shellfish; and 10.8 g of dairy products.

On comparing the food intake patterns between the 85-99
1286 ± 510 kcal/day in the 85-99 yr-old group, and 1,186 ± 418 kcal/day in centenarians were not significantly different; the 85-99 yr-old group and centenarians consumed significantly more dairy products (P < 0.05) and sweets (P < 0.001) than the 85-99 yr-old group did, because some centenarians enjoyed candy and consumed yogurt. However, no significant differences in the consumption of the other food groups or in the ratio of animal to plant food intake were found.

Energy intake and vitamin B12 Intakes

Daily energy and protein, fat, carbohydrate and vitamin B12 intake of subjects are shown in Table 3. The average energy intake was 1,229 ± 457 kcal/day which is 76.8% of the estimated energy requirement (EER) for Korean women aged 75 and over, 1,600 kcal/day [24]. The average energy intake in the 85-99 yr-old group and centenarians were not significantly different; 1286 ± 510 kcal/day in the 85-99 yr-old group, and 1,186 ± 418 kcal/day in centenarians. In total subjects, 52.7% consumed less than 1.0 µg/day, estimated average requirement (EAR) for vitamin B12 for Korean adults, 2.4 µg /day [24]. There was no significant difference in daily mean vitamin B12 intake between the two age groups (2.48 ± 3.00 µg /day in the 85-99 yr-old group and 3.73 ± 5.79 µg /day in centenarians). In total subjects, 52.7% consumed less than 2.0 µg/day, estimated average requirement (EAR) for vitamin B12 for Korean adults (Table 3).

Dietary source of vitamin B12

Dietary sources of vitamin B12 are listed in Table 4. The percentages of vitamin B12 intake derived from animal food and plant food were 69.4% and 30.6% of total vitamin B12 intake, respectively. The primary source of vitamin B12 was clearly meat, eggs, and fish, which provided 2.45 ± 4.52 µg/day (67.3% of total intake); the secondary was soybean-fermented foods, providing 3.17 ± 4.77 µg/day in centenarians. The subjects consumed 49.3 g of protein/day, 109.6% of the recommended intake (RI) of protein, 45 g/day [24], even though they consumed less animal foods. As shown in Fig. 1, 70.6% of energy was from carbohydrates (220.5 g/day); 15.5% from protein and 13.9% from fat, and these proportions were not significantly different between the two age groups.

In terms of vitamin B12, our subjects consumed 3.17 ± 4.77 µg /day of vitamin B12, which is about 132% of RI for Korean adults, 2.4 µg /day [24]. There was no significant difference in daily mean vitamin B12 intake between the two age groups (2.48 ± 3.00 µg /day in the 85-99 yr-old group and 3.73 ± 5.79 µg /day in centenarians). In total subjects, 52.7% consumed less than 2.0 µg/day, estimated average requirement (EAR) for vitamin B12 for Korean adults (Table 3).
However, vitamin B12 intake was significantly lower (vitamin B12 deficient. The prevalence of low serum vitamin B12 concentration was not significantly different between the two age groups (Table 5).

Table 5. Serum vitamin B12 concentration of subjects

| Serum B12 level (pg/mL) | Total (n = 83) | 85-99 yrs (n = 21) | ≥ 100 yrs (n = 62) | P-value |
|-------------------------|---------------|-------------------|-------------------|---------|
| Low (< 200)             | 8 (9.6)²      | 1 (4.8)           | 7 (11.3)          | 0.3810  |
| Normal (≥ 200)          | 75 (90.4)     | 20 (95.2)         | 55 (88.7)         |         |

² Mean ± SD

Table 6. Relation between serum vitamin B12 concentration and dietary intake

| Serum vitamin B12 (pg/mL) | Vitamin B12 intake (µg/day) | P-value |
|---------------------------|----------------------------|---------|
| Low (< 200)               | 0.79 ± 0.79³              | 0.0005***|
| Normal (≥ 200)            | 3.47 ± 5.72               |         |

³ Mean ± SD

It was not observed a significant correlation between serum vitamin B12 concentration and dietary intake (P = 0.4769). However, vitamin B12 intake was significantly lower (P < 0.001) in the subjects with low serum vitamin B12 (0.79 ± 0.79 µg/day) than those in normal subjects (3.47 ± 5.72 µg/day) (Table 6).

