Dietary intake and biochemical, hematologic, and immune status of vegans compared with nonvegetarians[1,2]

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ABSTRACT Dietary and nutritional status of individuals habitually consuming a vegan diet was evaluated by biochemical, hematologic, and immunologic measures in comparison with a nonvegetarian group. On the basis of 4-d dietary records, the intake of female and male vegans tended to be lower in fat, saturated fat, monounsaturated fat, and cholesterol and higher in dietary fiber than that of vegetarians. With computed food and supplement intakes, vegan diets provided significantly higher amounts of ascorbate, folate, magnesium, copper, and manganese in both female and male participants. The body mass index (BMI; in kg/m^2) of the vegans was significantly lower than that of the nonvegetarians and 9 of the 25 vegans had a BMI <19. Serum ferritin concentrations were significantly lower in vegan men but iron and zinc status did not differ between the sexes. Mean serum vitamin B-12 and methylmalonic acid concentrations did not differ; however, 10 of the 25 vegans showed a vitamin B-12 deficit manifested by macrocytosis, circulating vitamin B-12 concentrations <150 pmol/L, or serum methylmalonic acid >376 nmol/L. Vegans had significantly lower leukocyte, lymphocyte, and platelet counts and lower concentrations of complement factor 3 and blood urea nitrogen but higher serum albumin concentrations. Vegans did not differ from nonvegetarians in functional immunocompetence assessed as mitogen stimulation or natural killer cell cytotoxic activity. *Am J Clin Nutr* 1999;70(suppl):586S–93S.

KEY WORDS Vegans, dietary intake, iron, zinc, folate, vitamin B-12, methylmalonic acid, immunocompetence

INTRODUCTION

An extensive body of research documents the health benefits of vegetarian dietary practices and the lower incidence of chronic disease, especially heart disease, in vegetarians. Much of the data are derived from investigations in which Seventh-day Adventist vegetarians, most of whom consume a lactoovovegetarian diet, were examined. Strict vegetarian or vegan diets, which exclude all foods of animal origin, are increasingly being adopted. The adequacy and nutritional effect of diets based entirely on plant foods is still under investigation.

The early studies on vegan diets in adults concluded that daily intakes are nutritionally sufficient in protein and most vitamins except for vitamin B-12 (1–6). Since then, metabolic and neuropsychiatric abnormalities suggestive of vitamin B-12 deficiency have been observed in vegans (7, 8). Also, the high-fiber and -phytate content of plant-based foods has prompted questions about the iron and zinc adequacy of the diet (9–11). Adherence to largely vegan diets may compromise the immune status of individuals, including those living in developed countries (12).

This study was undertaken to assess the nutritional status of adults consuming only plant foods with respect to vitamin B-12, iron, zinc, and immune indicators. To do so, dietary intake and selected biochemical and hematologic measures in a group of vegans were compared with those of a similar group consuming nonvegetarian diets.

SUBJECTS AND METHODS

Subjects

Forty-five healthy adult volunteers were selected for the study. Participants were either students at Loma Linda University or employees of health-care facilities in the local area. To be included, participants had to *1*) be between the ages of 20 and 60 y, *2*) be within 120% of ideal body weight, and *3*) have followed a consistent dietary pattern for ≥1 y. Potential subjects were screened to exclude those with metabolic disease, those taking medications known to influence nutritional status, those who exercised >7 h/wk, those who smoked, or those who consumed more than the equivalent of 1 alcoholic drink/d. The study was approved by the Institutional Review Board of Loma Linda University and informed consent was obtained from the subjects at enrollment.

Dietary intake

Subjects were taught how to keep accurate food records. The first day of the record consisted of a 24-h recall completed by a trained interviewer to instruct participants in the degree of detail needed for the record. Participants recorded the type and quantity of food and beverages consumed for 2 weekdays and 1 weekend day; in total, 4-d food intake and supplement use

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TABLE 1
Characteristics of vegan and nonvegetarian subjects

