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Metabolic Vitamin B_{12} Status on a Mostly Raw Vegan Diet with Follow-Up Using Tablets, Nutritional Yeast, or Probiotic Supplements

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Key Words

Vegan · Vegetarianism · Raw food · Cobalamin · Vitamin B_{12} · Methylmalonic acid · Probiotic supplements · Nutritional yeast

Abstract

Background: Pure vegetarian diets might cause cobalamin deficiency due to lack of dietary intake. It was hypothesized that a population following a vegan diet consuming mostly raw fruits and vegetables, carrot juice, and dehydrated barley grass juice would be able to avoid vitamin B_{12} deficiency naturally. ***Methods:*** Subjects were recruited at a health ministers' reunion based on adherence to the Hallelujah diet for at least 2 years. Serum cobalamin and urinary methylmalonic acid (MMA) assays were performed. Follow-up with sublingual tablets, nutritional yeast, or probiotic supplements was carried out on subjects with abnormal MMA results. ***Results:*** 49 subjects were tested. Most subjects (10th to 90th percentile) had followed this diet 23–49 months. 6 subjects had serum B_{12} concentrations <147 pmol/l (200 pg/ml). 37 subjects (76%) had serum B_{12} concentrations <221 pmol/l (300 pg/ml). 23 subjects (47%) had abnormal urinary MMA concentrations above or equal to 4.0 μg/mg creatinine. Sublingual cyanocobalamin and nutritional yeast, but not probiotic supplements, significantly reduced group mean MMA concentrations (tablet $p < 0.01$; yeast $p < 0.05$, probiotic > 0.20). ***Conclusions:*** The urinary MMA assay is effective for identifying early metabolic cobalamin deficiency. People following the Hallelujah diet and other raw-food vegetarian diets should regularly monitor their urinary MMA levels, consume a sublingual cobalamin supplement, or consume cobalamin in their food.

Introduction

The vitamin B_{12} status of people who avoid eating all animal products remains an important scientific question. Vitamin B_{12} is important in DNA synthesis, erythropoiesis, and development and maintenance of the myelin sheath of nerves. Deficiency of vitamin B_{12} leads to pernicious anemia, gastrointestinal disorders, and neurological damage. Generous folate intake by health-conscious vegans prevents easy detection of pernicious anemia, with the first signs of vitamin B_{12} deficiency being neurological [1]. Symptoms of neurological damage due to poor vitamin B_{12} status include numbness and/or tingling in outer extremities, decreased vibration sense and/or position sense, decreased visual acuity, unsteadiness, poor muscular coordination with ataxia, moodiness, mental slowness, poor memory, confusion, agitation, depression, delusions, hallucinations, and even overt psychosis [1]. There is significant overlap in the symptom picture with Alzheimer-type dementia [2].

