Serum Vitamin B₁₂ Levels in Young Vegans Who Eat Brown Rice

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Summary A nutritional analysis was conducted on the dietary intake of a group of 6 vegan children aged 7 to 14 who had been living on a vegan diet including brown rice for from 4 to 10 years, and on that of an age-matched control group. In addition, their serum vitamin B₁₂ levels and other data (red blood cell count, hematocrit, hemoglobin, etc.) were determined in the laboratory. In vegans' diets, 2–4 g of *nori* (dried laver), which contained B₁₂, were consumed daily. Not a single case of symptoms due to B₁₂ deficiency was found. There were no statistically significant differences between the two groups with respect to any of the examination data, including B₁₂ levels ($p < 0.05$). Therefore, consumption of *nori* may keep vegans from suffering B₁₂ deficiency.

Key Words vitamin B₁₂, vegan, vegetarian, diet, brown rice, seaweed

In recent years vegetarianism has been attracting attention due to the increase of allergic diseases and adult diseases such as heart disease and diabetes which seem to be caused by an excessive intake of animal foods (1,2). Vegetarians are divided into three categories: Vegans, or pure vegetarians who abstain from all foods of animal origin; lacto-vegetarians who include milk and dairy products in their diets; and lacto-ovo-vegetarians who consume egg products as well as milk and dairy products. Vitamin B₁₂ deficiency has been reported in strict vegans (3–5).

Genmai-saishoku (literally brown rice-proper diet) which this study is concerned with, is a pure vegetarian diet that emphasizes eating brown rice and vegetables in proper yin-yang balance (6). It is practiced mainly in Japan and is characterized by its unique selection and preparation of foods. It finds its historical antecedent in the macrobiotic diet (7), but macrobiotics as currently practiced in the U.S. and European countries is rather different from *genmai-saishoku* as practiced in Japan (8). Therefore, the term *genmai-saishoku* will be used throughout this paper.

So far in Japan few studies have been made about serum vitamin B₁₂ levels in vegans, especially in young vegans. The author determined serum vitamin B₁₂ levels and other data (red blood cell count, hematocrit, hemoglobin, etc.) among strict vegans who have practiced *genmai-saishoku* for many years.

SUBJECTS AND METHODS

Blood was sampled on August, 1993 from 6 strict vegan children (two boys and four girls) ranging from 7 to 14 years old. Each of the children were asked how long s/he had been on a vegan diet, and what the diet consisted of. The control group consisted of 4 children, who were the subjects' friends and who were not vegans. It was confirmed that all the subjects were in good health, and had taken no chemicals including vitamin supplements at least for the past 4 years. The nutritional data are based on the diets during 7 days prior to blood sampling.

Blood samples of the 10 children were analyzed immediately after sampling for serum vitamin B₁₂ (B₁₂), red blood cell count (RBC), hematocrit (Ht), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC), platelet count (PLT), serum iron (Fe), total iron-binding capacity (TIBC), albumin (Alb), cholinesterase (chE), total cholesterol (TC), high-density lipoprotein cholesterol (HDL), and triglycerides (TG). All of these items were measured by Kyoto Medical Science Laboratory and serum vitamin B₁₂ levels were determined by protein binding assay. Blood was sampled, with the parents' permission, early in the morning before breakfast. Statistical analysis was performed with the unpaired Student's *t*-test.

RESULTS

Table 1 gives nutritional data of the vegan group and the control group (at the time when the data were taken, children had no school due to summer vacation. On school days, vegan children in the *genmai-saishoku* group carried their lunch and did not eat school lunch). The nutritional constitution of the vegan diet was as follows (the calculation was based on the data in Standard Tables of Food

Table 1. Daily dietary intakes in controls and *genmai-saishoku* vegans.