	Nonvegetarians (n = 20)	Vegans (n = 25)
Age (y)	33.5 ± 8.2[1]	36.0 ± 8.1
BMI (kg/m^2)	25.5 ± 3.1	20.5 ± 2.5[2]
Exercise		
(kcal/d)	145 ± 145	150 ± 225
(kJ/d)	607 ± 607	628 ± 941
Blood lipids (mmol/L)		
Total cholesterol	4.80 ± 0.95	4.30 ± 0.90
HDL cholesterol	1.50 ± 0.40	1.20 ± 0.30
Triacylglycerol	1.20 ± 0.60	1.00 ± 0.40
Duration of vegetarian diet (y)	0	12.1 (1–25)[3]
Duration of vegan diet (y)	0	4.2 (1–37)
Multivitamin-mineral supplement users	7	4
Single-nutrient (calcium, iron, or vitamin C) supplement users	1	6
Vitamin B-12 supplement users	0	9

[1] $\bar{x}$ ± SD.
[2] Significantly different from nonvegetarians, $P < 0.001$ (t test).
[3] $\bar{x}$; range in parentheses.

records were obtained from each participant.. Food records were analyzed by using NUTRITIONIST IV software (version 2.01 1993; N-Squared Computing, Salem, OR). Vitamin and mineral supplement use was documented and evaluated as appropriate.

Clinical and biochemical measures

Fasting, peripheral venous blood samples were collected in the morning between 0700 and 0900 by venipuncture. Complete blood counts, a chemistry panel, a serum immunoglobulin analysis, and a complement fraction analysis were performed by the Loma Linda University Medical Center Clinical Laboratory according to standardized procedures.

Serum ferritin was analyzed with an enzyme immunoassay kit (Milenia NKFE1; Diagnostic Products Corporation, Los Angeles) by using a microplate reader (model 2380; Bio-Tek, Winooski, VT). Serum folic acid and vitamin B-12 concentrations were determined by simultaneous radioassays (Quantaphase-II, 1911040; Bio-Rad Laboratories, Richmond, CA) using a gamma counter (model LB1213; EGNG Berthold, Wildbad, Germany). Trace element–free tubes (Becton Dickinson, Rutherfold, NJ) were used to collect blood for plasma zinc analysis by atomic absorption spectrophotometry (model AA-475; Varian, Sunneyvale, CA) (13). Standard reference material (bovine serum standard reference material no. 1598, National Institute of Standards and Technology, Gaithersburg, MD) was used to check the accuracy and precision of the determinations. Serum methylmalonic acid, 2-methylcitrate homocysteine, and cystathionine were measured by gas chromatography–mass spectrometry at Metabolite Laboratories, Inc, at the University of Colorado Health Sciences Center, Denver (14, 15).

Mitogen assay and natural killer cell activity

Lymphocytes for blastogenic response tests and killer cell assays were isolated from blood with heparin by using Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, NJ) and resuspended in RPMI-1640 culture medium (Gibco, Grand Island, NY). Lymphocyte proliferation was measured by [^{3}H]thymidine incorporation after stimulation with phytohemagglutinin, concanavalin A and pokeweed mitogens (16). The stimulation index (SI) was calculated as follows: SI = [cpm (mitogen stimulated)/cpm (control)].

Natural killer cytolytic activity was determined in peripheral blood mononuclear cells by using K562 target cells in a ^{51}Cr release assay (17). The percentage of ^{51}Cr release or percentage lysis at multiple effector-to-target ratios was determined by using the following equation: [(sample − spontaneous) cpm]/[(maximum − spontaneous) cpm] × 100. Cytotoxicity was expressed as lytic units (LU) and these were defined as the number of cells required to cause 20% target cell lysis calculated by an exponentially fit equation and expressed as LU/10^6 peripheral blood mononuclear leukocytes.

Statistical methods

Statistical analyses were done by using SPSS for WINDOWS (Statistical Package for the Social Sciences, version 6.0 1996; SPSS, Inc, Chicago). Group means and SDs were calculated. Independent-sample t tests were conducted to evaluate differences between the vegan and nonvegetarian groups. Multiple regression was used to evaluate the influence of diet, age, or body mass index (BMI; in kg/m^2) on selected immune measures.

