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Vitamin B-12 Status of Long-Term Adherents of a Strict Uncooked Vegan Diet ("Living Food Diet") Is Compromised[1,2]

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ABSTRACT The present study examined the vitamin B-12 status in long-term adherents of a strict uncooked vegan diet called the "living food diet." The study was comprised of two parts. In the cross-sectional part, the data on serum vitamin B-12 concentrations and dietary intakes in 21 (1 male, 20 females) long-term adherents (mean 5.2 y, range 0.7–14) of the "living food diet" were compared with those of 21 omnivorous controls matched for sex, age, social status and residence. In the longitudinal part of the study, food consumption data were collected and blood samples were taken from nine "living food eaters" (1 male, 8 females) on two occasions 2 y apart. The cross-sectional study revealed significantly ($P < 0.001$, paired t test) lower serum vitamin B-12 concentrations in the vegans (mean 193 pmol/L, range 35–408) compared with their matched omnivorous controls (311, 131–482). In the vegan group, total vitamin B-12 intake correlated significantly ($r = 0.63$, $P < 0.01$) with serum vitamin B-12 concentration. The vegans consuming Nori and/or Chlorella seaweeds ($n = 16$) had serum vitamin B-12 concentrations twice as high as those not using these seaweeds ($n = 5$) (mean 221 pmol/L, range 75–408, vs. 105, 35–252, $P = 0.025$). In the longitudinal study, six of nine vegans showed slow, but consistent deterioration of vitamin B-12 status over a 2-y observation period. On the basis of these results we conclude that some seaweeds consumed in large amounts can supply adequate amounts of bioavailable vitamin B-12. However, the average use of seaweeds and fermented foods by "living food eaters" will not supply enough vitamin B-12 to maintain the body vitamin B-12 status. J. Nutr. 125: 2511–2515, 1995.

INDEXING KEY WORDS:

- *vegan* • *vitamin B-12* • *raw food* • *humans*
- *fermented food* • *seaweed*

Strict vegans, called "living food eaters," consume all of their food uncooked, and most of the daily food items are fermented or sprouted (Rauma et al. 1993). These food preparation methods are thought to enhance the growth of vitamin B-12–producing bacteria (Ro et al. 1979, Van Veen and Steinkraus 1970), and therefore the advocates of the "living food diet" are convinced that their diet will supply more vitamin B-12 than an ordinary vegan diet. Furthermore, the advocates believe that this diet, which is rich in lactobacilli, will modify the microflora at the distal ileum, thereby enhancing the amount of bioavailable vitamin B-12. In fact, only minute amounts of vitamin B-12 are found in fermented foods (Österdahl and Johansson 1989), and most of vitamin B-12 in seaweeds are analogues of the true vitamin (Herbert and Drivas 1982, van den Berg et al. 1988). Therefore, it remains to be shown whether the long-term use of large amounts of uncooked fermented foods and seaweeds could actually supply enough biologically active vitamin B-12.

SUBJECTS AND METHODS

Subjects. The present study is comprised of two parts. In the cross-sectional part, 21 (1 male, 20 females) long-term adherents of a strict uncooked vegan diet ("living food diet") were recruited via an advertisement in a living food eaters' newsletter in Finland ('The Friend of Living Food'). In the longitudinal study, the subjects were nine "pioneers" who had participated in our earlier study two years before (Rauma et al. 1994). The average duration of the vegan diet in

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TABLE 1

Characteristics of the long-term adherents of a strict uncooked vegan diet ("living food diet") and of their matched omnivorous controls[1,2]

	Vegans		Controls	
	Females (20)	Male	Females (20)	Male
Age, *y*	46 (27–69)	40	44 (27–65)	38
Weight, *kg*	57* (45–90)	69	67 (56–87)	91
Height, *cm*	163 (156–172)	176	164 (153–171)	192
BMI, *kg/m*^{2}	21* (18–31)	22	25 (20–35)	25
Duration of vegan diet, *y*	5.2 (0.7–14)	6	—	—

[1] Values are means (range).
[2] Statistical significances: * $P < 0.01$ (Student's *t* test for paired samples).

the cross-sectional portion was 5.2 y (range 0.7–14), and in the longitudinal study, 8.3 y (range 5–14) (**Table 1**). All participants were volunteers. Those who smoked, suffered from the active phase of any illness, or used regular medication, were excluded from the study. None of the study subjects had used any vitamin B-12 supplementation. Each vegan subject was matched with an omnivorous control subject for sex, age, social status and residence. Informed consent was obtained from each subject before the study, and the study protocol was accepted by the Ethical Committee of Kuopio University and Central Hospital.