	<i>Genmai-saishoku</i> vegans	Controls
Total calorie intake (kcal)	1,486 ± 129	1,469 ± 137
Protein (% of total calories)	11.7 ± 1.0	19.5 ± 3.1
Animal protein (% of total protein)	0	45.1 ± 9.9
Plant protein (% of total protein)	100	54.8 ± 9.9
Carbohydrate (% of total calories)	68.3 ± 7.4	57.8 ± 5.1
Fat (% of total calories)	20.0 ± 7.7	19.3 ± 2.1
P/S ratio	2.3 ± 0.6	1.3 ± 0.5
Saturated fatty acid (g)	7.0 ± 5.4	8.3 ± 2.7
Polyunsaturated fatty acid (g)	13.7 ± 5.8	10.3 ± 3.1
Cholesterol (mg/day)	< 1 mg	280 ± 119

P/S ratio = ratio of polyunsaturated to saturated fat.

Composition in Japan) (9): The total caloric intake per day was $1,486 \pm 129$ kcal. Protein (all of plant origin) accounted for $11.7 \pm 1.0\%$, carbohydrate $68.3 \pm 7.4\%$, and fat $20.0 \pm 7.7\%$. Fat intake per day was 32 ± 13 g, the P/S ratio (ratio of polyunsaturated to saturated fat) was as high as 2.3 ± 0.6 (the control: 1.3 ± 0.5) with little cholesterol being included.

The diet of genmai-saishoku group had the following characteristics

1) The diet mainly consisted of brown rice and other grains, and no animal foods [meat, fish, eggs, dairy, or even *dashi* (stock)]. Sea vegetables were abundantly taken [*nori* (dried laver), *hijiki*, *wakame*, *konbu*]. Particularly all 6 vegan children consumed 2–4 g of *nori* per day. Natural sea salt, *miso*, *shoyu* or soy sauce, sesame oil, and *natane* (rape) oil were used as seasonings. No sugar or *mirin* (rice syrup) was used.

2) All meals were prepared using domestically grown foods that were organically grown and free of pesticides.

3) Brown rice was cooked using a pressure cooker. Vegetables were just scrubbed (not peeled) as they were rinsed under running water. They were then sautéed in sesame oil and/or boiled, and were seasoned with *miso* or sea salt.

4) They chewed each mouthful 50–100 times.

Tables 2 and 3 give the results of the *genmai-saishoku* group and the control group, respectively. Not a single case of symptoms due to vitamin B₁₂ deficiency (neurological disturbances, mental disorders, etc.) was found. Two girls in the *genmai-saishoku* group were exclusively breast-fed until 6 months by their mothers who had practiced the *genmai-saishoku* diet for 9 years 8 months and 6 years 6 months prior to conception, but they showed no symptoms due to B₁₂ deficiency. Both of the mothers consumed 2 g of *nori* per day.

There were no statistically significant differences ($p < 0.05$) in the examination data between the *genmai-saishoku* group and the control group, including serum vitamin B₁₂ levels.

DISCUSSION

The liver stores most of the body's vitamin B₁₂, which is said to be enough for 1,000 days (10). Therefore, people on a strict vegan diet are expected to deplete the stocks of vitamin B₁₂ in the liver in 4 years, since vitamin B₁₂ is generally supposed to exist only in animal foods. However, serum vitamin B₁₂ levels of 6 young vegans who had been on a vegetarian diet for more than 4 years were not significantly different from those of the control group. All 6 cases were within the normal limit (230–800 pg/ml), and neither megalocytic anemia nor neurological disturbances were observed among them.

Two of them were girls (B₁₂ level: 480 pg/ml and 300 pg/ml) whose mothers had been *genmai-saishoku* vegans for 9 years 8 months and 6 years 6 months prior to conception, respectively, and who were exclusively breast-fed until 6 months.

Table 2. Clinical and laboratory data in *genmai-saishoku* vegans.

Sex	Age	Height (cm)	Weight (kg)	Years as vegan	B ₁₂ (pg/ml)	RBC ($\times 10^4/\mu\text{l}$)	Hb (g/dl)	Ht (%)	MCV (fl)	MCH (pg)
1. F	7.1	108	15	4.4	520	402	11.4	35.0	87.0	28.2
2. M	7.7	114	20	4.4	720	422	14.8	43.9	103.8	35.1
3. F	8.6	125	25	8.6	480	466	13.5	40.5	86.9	29.0
4. F	8.8	123	20	8.8	300	475	13.5	41.1	86.4	28.4
5. M	12.7	165	46	10.0	320	464	14.2	43.4	93.5	30.6
6. F	14.6	160	43	10.0	320	473	13.4	40.6	85.6	28.3
M $\pm$ SD	9.9 $\pm$ 3.0	132.5 $\pm$ 24.1	28.2 $\pm$ 13.1		443 $\pm$ 164	450 $\pm$ 31	13.5 $\pm$ 1.1	40.8 $\pm$ 3.2	90.5 $\pm$ 7.1	29.9 $\pm$ 2.7
Normal range upper: Male lower: Female					230–800	410–550 380–500	13.5–17.5 11.6–15.0	38–54 34–47	83–99 80–99	27–35