RESULTS

Twenty-five vegans (10 men, 15 women) and 20 nonvegetarians (10 men, 10 women) who met the eligibility criteria were included in the study. Subject characteristics are summarized in **Table 1**. Vegans were defined as those who excluded meat, fish, poultry, dairy products, and eggs from their diets whereas nonvegetarians regularly included all food categories. There were no significant differences between the 2 groups in age, physical activity level, or blood lipid concentrations. The vegan group, however, had a significantly lower BMI than the nonvegetarian group. The average number of years of vegan diet was 4.2 with a range of 1–25 y. Most of the vegans had followed a vegetarian diet before becoming vegans. The average number of years of following vegetarian dietary practices was 12.1 y with a range of 1–37 y. Of the 20 nonvegetarians, 7 regularly used multivitamin-mineral supplements, compared with 4 of 25 in the vegan group. One nonvegetarian and 6 vegans took single-nutrient supplements of iron, calcium, or vitamin C. None of the nonvegetarians took a separate vitamin B-12 supplement, whereas 9 of the 25 vegans reported that they did so.

Dietary pattern and nutrient intake

The food pattern of the vegans compared with that of the nonvegetarians is shown in **Figure 1**. Vegans consumed no flesh foods and practically no dairy foods or eggs. Vegans, however, consumed more servings per day of grains and breads, vegetables, fruit, legumes, and nuts and seeds. Only vegans consumed soymilk, tofu, and meat analogs. Meat analogs are commercially available foods prepared from soy protein, wheat gluten, legumes, and other ingredients and designed to substitute for meat in the diet.

Results from 4 d of food records were averaged for each person and used for group comparisons as shown in **Table 2**. The intake of female vegans compared with that of female nonvegetarians was significantly lower in protein, total fat, saturated fat, and monounsaturated fat both in quantity and as a percentage of energy. Female vegans consumed more dietary fiber and less dietary cholesterol. Nutrient values of food intake and of food plus supplement intake were computed. Foods consumed by female vegans provided significantly higher amounts of ascorbate, thiamine, folate, magnesium, and copper, and lower amounts of vitamin B-12. When supplement use was included, intakes of ascorbate, thiamine, folate, magnesium, and copper remained significantly different, but that of vitamin B-12 did not. The intakes of male vegans showed similar trends with percentage energy as fat, saturated fat, percentage energy as saturated fat, percentage energy as monounsaturated fat, and dietary cholesterol being lower, and dietary fiber being higher than that of male nonvegetarians. The diets of male vegans provided significantly higher amounts of vitamin A, ascorbate, thiamine, vitamin B-6, folic acid, magnesium, iron, copper, manganese, and dietary fiber, and lower amounts of vitamin B-12. When supplements were included, significantly higher intakes were observed for ascorbate, folate, magnesium, copper, and manganese in male vegans.

Iron and zinc

Hematologic and zinc nutritional status indicators in male and female vegans compared with nonvegetarians are shown in **Table 3**. Male vegans had a significantly greater mean cell volume and lower ferritin concentration than did nonvegetarians. Low hemoglobin concentrations were observed only in female participants with 1 of the 10 nonvegetarian and 2 of the 15 vegan females having a concentration ≤120 g/L, which suggests borderline iron deficiency anemia. Plasma ferritin is a sensitive indicator of iron storage and a value ≤12 μg/L indicates depletion of iron stores (18). The compromised iron stores of female subjects, as reflected in plasma ferritin results, showed that 2 of the 10 nonvegetarians and 4 of the 15 vegans had concentrations ≤12 μg/L.

Vitamin B-12 and folate

Biomarkers for vitamin B-12 and folate status are shown in **Table 4**. Mean serum vitamin B-12 and methylmalonic acid concentrations did not differ significantly between groups. However, of 25 vegan participants, 2 had macrocytosis (mean red cell volume ≥98 fL), 3 had circulating concentrations of vitamin B-12 <150 pmol/L, and 5 had methylmalonic acid concentrations >376 nmol/L, which is the critical cutoff point that represents 3 SDs above the population mean.

The vegan group had a significantly lower mean serum 2-methylcitric acid concentration than did the nonvegetarian group. As expected, vegans also had significantly higher mean serum folate concentrations.

Immune variables and immunocompetence

White blood cell counts and immune status measures in nonvegetarian and vegan participants are shown in **Table 5**. The vegan group had significantly lower numbers of leukocytes, lymphocytes, and platelets and lower complement factor 3. Mean albumin concentration was significantly higher in the vegan group and blood urea nitrogen was significantly lower. There were no significant differences between the groups in natural killer cell activity or in the mitogen stimulation indexes.