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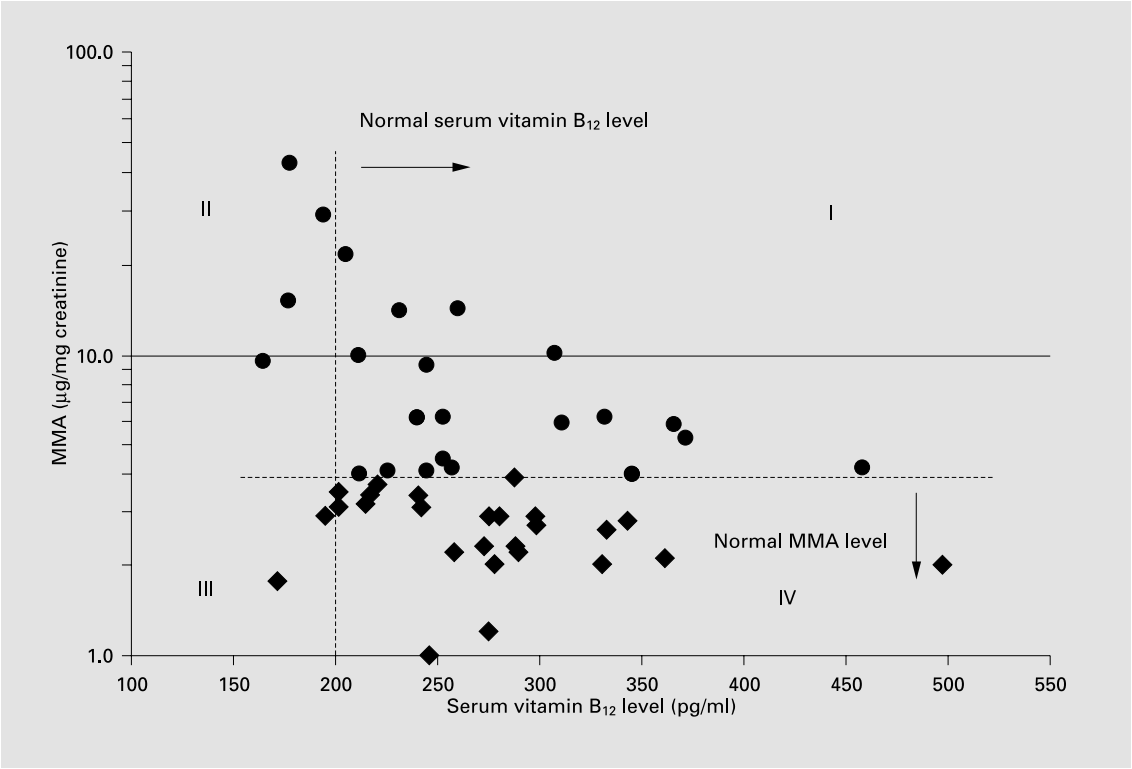

Fig. 1. Initial vitamin B_{12} status. The graph is divided into four quadrants (I–IV), based on the normal ranges for serum B_{12} and urinary methylcobalamin. ● = Subjects with elevated concentrations; ◆ = subjects with normal MMA concentrations.

Since true cobalamin is not found in any appreciable amount in plant foods [3], it would be expected that all people avoiding animal products would eventually develop symptoms of vitamin B_{12} deficiency. However, it has been reported that vitamin B_{12} can be synthesized by bacteria residing in the small intestine [4]. Furthermore, dehydrated cereal grasses have been reported to contain a small amount of vitamin B_{12}. Therefore, it was hypothesized that a group of people following a pure vegetarian diet consuming mostly raw fruits and vegetables, carrot juice, and dehydrated barley grass juice would be able to avoid vitamin B_{12} deficiency through natural means without the use of supplementary vitamin B_{12}.

Subjects and Methods

The subjects were recruited at an annual meeting of health ministers, sponsored by Hallelujah Acres. The meeting was a health education seminar open to people who had previously attended a seminar on implementing the Hallelujah diet. Most of the attendees were active in promoting the Hallelujah diet in their communities.

The main selection criterion for this study was adherence for at least 2 years, preferably over 3 years, to a diet popularly referred to as the Hallelujah diet. A second citerion was that the subjects not be consumers of vitamin B supplements. The Hallelujah diet is a pure vegetarian diet based on consumption of raw vegetables and fruit, carrot juice, dehydrated juice from young barley plants, raw nuts and seeds, and limited amounts of cooked tubers and whole-grain products [5]. Two years was chosen as the minimal time to see vitamin B_{12} deficiency based on previous reports [6; see figure 1 in 7].

A short food frequency questionnaire was used to test for correlations between food consumption and B_{12} status. This questionnaire included questions on frequency and amounts of foods suspected to be sources of vitamin B_{12} (dehydrated juice from young barley plants, seaweed, nutritional yeast, fortified vegetarian foods) as well as questions regarding animal product consumption and vitamin and mineral supplements. Adherence to the Hallelujah diet was also assessed using this questionnaire. Exposure to chlorine through municipal water supplies and swimming pools was also assessed.