Health assessment. The general health status of subjects was assessed by routine clinical blood chemistry (blood cell counts and hemoglobin concentration, serum aminotransferase and alkaline phosphatase activities, and serum concentrations of bilirubin, creatinine, albumin, transferrin and ferritin). The participants were also asked to report the consumption of alcohol and health supplements, physical activities, menstrual cycle and compliance to the vegan diet (vegans only).

Dietary assessment. Dietary information was obtained using 5-d food records (the cross-sectional study) and 7-d food records (the longitudinal study). Nutrient intakes were calculated by the Nutrica Computer Program (Social Insurance Institution, Finland) using the Finnish nutrient database (Rastas et al. 1993), supplemented with nutrient values of the vegetable foods commonly used by the vegans. The database contains the vitamin B-12 content of foods of animal origin but not of the ingredients of the vegan diet, other than those of the seaweeds Chlorella and Nori.

Biochemical measurements. Blood samples were collected by venipuncture after an overnight fast. Blood hemoglobin concentration and erythrocyte mean corpuscular volume were analyzed using Coulter Counter[R] Model S-PLUS VI analyzer, (Hialeah, FL).

Twenty-four-hour urine samples were collected in plastic containers and aliquots were stored frozen until determination of sodium concentration. Sodium was analyzed using an ion-selective electrode in a Hitachi 717 analyzer (Hitachi, Tokyo, Japan).

In the cross-sectional study, serum vitamin B-12 and folic acid concentrations were determined using the Vitamin B-12/Folate Dual Radioassay Kit (Kodak, Clinical Diagnostics, Amersham, UK). Because the serum vitamin B-12 concentrations of the nine subjects of the earlier study (Rauma et al. 1994) had been determined using the Quantaphase II B-12/Folate Radioimmunoassay Kit (Bio-Rad, Diagnostics Group, Hercules, CA), the samples of the second collection from the same nine subjects (cross-sectional study) were reanalyzed using the Bio-Rad kit to allow longitudinal comparison.

Statistical analyses. The SPSS/PC computer program was used in the statistical analyses. Statistical significances of the differences between the matched pairs (vegan vs. omnivorous control) was assessed by Student's *t* test for paired samples. Pearson's correlation coefficients were used to establish the correlations between the serum vitamin B-12 concentrations and the calculated dietary intakes of vitamin B-12, the duration of the "living food diet" and the hematological variables (hemoglobin, mean corpuscular volume and erythrocyte folate). The Mann-Whitney U-test was used to statistically compare the data between the vegan subgroups of seaweed users ($n = 16$) and those not using Chlorella and/or Nori seaweed ($n = 5$).

RESULTS AND DISCUSSION

Mean values for age and height were similar within the pairs, but the vegans had significantly lower body weights and body mass indices (BMI) (Table 1). As judged by the routine clinical blood chemistry, all participants were healthy.

In the cross-sectional study, serum vitamin B-12 concentrations of the "living food eaters" (mean 193 pmol/L, range 35–408) were significantly lower (*P*

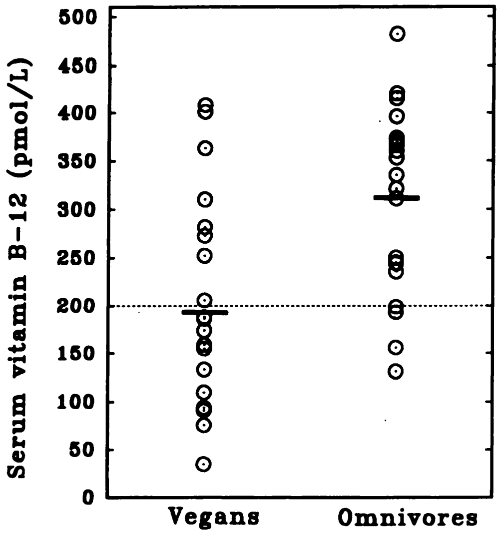

FIGURE 1 Cross-sectional data on serum vitamin B-12 concentrations in long-term adherents of a strict uncooked vegan diet ("living food diet") and in their matched omnivorous controls. The bar represents the mean value of the group (n = 21). The dashed line is the lower reference limit for serum vitamin B-12 concentration (The Deaconess Laboratory, Helsinki).