Multiple regression coefficients for diet (vegan = 1, nonvegetarian = 2), BMI, and age as predictors for leukocyte count, lymphocyte count, and complement factor 3 concentration are shown in **Table 6**. These variables were entered because they were found to contribute significantly to the variation in one or more of these measures in initial univariate analysis. A second analysis was conducted to evaluate whether BMI or age were significant predictors over and above the effect of diet. The change in

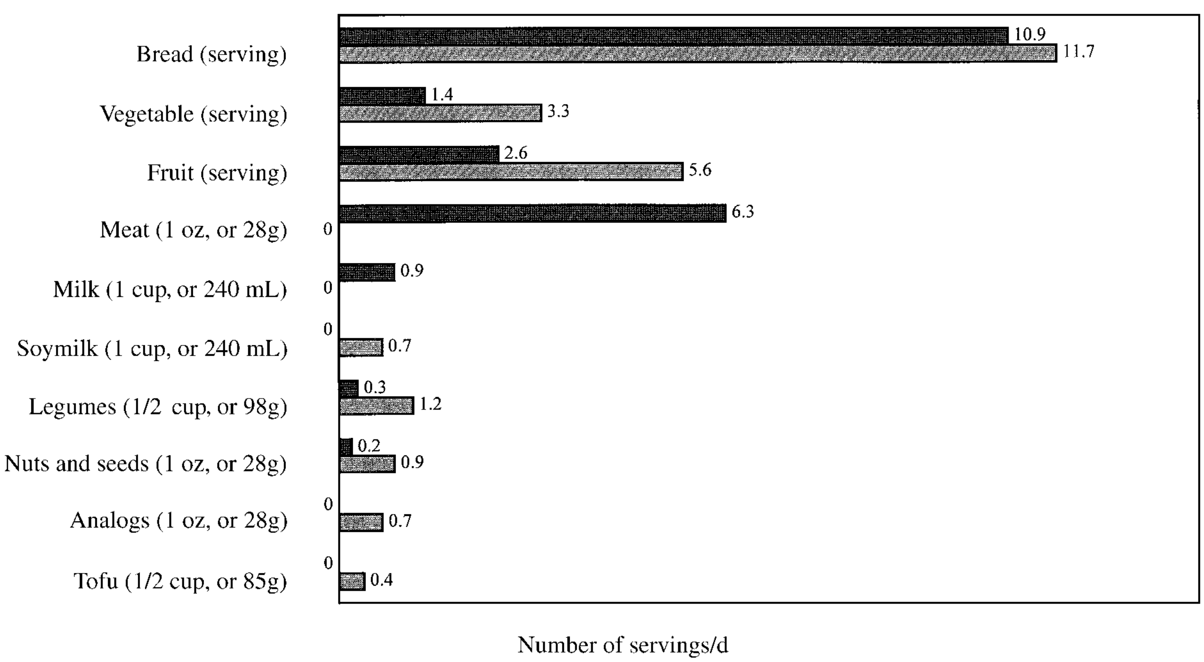

FIGURE 1. Food patterns of vegan (□) compared with nonvegetarian (■) diets based on 4-d food records. The number of servings of meat, bread, vegetable, fruit, and milk were computed by using NUTRITIONIST IV (version 2.01). Serving of legumes, nuts, seeds, analogs (meat substitutes), and tofu were computed manually on the basis of the serving sizes shown.

TABLE 2
Estimated mean nutrient intakes of nonvegetarian and vegan females and males based on 4-d food records[1]