Blood samples were drawn and processed in accordance with laboratory guidelines. Serum vitamin B_{12} concentrations were assayed using an immunochemiluminometric method (Labcorp, Charlotte, N.C., USA). A spot urine sample was also collected from each subject. Urine samples were analyzed by stable isotope dilution gas chromatography mass spectrometry for the methylmalonic acid (MMA) concentration, on the basis of creatinine content (Norman Clinical

Laboratories, Cincinnati, Ohio, USA). The usefulness of serum cobalamin levels for diagnosing vitamin B_{12} deficiency has been questioned due to the low specificity of the cobalamin assay; the preferred test for diagnosis is the MMA assay [2, 8–12]. In a study of hospital patients [9], the urinary MMA assay was 100% sensitive and 99% specific in identifying patients who were clinically vitamin B_{12} deficient.

Subjects who had elevated urinary MMA concentrations were asked to participate in a follow-up of this initial screening. Subjects were randomly assigned to three treatment groups. Men and women were randomized separately to evenly divide each sex into the three groups. The first group consumed a sublingual cyanocobalamin tablet (500 μg; Twinlabs, Minneapolis, Minn., USA) for 3 days a week. Sublingual tablets have been shown to be very effective in improving vitamin B_{12} status [6, 13]. The second group daily consumed 1 tablespoon of nutritional yeast (Red Star Vegetarian Support Formula; Universal Foods, Milwaukee, Wisc., USA). This was an attempt to identify an acceptable food that would supply vitamin B_{12}. One tablespoon contained about 5 μg of cyanocobalamin. The third group daily consumed 2 capsules of one of two selected probiotic supplements: Probiotic Formula (Natural Choice Products, Frenchtown, Mont., USA) and Flora Food (AIM International, Nampa, Idaho, USA). The Probiotic Formula contains the five bacteria *Lactobacillus plantarum, Lactobacillus salivarius, Lactobacillus acidophilus, Bifidobacterium bifidus* and *Bacillus subtilis*. Flora Food contains *L. salivarius* and *L. plantarum* variant OM. The probiotics were used to test whether the bowel flora could be modified to produce vitamin B_{12} at physiologically relevant amounts. It was estimated that 3 months would be sufficient time to see a significant effect of the probiotic supplements. Compliance was checked by self-report at the end of the follow-up period. After 3 months of follow-up, the subjects submitted a urine sample for another MMA determination.

An informed consent document explaining the study and the risks and benefits of participation was given to each volunteer before sampling. Written consent was obtained from subjects before participation. The study was conducted in accord with the ethical standards of the Helsinki Declaration of 1975, as revised in 1983.

Statistics

The Mann-Whitney rank sum test [14] was used to test whether vitamin B_{12} intake (<0.10 μg/month vs. >0.10 μg/month) was correlated with the urinary MMA concentration. Group mean values before and after supplementation were compared by paired Student's t test.

Results

49 subjects (17 men and 32 women) participated in the study. The average age was 55 ± (SD) 9 years. Subjects had adhered to this dietary approach for a mean of 39 (range 22–180) months. Most subjects (10th to 90th percentile) had followed this diet for 23–49 months. The median vitamin B_{12} intake was 0.7 μg/month, while the average intake was 5 ± 11 μg/month. No subjects were aware of any signs of cobalamin deficiency.

In the initial screening, it was found that 2 men and 4 women had serum B_{12} concentrations <147 pmol/l (200 pg/ml). All concentrations were >118 pmol/l (160 pg/ml). 37 and 44 subjects had serum B_{12} concentrations <221 and 257 pmol/l, respectively.

8 men and 15 women had urinary MMA concentrations above or equal to 4.0 μg/mg creatinine (fig. 1). 16 subjects had urinary MMA concentrations equal to or above 5.0 μg/mg creatinine. 2 subjects had serum B_{12} concentrations <147 pmol/l, but had normal urinary MMA concentrations. Only 4 individuals had a combination of elevated MMA concentrations and low serum B_{12} concentrations. There was no correlation between MMA and intake of vitamin B_{12} from foods, length of time following a meatless diet, length of time following the Hallelujah diet, or exposure to chlorinated water.