Sex	MCHC (%)	WBC ($/\mu\text{l}$)	PLT ($\times 10^4/\mu\text{l}$)	Fe ($\mu\text{g/dl}$)	TIBC ($\mu\text{g/dl}$)	Alb (g/dl)	chE (IU/liter)	TC (mg/dl)	HDL (mg/dl)	TG (mg/dl)
1. F	32.4	4,600	30.0	175	365	4.1	163	136	55	32
2. M	33.8	5,900	21.4	89	340	4.3	198	120	52	24
3. F	33.3	6,400	38.6	192	347	4.3	179	165	58	38
4. F	32.9	5,500	33.1	152	350	4.5	201	128	49	26
5. M	32.7	4,100	22.2	102	362	4.6	274	105	32	80
6. F	33.0	5,000	22.2	167	372	4.4	228	121	37	88
M $\pm$ SD	33.0 $\pm$ 0.5	5,250 $\pm$ 850	27.9 $\pm$ 7.1	146 $\pm$ 42	356 $\pm$ 12	4.4 $\pm$ 0.2	207 $\pm$ 39	129.2 $\pm$ 20.3	47.2 $\pm$ 10.4	48.0 $\pm$ 28.4
Normal range upper: Male lower: Female	32–36	4,000–9,000	15–35	70–160 50–140	250–370	3.7–5.2	100–240	130–230	35–60 40–68	35–170

1) The mothers of No. 3 and No. 4 had observed a strict *genmai-saishoku* diet prior to conception for 9 years 8 months and 6 years 6 months, respectively. 2) No. 5 and No. 6 are siblings.

Table 3. Clinical and laboratory data in controls.

Sex	Age	Height (cm)	Weight (kg)	B ₁₂ (pg/ml)	RBC (×10 ⁴ /μl)	Hb (g/dl)	Ht (%)	MCV (fl)	MCH (pg)	MCHC (%)
1. F	10.3	134	26	370	464	13.8	41.5	89.4	29.7	33.2
2. F	10.3	143	29	820	463	13.3	39.0	84.2	28.7	34.0
3. F	12.1	143	28	510	476	13.3	39.3	82.5	27.9	33.9
4. F	12.8	155	45	720	455	13.4	40.3	88.5	29.6	33.4
M±SD	11.4±1.3	143.8±8.6	32.0±8.8	605±203	465±9	13.5±0.2	40.0±1.1	86.2±3.3	29.0±0.8	33.6±0.4

Sex	WBC (/μl)	PLT (×10 ⁴ /μl)	Fe (μg/dl)	TIBC (μg/dl)	Alb (g/dl)	chE (IU/liter)	TC (mg/dl)	HDL (mg/dl)	TG (mg/dl)
1. F	6,200	24.6	179	379	4.4	216	137	53	35
2. F	4,200	20.8	70	357	4.5	181	120	43	60
3. F	4,500	22.0	168	349	4.0	130	138	47	40
4. F	4,500	32.6	159	384	4.7	144	141	47	53
M±SD	4,850±911	25.0±5.3	144±50	367±17	4.4±0.3	168±39	134.0±9.5	47.5±4.1	47.0±11.5

However, girls and their mothers showed no signs of vitamin B₁₂ deficiency. This contrasts with a number of studies reporting cases of neurological disturbances, mental disorders, or intellectual disorders caused by vitamin B₁₂ deficiency (3–5).