	Females		Males	
	Nonvegetarian (*n* = 10)	Vegan (*n* = 15)	Nonvegetarian (*n* = 10)	Vegan (*n* = 10)
Energy (MJ/d)	8.24 ± 2.18	7.09 ± 1.82	9.04 ± 2.76	9.29 ± 2.17
Protein				
(g/d)	74 ± 14	52 ± 13[2]	85 ± 23	75 ± 18
(% of energy)	15 ± 3	12 ± 1[3]	16 ± 6	13 ± 2
Fat				
(g/d)	76 ± 27	52 ± 20[3]	80 ± 24	67 ± 14
(% energy)	34 ± 5	25 ± 7[2]	32 ± 5	26 ± 4[4]
Saturated fat				
(g/d)	27 ± 12	12 ± 7[4]	25 ± 8	13 ± 7[4]
(% energy)	12 ± 3	6 ± 4[2]	10 ± 2	5 ± 2[2]
MUFA				
(g/d)	30 ± 13	19 ± 10[3]	31 ± 10	23 ± 8
(% energy)	14 ± 3	10 ± 4[3]	13 ± 3	9 ± 3[3]
PUFA				
(g/d)	15 ± 6	14 ± 5	16 ± 6	21 ± 8
(% energy)	7 ± 2	8 ± 2	7 ± 1	9 ± 3
Dietary Fiber (g)	15 ± 6	38 ± 11[2]	20 ± 7	48 ± 11[2]
Cholesterol (mg)	235 ± 65	20 ± 30[2]	260 ± 120	3 ± 4[2]
Vitamin A (RE)				
Food	1310 ± 955	2210 ± 3920	875 ± 460	2040 ± 1660[3]
Food + supplements	1475 ± 905	2420 ± 3965	1200 ± 715	2040 ± 1660
Vitamin E (TE)				
Food	23 ± 12	17 ± 7	18 ± 8	21 ± 9
Food + supplements	25 ± 11	19 ± 8	21 ± 10	21 ± 9
Ascorbate (mg)				
Food	115 ± 60	230 ± 150[3]	120 ± 55	240 ± 125[3]
Food + supplements	125 ± 60	275 ± 230[3]	140 ± 75	240 ± 125[3]
Thiamine (mg)				
Food	1.40 ± 0.42	1.97 ± 0.64[3]	1.62 ± 0.67	3.47 ± 2.05[3]
Food + supplements	1.63 ± 0.59	2.28 ± 0.77[3]	2.11 ± 1.25	3.47 ± 2.05
Riboflavin (mg)				
Food	1.65 ± 0.51	1.36 ± 0.29	1.67 ± 0.63	1.85 ± 0.87
Food + supplements	1.92 ± 0.56	1.72 ± 0.70	2.23 ± 1.37	1.85 ± 0.87
Niacin (mg)				
Food	22.7 ± 8.5	17.3 ± 4.7	24.2 ± 7.2	26.3 ± 9.2
Food + supplements	25.8 ± 8.2	21.5 ± 8.0	30.7 ± 13.7	26.3 ± 9.2
Vitamin B-6 (mg)				
Food	1.68 ± 0.49	2.17 ± 0.75	1.74 ± 0.48	3.21 ± 1.33[4]
Food + supplements	2.00 ± 0.56	2.59 ± 0.95	2.39 ± 1.20	3.21 ± 1.33
Folate (μg)				
Food	240 ± 115	435 ± 155[4]	275 ± 175	640 ± 250[2]
Food + supplements	300 ± 140	520 ± 205[4]	400 ± 275	640 ± 250[3]
Vitamin B12 (μg)				
Food	4.6 ± 2.9	1.4 ± 1.2[2]	3.3 ± 1.5	2.9 ± 3.9
Food + supplements	5.7 ± 3.0	6.0 ± 5.1	5.3 ± 3.6	5.0 ± 4.4
Calcium (mg)				
Food	830 ± 375	590 ± 195	670 ± 325	715 ± 395
Food + supplements	855 ± 355	710 ± 280	720 ± 375	840 ± 750
Magnesium (mg)				
Food	300 ± 120	420 ± 125[3]	330 ± 70	605 ± 170[4]
Food + supplements	315 ± 105	440 ± 130[3]	365 ± 100	605 ± 702[4]
Iron (mg)				
Food	15.3 ± 9.1	17.6 ± 6.1	15.0 ± 5.7	26.4 ± 12.3[2]
Food + supplements	20.2 ± 11.6	22.6 ± 10.0	20.9 ± 13.2	43.4 ± 41.2
Zinc (mg)				
Food	10.9 ± 4.6	7.7 ± 1.9	10.1 ± 1.8	12.2 ± 4.7
Food + supplements	13.2 ± 5.2	10.8 ± 6.4	15.0 ± 8.8	12.2 ± 4.7
Copper (mg)				
Food	1.5 ± 0.8	2.2 ± 0.6[3]	1.3 ± 0.3	3.1 ± 0.9[2]
Food + supplements	1.8 ± 0.8	2.6 ± 0.9[3]	2.0 ± 1.2	3.1 ± 0.9[3]
Manganese (mg)				
Food	2.3 ± 1.3	4.1 ± 2.5[3]	2.8 ± 1.6	5.6 ± 2.0[4]
Food + supplements	2.7 ± 1.4	4.7 ± 3.0[3]	3.6 ± 2.1	5.6 ± 2.0[3]

[1] $\bar{x}$ ± SD. MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; RE, retinol equivalents; TE, tocopherol equivalents.
[2–4] Significantly different from the nonvegetarian group of the same sex: [2] $P < 0.001$, [3] $P < 0.05$, [4] $P < 0.01$.