Subjects who had elevated urinary MMA concentrations were asked to participate in a follow-up of this initial screening. 24 subjects (23 subjects with elevated MMA concentrations, and 1 subject with MMA equal to 3.9 μg/mg) were randomly assigned to three treatment groups. Men and women were randomized separately to evenly divide each sex into the three groups as detailed above.

After 3 months of follow-up, the subjects submitted a urine sample for MMA determination. As shown in figure 2, sublingual cyanocobalamin and nutritional yeast significantly reduced all MMA concentrations (tablet $p = 0.01$; yeast $p = 0.05$). 7 of 8 subjects' MMA concentrations normalized using the sublingual tablet; one was slightly elevated at 4.1 μg/mg creatinine. 5 of 8 subjects' MMA concentrations returned to normal using the nutritional yeast, and all 8 subjects showed improvement. Failure to normalize the MMA concentration was not related to poor compliance. 3 of 4 subjects using the Probiotic Formula decreased their MMA concentration; only 1 of 4 subjects using the Flora Food showed slight improvement. None of the subjects normalized their MMA concentration after consistent use over a 3-month period, and the difference in the group mean MMA level before and after supplementation with probiotics was not significant ($p > 0.20$).

6 additional people following the Hallelujah diet and 1 subject following the same diet except for the addition of about 6 eggs per week also submitted urine samples for the MMA assay. 3 of the 5 subjects following the vegan diet had elevated MMA concentrations. The 6th subject, consuming an ovovegetarian diet, had the lowest MMA concentration (1.2 μg/mg creatinine). One subject then consumed the Flora Food probiotic for 3 months, resulting in a more elevated MMA concentration. Subsequent-

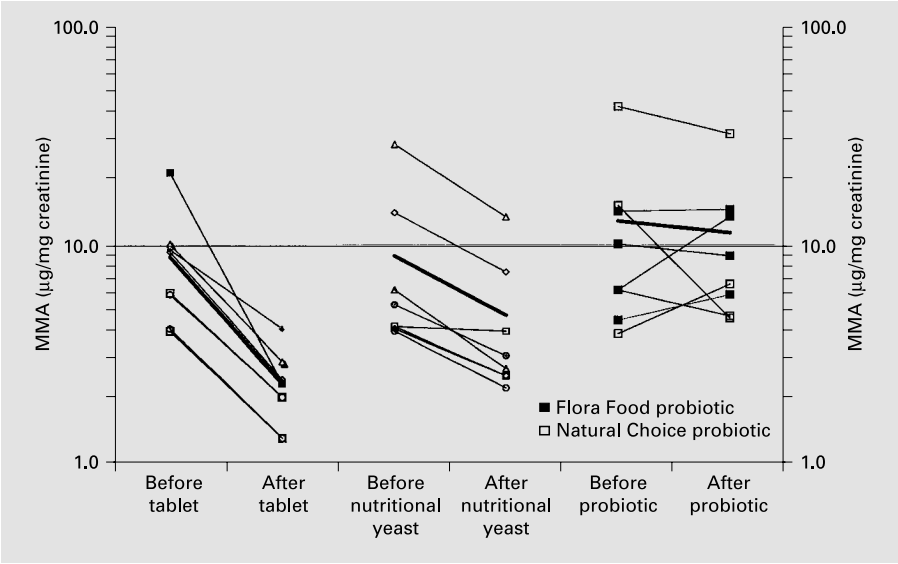

Fig. 2. Follow-up of subjects with elevated MMA using sublingual tablets, nutritional yeast, or two formulations of probiotic bacteria. The thick line indicates the group mean value. Significant changes in group mean concentrations seen with tablets ($p = 0.01$) and nutritional yeast ($p = 0.05$).

ly, all 3 subjects daily consumed 1–2 sublingual tablets (0.5–1 tablet twice daily) of methylcobalamin (Enzymatic Therapy, Green Bay, Wisc., USA). After 3 weeks, their MMA concentrations were retested. In 2 of the subjects, the MMA concentrations were in the normal range, while in 1 it remained slightly elevated (4.1 µg/mg creatinine).