Discussion

It is well known that the metabolism of vitamin B12, folate, and homocysteine are associated in humans and play very important roles in preventing cognitive impairment in the elderly [27-29]. Risk factors for vitamin B12 deficiency include low animal protein intake, malabsorption associated with atrophic gastritis or Helicobacter pylori infection, pancreatic or intestinal pathology, and gastric acid-reducing medications [6,30-32]. Malabsorption of vitamin B12 from food is the main cause of deficiency in the elderly and explains why depletion occurs with aging. The condition is caused by atrophy of gastric mucosa and the gradual loss of gastric acid, which releases the vitamin from food. The low gastric pH that occurs as a result of gastric atrophy can also increase bacterial overgrowth in the upper intestine, which results in less absorption of protein-bound vitamin B12.

Approximately, 10-30% of older adults suffer from malabsorption of protein-bound vitamin B12 [6].

Serum vitamin B12 concentrations < 150 pmol/L (200 pg/mL) indicates frank vitamin B12 deficiency, but there is no widely accepted biochemical cutoff for marginal or preclinical vitamin B12 deficiency or vitamin B12 adequacy [6]. The use of both serum vitamin B12 and methylmalonic acid (MMA) or holotranscobalamin is more recommended to improve diagnosis of vitamin B12 deficiency [25,26]. However, it is limitation s to use MMA level for simple screening of the vitamin B12 status because MMA measurement needs mass spectrometry which is not easily available and needs trained labor and high cost.

It has been reported that, depending on the biochemical criteria for vitamin B12 and/or MMA, approximately 5 - 20% of elderly individuals are deficient in vitamin B12 in the Americans [1,6,30,33]. Pfeiffer et al. [34] reported that the prevalence of vitamin B12 deficiency (serum concentration < 200 pg/mL) in US population varied by age group and affected ≤ 3% of those aged 20-39 y, ~ 4% of those aged 40-59 y and ~6% of persons aged ≥ 70 y, and plasma MMA concentration were markedly higher after > 60 y. The prevalence of vitamin B12 deficiency increased substantially after 69 y in 3 UK surveys; it affected about 1 in 20 people aged 65-74 y and at least 1 in 10 of those aged ≥ 75 y [1].

Originally, we expected that vitamin B12 deficiency would be more prevalent in our subjects than in subjects of Western countries because our cohort were included many centenarians and had been eating a greater proportion of plant foods. However, only 9.6% of our subjects showed low vitamin B12 concentration (< 200 pg/mL), which was lower than those of Western cohorts. So far, there were very few reports on vitamin B12 status or intake in subjects aged 85 and more. The mean serum vitamin B12 concentration of our subjects, 450.5 pg/mL (337 pmol/L), was similar to 332 pmol/L of an elderly cohort (mean age 76.4 years) in the US [30], but lower than 358 pmol/L of 71-74 y-old female Norwegian cohort [35].

The relation between dietary intake and vitamin B12 status has
been investigated in different populations, with conflicting results. One study concluded that low vitamin B$_12$ status in the elderly was not related to inadequate intake [36], whereas other reports showed significant associations between intake of vitamin B$_12$ and plasma concentrations [37,38]. Recently, Vogiatzoglou et al. [35] reported that the association of plasma vitamin B$_2$ with food intake was weaker in older subjects than in younger subjects, and that plasma vitamin B$_{12}$ was associated with intakes of increasing amounts of vitamin B$_{12}$ from dairy products or fish but not with intakes of vitamin B$_{12}$ from meat or eggs. In the present study, it was not observed significant correlation between dietary intake and serum vitamin B$_{12}$ concentration ($P=0.4769$), however, the dietary intake of the subjects with low serum vitamin B$_{12}$ (< 200 pg/mL) was significantly lower ($P=0.0005$) than that of the subjects with normal serum normal serum vitamin B$_{12}$.

Our subjects consumed dietary vitamin B$_{12}$ (3.17 µg/day) less than that in female subjects aged 85 and older in Austria (3.9 µg/day) or the UK (4.3 µg/day) [39]. Interestingly, the dietary source of vitamin B$_{12}$ intake was totally different. Whereas our subjects were taking 30.6% of total vitamin B$_{12}$ intake from plant-origin foods, mainly soybean-fermented foods, seaweeds and kimchi, female Norwegian aged 71-74 y were taking 5.0 µg/day of vitamin B$_{12}$, which was entirely from animal foods; 52.7% from meat, fish and eggs and 47.3% from milk and dairy products [35].

Most of Koreans enjoy fermented foods, such as doenjang (soybean-fermented paste), ganjang (soy sauce) and gochujang (Red pepper, soybean & starch-fermented paste), and kimchi (vegetable-fermented foods) every day, and seaweeds very often. Therefore, it is considered that these foods are very helpful in protecting the elderly Koreans from vulnerability to vitamin B$_{12}$ deficiency. Some edible algae, including laver, have already been investigated in different populations, with conflicting results. One study concluded that low vitamin B$_12$ status in the elderly was not related to inadequate intake [36], whereas other reports showed significant associations between intake of vitamin B$_12$ and plasma concentrations [37,38].