TABLE 3
Iron and zinc nutritional status indicators in male and female vegans compared with nonvegetarians

	Males		Females	
	Nonvegetarians (n = 10)	Vegans (n = 10)	Nonvegetarians (n = 10)	Vegans (n = 15)
Hemoglobin (g/L)	156 ± 7[1]	154 ± 7	133 ± 10	132 ± 10
Subjects with hemoglobin ≤ 120 g/L	0	0	1	2
Hematocrit	0.45 ± 0.02	0.45 ± 0.02	0.40 ± 0.02	0.39 ± 0.03
Mean cell volume (fL)	88.2 ± 2.6	91.5 ± 3.8[2]	90.1 ± 4.0	90.7 ± 4.4
Ferritin (μg/L)	141 ± 93	72 ± 32[2]	22 ± 13	27 ± 16
Subjects with ferritin ≤ 12 μg/L	0	0	2	4
Plasma zinc (μmol/L)	16.2 ± 2.6	15.1 ± 2.7	13.8 ± 1.1	13.7 ± 1.4

[1] $\bar{x}$ ± SD.
[2] Significantly different from nonvegetarians, $P < 0.05$.

variance explained by the addition of a variable in the regression analysis is defined as ΔR^2. After diet was controlled for, only age was a significant predictor of lymphocyte count. BMI did not account for a significant proportion of the variance seen in leukocyte count or complement factor 3 concentrations. These results suggest that individuals with similar BMIs are more likely to have lower leukocyte counts and complement 3 concentrations if they are following a vegan diet.

DISCUSSION

The objective of this study was to assess the nutritional status of vegans compared with that of nonvegetarians. Interpretation of the findings must consider the relative body weights of the participants in the groups. Although severely overweight individuals were excluded from the study, the mean BMI of the vegans was significantly lower than that of the nonvegetarians. Of the 25 vegan participants, 9 had BMIs < 19. The reported energy intakes calculated from 4-d records were lower in the female vegans and higher in the male vegans than in nonvegetarians of the respective sex, but the differences were not significant. Leanness in vegans may be due to reduced food intake not reflected in the 4-d records, habitually low fat intake, or other factors.

Dietary intake data obtained in this study were similar to those observed by others who have assessed vegan diets (20–25). According to the 4-d records, the protein contents of the vegan diets of women were significantly lower than those of the nonvegetarians, and 10 of the 25 vegan women failed to meet the recommended dietary allowance of 0.8 g/kg body wt for daily protein intake. Diets based entirely on plant foods tend to be lower in total fat, saturated fat, monounsaturated fat, and cholesterol. They also tend to be higher in dietary fiber and most nutrients, including many of the mineral elements, except for vitamin B-12. Although plant foods do not contain vitamin B-12, some of the vegan participants consumed food items fortified with vitamin B-12, such as ready-to-eat breakfast cereal, soymilk, and meat analogs, or took vitamin B-12 supplements.

Iron

One concern with vegetarian diets has been the possibility of iron deficiency and consequent anemia. Iron bioavailability from foods of plant origin is low compared with that from meat. Inorganic iron binds to phytates, tannins, and phosphates in plant foods and these may have an inhibitory effect on iron absorption (26). On the other hand, vegetarian diets provide ample quantities of vitamin C, which is known to enhance the absorption of iron (27).

Vegan men had a relatively high intake of dietary iron but their mean ferritin concentrations were significantly lower than those of nonvegetarians. This is consistent with other studies that showed hemoglobin to be within the normal range and ferritin concentrations to be lower in male vegetarians (28, 29). In population studies, lower ferritin concentrations have been associated with a lower risk of heart disease (30) and may be thought of as a beneficial consequence of vegetarian and vegan diets. Our results show that marginal iron status is a potential problem for women whether they follow vegan or nonvegetarian diets.