Discussion

Vegans eating large amounts of unprocessed, raw foods rely on the work by Albert et al. [4] that demonstrated that bacteria (*Pseudomonas* and *Klebsiella* species) isolated from the small intestine of a few volunteers in southern India could produce cobalamin in vitro. Based on this finding it is claimed by some advocates that natural, mostly raw-food vegans do not need to supplement their diets with cobalamin, since their intestinal flora will make it for them. There has been no further confirmation in the literature of this report, either in vitro or in vivo.

To the contrary, every study of vegetarian, and especially vegan, populations reveals that a substantial proportion of the tested group has below-normal levels of vitamin B_{12} or elevated levels of MMA [6–7, 15–16, 22–28] (table 1). The two analyzed populations ('living-food' vegans and Natural Hygiene vegans), similar to the population studied here, also had high rates of below-normal serum cobalamin concentrations [15, 16]. Our report here is in agreement with the average of all of the other studies, that about 50% of vegans, measured cross-sectionally, have below-normal cobalamin status. The mounting evidence points toward the need for all vegans to supplement their diets with cobalamin or to monitor their vitamin B_{12} status.

It has not been determined why some people, but not others, suffer from vitamin B_{12} deficiency, when they eliminate all animal products from their diet. There was no correlation with length of time following a vegan diet here or with intake of cobalamin. However, all intakes of cobalamin were very low, making detection of a correlation with intake difficult in this study. One longitudinal study [17] concluded that all vegans eventually suffer from vitamin B_{12} deficiency. Generally, the vitamin B_{12} status is directly correlated with dietary intake, with other factors being much less important. For vegans, slight differences in the efficiency of enterohepatic circulation of vitamin B_{12} may be very important.

Differences in bowel flora may contribute to the cobalamin status of the host. Here, we present data reporting that one formula of probiotic supplements (Natural Choice Probiotic Formula) can improve the vitamin B_{12} status at the metabolic level. The small number of subjects taking this supplement prevented the improvement from being statistically significant.

How does a probiotic supplement improve B_{12} status? First, we do not deny that uptake occurs in the ileum, so that colonic synthesis of B_{12} would be useless to the host. There is a small amount of bacteria resident in the small intestine. It is possible that some of the bacteria from the probiotic supplement took up residence in the small intes-

Table 1. Comparison of vegetarian vitamin B_{12} status

Diet	Subjects	Assay[1]	Normal test cutoff	Abnormal tests	Abnormal tests, %	Reference
Hallelujah diet	54	uMMA	≥4 μg/mg	23	47	this study
	49	B_{12}	<147 pmol/l	6	12	
Vegan, living food	21 adults	B_{12}	<147 pmol/l	12	57	16
Vegan, Natural Hygiene	13 adults	B_{12}	<147 pmol/l	12	92	15
Lactovegetarian, Natural Hygiene	28 adults	B_{12}	<147 pmol/l	18	64	15
Lacto-ovovegetarian, Natural Hygiene	15 adults	B_{12}	<147 pmol/l	7	47	15
Vegan, Adventist	9	B_{12}	<147 pmol/l	8	89	22
	9	uMMA	>4 μg/mg	8	89	
Vegan, Adventist	78 adults	B_{12}	<147 pmol/l	47	60	
	78 adults	B_{12}	<118 pmol/l	36	46	6
	29 adults	uMMA	>4 μg/mg	8	28	
Lacto-ovovegetarian, Adventist	245 adults	B_{12}	<171 pmol/l	130	53	
			<221 pmol/l	179	73	23
Vegan	36 adults	B_{12}	<147 pmol/l	26	72	24
Vegan	25	sMMA	>376 nmol/l	5	20	25
		B_{12}	<150 pmol/l	3	12	
Lacto-ovovegetarian	120	B_{12}	<147 pmol/l	16	13	26
Macrobiotic	110 adults	B_{12}	<147 pmol/l	56	51	7
	25 adults	uMMA	>4 μg/mg	14	56	
Macrobiotic	42 children	uMMA	>5 μg/mg	23	55	7
		sMMA	>0.43 μmol/l	35	85	
Macrobiotic	41 infants	B_{12}	<218 pmol/l	34	83	27
		tHcy	>10.35 μmol/l	34	83	
Macrobiotic	17 infants	uMMA	>4 μg/mg	16	94	
	16 women	uMMA	>4 μg/mg	12	75	28
	16 women	B_{12}	<147 pmol/l	9	56	
Total tests	1,100			550	50	