Only a small number of reports have been made of serum vitamin B₁₂ levels of adolescent vegans. Ashkenazi et al. reported the case of a 14-year-old girl who exhibited symptoms of megalocytic anemia (serum vitamin B₁₂ level, 50 pg/ml; Hb, 11.5 g/dl; MCV, 109–153) and neurological disturbances after 8 years of strict vegan diet (3). In contrast, Bar-Sella et al. reported on five young vegans (8–19 years old), who showed no significant differences from the control group with respect to serum vitamin B₁₂ level, Hb, and MCV, and who had neither megalocytic anemia nor neurological disturbances (11). Elliss and Montegriffo reported on a 14-year-old and an 18-year-old boy, whose serum vitamin B₁₂ levels were within the normal limit (275 pg/ml and 375 pg/ml, respectively) (12). One thing to note about these reports is that none of them mentions the contents of the vegan diet (nutritional data, preparation of foods, etc.).

How is it that *genmai-saishoku* people can maintain normal serum vitamin B₁₂ levels without consuming animal foods, then? The following possibilities suggest themselves:

(1) According to Van Den Berg et al., 100 g of *nori* contain 68.8 μ g of vitamin B₁₂ (13). Since the daily intake of vitamin B₁₂ recommended by the joint research group of FAO and WHO is 1 μ g, a piece of *nori* per day is sufficient. This suggests that consumption of *nori* may keep vegetarians from suffering vitamin B₁₂ deficiency. As a matter of fact, *genmai-saishoku* children as well as their mothers consumed at least 2 g of *nori* every day. The Japanese diet customarily includes *nori*.

(2) The elimination of vitamin B₁₂ is controlled by enterohepatic circulation. About 4 μ g of vitamin B₁₂ per day are combined with R protein and are then eliminated into bile, some of which are reabsorbed. The vegetarian may get vitamin B₁₂ efficiently by reabsorption from bile thereby preventing vitamin B₁₂ deficiency (14).

(3) The vitamin B₁₂ in foods is combined with protein contained therein. It becomes free through the action of pepsin and gastric acid. Under acidic conditions, R protein, which is a non-intrinsic factor found in saliva that easily combines with vitamin B₁₂, has a stronger affinity for vitamin B₁₂ than does intrinsic factor. Therefore, in the stomach, vitamin B₁₂ combines with R protein and is thereby protected (15).

Genmai-saishoku people chew each mouthful 50–100 times. Good chewing brings about more R protein secretion in saliva, which will make it possible to use efficiently vitamin B₁₂ obtained from *nori*.

(4) In *genmai-saishoku* style preparation of foods, vegetables are just rinsed with running water and are not peeled prior to cooking. Thus vitamin B₁₂ synthesized by microorganisms is left on the vegetables, which may be available to vegetarians. However, this might not account for a large amount of vitamin B₁₂.

Since *genmai-saishoku* people rarely eat vegetables raw, the author thinks it possible that much of the vitamin B₁₂ will be destroyed during the cooking process.

(5) Cows and sheep make use of vitamin B₁₂ synthesized from cobalt by enterobacterium. In humans, vitamin B₁₂ is synthesized by enterobacterium as well, but is passed out in feces without being absorbed by the large intestine (16).

Halsted et al. contended that vegans in one area of Iran are untroubled by vitamin B₁₂ deficiency because they eat vegetables grown in human manure without cleansing them (17). Elliss and Montegriffo report that serum vitamin B₁₂ levels of the 4 vegans who had been on the diet for more than 13 years were within the normal limit, suggesting the possibility that human beings absorb vitamin B₁₂ synthesized by enterobacterium (12).

Foods rich in cobalt include buckwheat, adzuki beans, kidney beans, *shiitake*, *hijiki* etc., all of which *genmai-saishoku* people consume regularly (18). It is thus conceivable that vegans get vitamin B₁₂ which enterobacteria synthesize from cobalt.

In the present study, serum vitamin B₁₂ levels of children aged 7 to 14 who had been on a vegan diet for up to 10 years were within the normal limit. This suggests the possibility of preventing vitamin B₁₂ deficiency by consuming *nori*, although this possibility needs to be tested by long-term follow-up research and examination of infants.

Many previous studies of serum vitamin B₁₂ levels among vegans do not mention the contents of the diet (nutritional data, how foods are prepared, etc.). In order to assess the safety and validity of the vegetarian diet, more research needs to be done taking into account the content of the diet.

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