References

1. Allen LH. How common is vitamin B$_{12}$ deficiency? Am J Clin Nutr 2009;89:693-9.
2. Masalha R, Rudoy I, Volkov I, Yusuf N, Wirguin I, Herishana Y. Symptomatic dietary vitamin B$_{12}$ deficiency in a nonvegetarian population. Am J Med 2002;112:413-6.
3. Allen LH. Folate and vitamin B$_{12}$ status in Americans. Nutr Rev 2004;62:29-33.
4. Stabler SP, Allen RH. Vitamin B$_{12}$ deficiency as a worldwide problem. Annu Rev Nutr 2004;24:299-326.
5. Volkov I, Rudoy I, Machagna M, Gleizer I, Ganel U, Orenshtein A, Press Y. Modern society and prospects of low vitamin B$_{12}$ intake. Ann Nutr Metab 2007;51:468-70.
6. Baik HW, Russell RM. Vitamin B$_{12}$ deficiency in the elderly. Annu Rev Nutr 1999;19:357-77.
7. Miller A, Korem M, Almqvist C, Galboiz Y. Vitamin B$_{12}$, demyelination, remyelination and repair in multiple sclerosis. J Neurol Sci 2005;233:93-7.
8. Herbert V. Vitamin B$_{12}$: Plant sources, requirements and assay. Am J Clin Nutr 1988;58:852-8.
9. Reynish W, Andrieu S, Nourhashemi, Vella B. Nutritional factors and Alzheimer’s disease. J Gerontol A Biol Sci Med Sci 2001;56:675-80.
10. Lewis MS, Miller LS. Elevated methylmalonic acid is related to cognitive impairment in older adults enrolled in an elderly nutrition program. J Nutr Elder 2005;24:47-65.
11. Pennix BW, Guralnik JM, Ferrucci L, Fried LP, Allen RH, Stabler SP. Vitamin B$_{12}$ deficiency and depression in physically disabled older women: Epidemiologic evidence from the Women’s Health and Aging Study. Am J Psychiatry 2000;157:715-21.
12. Houston DK, Johnson MA, Nozza RJ, Gunter EW, Shea KJ, Cutler GM, Edmonds JT. Age-related hearing loss, vitamin B$_{12}$ and folate in elderly women. Am J Clin Nutr 1999;69:564-71.
13. Ames BN, Wakimoto P. Are vitamin and mineral deficiencies a major cancer risk? Nat Rev Cancer 2002;2:694-704.
14. Morris MS, Jacques PF, Selhub J. Relation between homocysteine and B-vitamin status indicators and bone mineral density in older Americans. Bone 2005;37:234-42.
15. Dhonukshe-Rutten RAM, Pluijm SMF, de Groot LCPGM, Lips P, Smits JH, van Staveren WA. Homocysteine and vitamin B$_{12}$ status relate to bone turnover markers, broadband ultrasound attenuation, and fractures in healthy elderly people. J Bone Miner Res 2005;20:921-9.
16. Herbert V. Staging Vitamin B$_{12}$ status in vegetarians. Am J Clin Nutr 1994;59:1213-22.
17. Liem ITH, Steinkraus KH, Cronk TC. Production of vitamin B$_{12}$ in Tempe, a fermented soybean food. Appl Environ Microbiol 1977;34:773-6.
18. Watanabe F, Takenaka S, Katsura H, Zakir Hussain Masumder SAM, Abe K, Tamura Y, Nakano Y. Dried green and purple lavers (Nori) contain substantial amounts of biologically active vitamin B$_{12}$ but less of dietary iodine relative to other edible seaweeds. J Agric Food Chem 1999;47:2341-3.
19. Watanabe F. Vitamin B$_{12}$ source and bioavailability. Exp Biol Med 2007;232:1266-74.
20. Kwak CS, Hwang JY, Watanabe F, Park SC. Vitamin B$_{12}$ content in some Korean fermented foods and edible seaweeds. The Korean Journal of Nutrition 2008;41:439-47.
21. Lim HS, Lee Nam K, Heo Y. The relationships of health-related lifestyles with homocysteine, folate, and vitamin B$_{12}$ status in Korean adults. Korean Journal of Community Nutrition 2001;6:507-15.
22. Kang HS, Lee MC, You YC, Chang N. Effect of endurance
training on the plasma homocysteine and vitamin B levels in male adolescent field hockey players. The Korean Journal of Nutrition 2004;37:881-7.