[1] uMMA = Urinary MMA assay, normalized per milligram of creatinine; B_{12} = serum cobalamin; sMMA = serum MMA; tHcy = serum total homocysteine.

tine, at least transiently, and produced cobalamin for the host. Another possibility is that a small amount of cobalamin was produced by the bacteria in the probiotic supplement before reaching the large intestine. This would only be a transient effect while the supplement is being taken. We did not measure whether the status would change after ceasing intake of the supplement.

Bacteria in the small intestine are more commonly associated with vitamin B_{12} deficiency due to uptake of vitamin B_{12} by the bacteria. Overgrowth of bacteria is to be avoided, but a small amount of the right strains of bacteria in the small intestine may well be beneficial. For a person consuming no vitamin B_{12} (a vegan not using any B_{12}-fortified foods or supplements), the bowel flora is the sole source of cobalamin, and any loss of this function would be detrimental to this person's long-term health prospects. More study is needed to determine whether a population of bowel flora could be maintained in adult subjects that continually produces enough cobalamin to satisfy nutritional requirements.

Nutritional yeast appears to be an effective mode of delivery of cobalamin for many individuals. The cobalamin in Red Star nutritional yeast is due to fortification and does not occur naturally in the yeast. Many vegans will find this to be an acceptable food based on its taste and nutrition properties. Nutritional yeast is an excellent source of zinc and selenium which are not found in generous concentrations in fruits and vegetables. Seaweed has been claimed to be a source of cobalamin, but the seaweeds dulse and spirulina were ineffective in improving

cobalamin status in an intervention trial [18]. The only proven method for normalizing cobalamin status is by supplying dietary cobalamin or by supplementation. Cyanocobalamin or methylcobalamin sublingual tablets were very effective in this study. There is some evidence that methylcobalamin, an active form of vitamin B_{12} is more effective than cyanocobalamin in relieving neurological symptoms [19, 20].

We showed here that the urinary MMA assay was much more effective than the serum cobalamin assay in identifying individuals at risk of vitamin B_{12} deficiency and in monitoring improvement of cobalamin status. Only 6 subjects had below-normal levels of serum cobalamin, but 23 subjects had abnormally elevated urinary concentrations of MMA. Even though 19 subjects in this study had normal concentrations of serum cobalamin, their urine MMA concentration indicated early signs of metabolic insufficiency of cobalamin.

Cobalamin deficiency may take many years to develop symptoms, as many authors state, but metabolic evidence of insufficiency can be detected with the urinary MMA assay within a few years of ceasing cobalamin consumption. Our results indicate that shortly after ceasing intake of vitamin B_{12}, its metabolism is unfavorably altered by the lack of B_{12} at the cellular level, though liver stores are not depleted. This information has not been widely recognized, yet our results agree with other reports of signs of vitamin B_{12} deficiency in adults after just 2 years without consumption [6, 7, 21]. Early detection and supplementation among pure vegetarians is the best way to circumvent permanent neurological damage and disorders.

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