Zinc

The vegan diet has the potential to be low in zinc. In the United States, 65% of dietary zinc comes from animal products such as meat, poultry eggs, oysters, and other seafood. Vegan diets contain large amounts of fiber and phytate and it was found that the crude fiber intake of vegetarian diets negatively correlated with plasma zinc (10). Freeland-Graves et al (31, 32) found that vegan women had low dietary intake of zinc and although their serum zinc concentration was lower than that of nonvegetarians, the difference was not significant. Anderson et al (28) examined Canadian Seventh-day Adventist lactoovovegetarian women and found that plant foods provided 77% of their zinc intake and their serum zinc concentrations were not significantly different from those of nonvegetarians.

There is no agreement on the best way to assess zinc status. Plasma zinc concentrations of vegans in this study tended to be lower than those of nonvegetarians, however, not significantly so. Because the dietary intakes of the 2 groups were approximately equal, the data are consistent with lower absorption of zinc from plant foods. The vegans in this study did not appear to have impaired zinc status.

Vitamin B-12

Vitamin B-12 is present only in animal foods; diets based entirely on plant foods are devoid of the vitamin unless they are supplemented or contaminated. Depletion of vitamin B-12 stores is thought to be rare in healthy young- and middle-aged individuals who adopt vegan diets, and, even if no dietary source is consumed, depletion may take many years to occur if at all. Although the average time subjects consumed a vegan diet was 4.2 y in this study, the data showed that 10 of the 25 vegans had at least one indicator of

TABLE 4
Vitamin B-12 and folate status

Status indicator	Nonvegetarians (n = 20)	Vegans (n = 25)
Serum component		
Vitamin B-12 (pmol/L)	313 ± 99[1]	312 ± 125
Folate (nmol/L)	25 ± 10	38 ± 15[2]
Methylmalonic acid (nmol/L)	262 ± 53	316 ± 152
2-Methylcitric acid (nmol/L)	165 ± 31	140 ± 30[2]
Total homocysteine (μmol/L)	8.0 ± 1.9	7.9 ± 1.5
Cystathionine (nmol/L)	124 ± 41	105 ± 54
Subjects with indicators of vitamin B-12 deficit		
Serum vitamin B-12 < 150 pmol/L	0	3
Serum methylmalonic acid > 376 nmol/L[3]	0	5
Mean cell volume > 98 fL	0	2

[1] $\bar{x}$ ± SD.
[2] Significantly different from nonvegetarians, $P < 0.01$.
[3] Methylmalonic acid > 376 nmol/L is 3 SDs above the population mean (19).

TABLE 5
White blood cell counts and immune status indicators in nonvegetarians and vegans[1]

	Nonvegetarians (n = 20)	Vegans (n = 25)
White blood cell counts (× 10^9/L)		
Leukocytes	5.83 ± 1.51	4.96 ± 0.91[2]
Lymphocytes	1.90 ± 0.59	1.56 ± 0.39[2]
Neutrophils	3.47 ± 1.02	3.04 ± 0.83
Monocytes	0.24 ± 0.08	0.19 ± 0.09
Eosinophils	0.17 ± 0.09	0.14 ± 0.10
Basophils	0.05 ± 0.03	0.04 ± 0.02
Platelets	270 ± 55	235 ± 60[2]
Albumin (g/L)	46.9 ± 3.8	49.3 ± 2.9[2]
Blood urea nitrogen (mmol/L)	4.78 ± 1.0	4.03 ± 1.0[2]
Immune globulins (g/L)		
Immunoglobulin G	13.5 ± 2.2	13.6 ± 2.9
Immunoglobulin A	29.0 ± 1.3	23.0 ± 7.0
Immunoglobulin M	18.5 ± 9.5	20.0 ± 8.5
Complement factors (g/L)		
Complement factor 3	0.75 ± 0.11	0.63 ± 0.09[3]
Complement factor 4	0.27 ± 0.08	0.23 ± 0.08
Complement factor 50	202 ± 74	195 ± 61
C-reative protein	0.282 ± 0.10	0.286 ± 0.13
Natural killer cell cytotoxic activity		
20:1, Effector-to-target ratio (% lysis)	31.6 ± 10.7	33.5 ± 17.9
Lytic units (LU/10^6 cells)	15.5 ± 11.9	16.6 ± 17.8
Mitogen stimulation (SI)[4]		
Phytohemagglutinin	80 ± 45	106 ± 70
Concanavalin A	60 ± 33	73 ± 51
Pokeweed	13 ± 7	16 ±13