23. Kim KN, Kim YJ, Chang N. The interaction of the 5,10-methylenetetrahydrofolate reductase (MTHFR) polymorphism with folate and vitamin B12 intake and serum homocysteine concentrations in pregnant women. The Korean Journal of Nutrition 2002;35:1045-52.

24. The Korean Nutrition Society. Dietary Reference Intakes for Koreans. Seoul: Kookjin Publishing Co.; 2005.

25. Carmel R. Current concepts in cobalamin deficiency. Annu Rev Med 2000;51:357-75.

26. Park S, Johnson MA. What is an adequate dose of oral vitamin B12 in older people with poor vitamin B12 status? Nutr Rev 2006;64:373-8.

27. Kado DM, Karlamangla AS, Huang MH, Dphil AT, Rowe JW, Selhub J, Seeman T. Homocysteine versus the vitamins folate, B6, and B12 as predictors of cognitive function and decline in older high-functioning adults: MacArthur Studies of Successful Aging. Am J Med 2005;118:161-7.

28. Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L, Ueland PM. Folate, vitamin B12 and serum total homocysteine levels in confirmed Alzheimer disease. Arch Neurol 1998;55:1449-55.

29. Hulberg B, Jensen E, Dehlin O, Hagberg B, Samuelsson G, Svensson T. Concentrations of plasma methylmalonic acid in 80-year-olds show only weak relation to psychological performance. Clin Chem Lab Med 1999;37:963-7.

30. Johnson MA, Hawthorne NA, Brackett WR, Fisher JG, Gunter EW, Allen RH, Stabler S. Hyperhomocysteinemia and vitamin B12 deficiency in elderly using Title III C nutrition services. Am J Clin Nutr 2003;77:211-20.

31. Garcia A, Paris-Pombo A, Evans L, Day A, Freedman M. Is low-dose oral cobalamin enough to normalize cobalamin function in older people? J Am Geriatr Soc 2002;50:1401-4.

32. Rajan S, Wallace JL. Screening for cobalamin deficiency in geriatric outpatients: Prevalence and influence of synthetic cobalamin intake. J Am Geriatr Soc 2002;50:624-30.

33. Hin H, Clark R, Sherliker P, Atoyebi W, Emmens K, Birks J, Schneede J, Ueland PM, Nexø E, Scott J, Molloy A, Donaghy M, Frost C, Evans G. Clinical relevance of low serum vitamin B12 concentrations in older people: The Banbury B12 study. Age Ageing 2006;35:416-22.

34. Pfiffner CM, Caudill SP, Guenther EW, Osterloh J, Sampson EJ. Biochemical indicators of B vitamin status in the US population after folic acid fortification: results from the National Health and Nutrition Examination Survey 1999-2000. Am J Clin Nutr 2005;82:442-50.

35. Vogiatzoglou A, Smith AD, Nurk E, Berstad P, Drevon CA, Ueland PM, Vollset SE, Tell GS, Refsum H. Dietary sources of vitamin B12 and their association with plasma vitamin B12-concentrations in the general population: the Hordaland Homocystein Study. Am J Clin Nutr 2009;89:1078-89.

36. Howard JM, Azén C, Jacobsen DW, Green R, Carmel R. Dietary intake of cobalamin in elderly people who have abnormal serum cobalamin, methylmalonic acid and homocysteine levels. Eur J Clin Nutr 1998;52:582-7.

37. Tucker KL, Rich S, Rosenberg I, Jacques P, Dallal G, Wilson PWF, Selhub J. Plasma vitamin B12 concentrations related to intake source in the Framingham Offspring study. Am J Clin Nutr 2000;71:514-22.

38. Bor MV, Lydeking-Olsen E, Møller J, Nexø E. A daily intake of approximately 6 microg vitamin B12 appears to saturate all the vitamin B12 related variables in Danish postmenopausal women. Am J Clin Nutr 2006;83:52-8.

39. Fabian E, Elmadfa I. Nutritional situation of the elderly in the European Union: Data from the European nutrition and health report (2004). Ann Nutr Metab 2008;52:57-61.

40. Watanabe F, Takenaka S, Kittaka-Katsura H, Ebara S, Miyamoto E. Characterization and bioavailability of vitamin B12-compounds from edible algae. J Nutr Sci Vitaminol 2002;48:325-31.

41. Takenaka S, Sugiyama S, Ebara S, Miyamoto E, Abe K, Tamura Y, Watanabe F, Tsuyama S, Nakano Y. Feeding dried purple laver (nori) to vitamin B12-deficient rats significantly improves vitamin B12 status. Br J Nutr 2001;85:699-703.