< 0.01) than those of the omnivorous controls (311, 131–482) (**Fig. 1**). In the vegan group, 57% of the subjects had values below the lower reference limit (200 pmol/L, Kodak kit) used by The Deaconess Laboratory, Helsinki, whereas the respective percentage in the control group was 19. Serum vitamin B-12 concentration was not associated with duration of the diet (r = 0.07, n = 21) (**Fig. 2**). The lowest values (35 pmol/L) were observed in a married couple who had adhered to the "living food diet" for 6 y, and the highest value (408 pmol/L) in a subject who had consumed this diet for 5 y.

Serum vitamin B-12 concentration was not associated with the mean corpuscular volume (MCV) of erythrocytes, blood hemoglobin (B-Hb) concentration, nor with erythrocyte folate concentration. Moreover, the concentrations of these variables in the vegans did not differ significantly from those of the omnivorous controls, and all values were within the appropriate reference ranges of The Deaconess Laboratory, Helsinki (B-Hb: female 125–160, male 135–180 g/l; E-MCV: 76–96 fl; E-Folate: 350–1000 nmol/L).

The urinary 24-h excretion of sodium by the vegans (mean 50 mmol/day, range 14–88) was considerably lower than that of the omnivorous controls (151 mmol/day, 78–391). The low urinary sodium indicates a good compliance to the vegan diet (Hänninen et al. 1993). The average daily consumption of fermented foods by the vegan subjects was ~2 kg, including wheat drink (1 L/d), oat yogurt, sauerkraut and other fermented vegetables.

The mean (range) dietary intake of vitamin B-12 (including vitamin B-12 from both animal foods and from seaweeds) in the vegan group was 1.8 (0–12.8) μg/d, and in the omnivorous control group 6.2 (2.2–12.8) μg/d (P < 0.001). The majority of the vegan group (n = 16) received vitamin B-12 from Chlorella and Nori seaweeds (mean 2.2 μg/d, range 0.2–12.6). These seaweeds contained 125.9 (Japan Dairy Technical Association, Kioicho, Chiyo-Daku, Tokyo, Japan) and 68.8 μg (van den Berg et al. 1988) vitamin B-12/100 g, respectively. Because no analytical data on vitamin B-12 content of the other ingredients of the "living food diet" were available, the calculated daily vitamin B-12 intake by the vegans was less than the true vitamin B-12 intake. However, the vegans using seaweed (n = 16) had serum vitamin B-12 concentrations twice as high as those not using the above-mentioned seaweeds (n = 5) (mean 221 pmol/L, range 75–408 vs. 105, 35–252, P = 0.025), indicating that ingredients other than seaweed did not provide substantial amounts of bioavailable vitamin B-12. Total vitamin B-12 intake in the vegan group (n = 21) correlated significantly with the serum vitamin B-12 concentration (r = 0.63, P < 0.01), but not in the controls (r = 0.31).

The longitudinal study revealed a decrease in serum vitamin B-12 concentrations with time in six of nine subjects, indicating that the supply of vitamin B-12 from the "living food diet" is inadequate to maintain the serum vitamin B-12 concentration (**Table 2**). The concentrations of vitamin B-12 in the serum of the subjects using large amounts of Chlorella (subject #9 or Nori (subject #6) were clearly above the mean of the vegan group, indicating that consumption of these seaweeds in larger amounts than recommended by the importer (Chlorella 1.7 g/d) or by the "living food" therapeutists (Nori 0.25 g/d) will supply biologically available vitamin B-12. However, this practice is not recommended, because excessive use of these seaweeds will concomitantly lead to harmful amounts of dietary

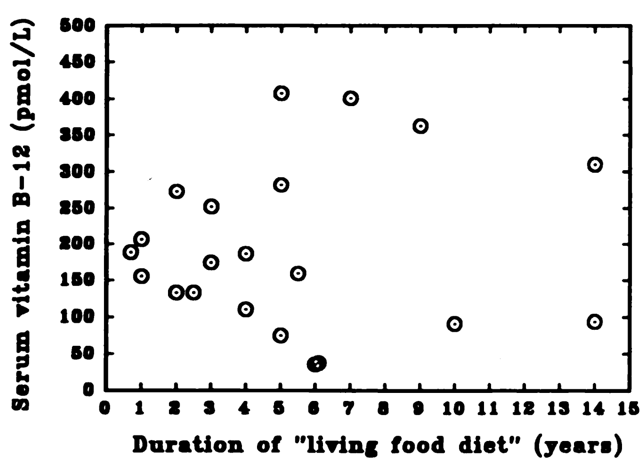

FIGURE 2 Serum vitamin B-12 concentrations in long-term adherents of a strict uncooked vegan diet ("living food diet") as a function of years consuming the diet (r = 0.07, n = 21).

TABLE 2

Serum vitamin B-12 concentrations and use of seaweeds in long-term adherents of a strict uncooked vegan diet ("living food diet")

Subject	Duration of vegan diet	Serum vitamin B-12			Seaweed intake	
		1992[1]	1994[1]	1994[2]	1992	1994
	y		*pmol/L*		*g/d*	
1	10	136*	133*	91*	Nori 1.0	Dulce 0.3
2	6	84*	78*	35*	Nori 1.0 AKS[3]	Arame 2.0
3	5	112*	99*	75*	Nori 0.7	Spirulina 3.2 Nori 0.5 Arame 0.3
4	14	151	135*	94*	Nori 1.0	Spirulina 3.0 Arame 1.8 Nori 0.5
5	5	360	328	282	Nori 1.3	Arame 0.6 Nori 0.5
6	14	825*	640	310	Nori 5.0	Nori 3.0
7	6	84*	85*	35*	Nori 1.0 AKS[3]	Arame 2.0
8	6	173	190	159*	Nori 0.6	Nori 0.7 Arame 0.6
9	9	217	393	363	Chlorella 6.9	Chlorella 5.2 Arame 1.0 Nori 0.5
Mean	8.3	238	231	160		
SD	3.6	236	189	126		

[1] Quantaphase II B-12/Folate Radioimmunoassay Kit, Bio-Rad Diagnostics Group, Hercules, CA (* outside of the reference range, 150–650 pmol/L).
[2] Vitamin B-12/Folate Dual Radioassay Kit, Kodak, Clinical Diagnostics, Amersham, UK (* outside of the reference range, 200–800 pmol/L).
[3] Arame-Kelbamare-Spirulina.

iodine (Rauma et al. 1994). The reason for the very high serum vitamin B-12 concentration of subject #6 (Table 2) is unknown. Her vitamin B-12 intake was 3.4 μg/d, which is nearly twice the current recommended daily allowance for vitamin B-12 (Recommended Dietary Allowances 1989).

The present data support the earlier findings of poor vitamin B-12 status in vegetarians (Bar-Sella et al. 1990, Dong and Scott 1982, Immerman 1981, Miller et al. 1991), and indicate that the "living food eaters" should not rely on the use of fermented foods for supplying adequate amounts of vitamin B-12 to maintain the serum vitamin B-12 concentration within the reference range. However, unlike the earlier studies (Miller 1991, van den Berg 1988), the present study demonstrated clearly that excess consumption of some seaweeds can preserve the body vitamin B-12 status.

The depletion of vitamin B-12 stores in the vegans was slow, and varied among individuals due to the observed differences in seaweed intake and presumably also due to the initial body vitamin B-12 pool when starting the vegan regimen. The other factors known to influence the depletion of serum vitamin B-12 concentration are the enterohepatic circulation of vitamin B-12, and the overall health and nutritional status of the vegetarians (Herbert 1994). Especially vulnerable groups are pregnant vegan women, and infants and children of vegan mothers with low vitamin B-12 stores (Higginbottom 1978, Miller 1991). According to Herbert (1994), a low total serum vitamin B-12 concentration is an indicator of deteriorated vitamin B-12 status, not merely that of a short-term negative balance. Low serum vitamin B-12 concentrations may have deleterious effects on DNA synthesis and nerve cell myelination in spite of the absence of hematological abnormalities (Herbert 1994), and hence the occurrence of low serum vitamin B-12 concentrations in over 50% of the long-term adherents of this diet warrants further study of the possible health risks involved.

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