[1] $\bar{x}$ ± SD.
[2,3] Significanlty different from nonvegetarians: [2] $P < 0.05$, [3] $P < 0.001$.
[4] SI, stimulation index = [cpm (mitogen stimulated)/cpm (control)].

vitamin B-12 deficiency, either macrocytosis, low serum vitamin B-12, or elevated methylmalonic acid concentration (Table 4).

Vitamin B-12 is required for DNA synthesis and erythropoiesis and a deficiency may result in higher proportions of immature, enlarged red blood cells. It is also an enzymatic cofactor for the action of methylmalonyl-CoA mutase in the conversion of methylmalonyl-CoA to succinyl-CoA. If vitamin B-12 status is inadequate, mutase is inhibited and the metabolite methylmalonic acid accumulates (33). Increased serum methylmalonate is a sensitive early indicator of vitamin B-12 deficiency. Vegan subjects in this study had elevated serum methylmalonic acid concentrations with 5 having concentrations >376 nmol/L, a definitive cutoff value 3 SDs above the mean of a healthy population (19, 34).

Studies have reported elevations in serum 2-methylcitrate in vitamin B-12 deficiency (14). This metabolite is a product of the condensation of propionyl-CoA and oxaloacetate, and in vitamin B-12 deficiency, propionyl Co-A may accumulate and result in increased synthesis of 2-methylcitric acid. In this study, however, the vegan group had a significantly lower mean serum 2-methylcitrate concentration and all vegan participants had values within the normal range. There were no differences between vegans and nonvegetarians in serum homocysteine concentration and, with one exception, homocysteine concentrations were within or below normal limits for all participants.

Correlational analysis and group comparisons did not show a relation between supplemental vitamin B-12 consumption and any of the metabolites assessed. There was, however, a significant correlation ($P < 0.05$) between vitamin B-12 supplement intake and serum B-12 concentrations. Marginal vitamin B-12 intake can cause the development of neuropsychiatric disorders such as paresthesia, weakness, fatigue, and poor mental concentration in the absence of abnormal manifestations in the usual indicators such as very low serum concentrations of the vitamin, macrocytosis, or the resulting anemia (7, 8). These changes are serious and could result in irreversible functional deterioration.

Our results are consistent with those of others that showed that vegans adhering to entirely plant-based diets are at risk of developing vitamin B-12 deficiency (35). Although group means did not show differences in dietary plus supplemental vitamin B-12 intakes between groups, several individuals in the vegan group did not regularly consume vitamin B-12–fortified foods or supplements. It is important to emphasize that several indicators must be evaluated to assess status because individuals respond differently to low intakes. Serum vitamin B-12 concentrations are helpful in diagnosis of vitamin B-12 deficiency but serum methylmalonic acid concentration is a sensitive and specific early indicator of deficit.

Immune status

Results of this study showed lower leukocyte, lymphocyte, and platelet counts and complement factor 3 concentrations in vegans than in nonvegetarians. Similar reductions in these measures have been observed in protein-energy malnutrition (36–38) and as a consequence of energy restriction for the purposes of weight control (39).

The vegan group had significantly higher mean serum albumin and lower blood urea nitrogen concentrations. The lower blood urea nitrogen reflects the lower dietary protein intake of vegans. Although serum albumin may not be a sensitive indicator of protein nutriture, the higher concentrations suggest that the diets of the vegan participants were adequate in protein. A dietary intervention study of young men before and after 12 wk of a low-fat diet resulted in an increase in natural killer cell activity (40). Even though the dietary fat intake of vegans in this study was substantially lower, their natural killer cell activity was not different from that of nonvegetarians.

TABLE 6
Standardized regression coefficients, regression coefficients, changes in R^2 (ΔR^2), and significance of diet, age, or BMI as predictors of leukocyte count, lymphocyte count, and plasma complement factor 3 concentration

Variable	β	R^2	ΔR^2	P
Leukocyte count				
Diet	0.34	0.12	—	0.02
BMI	0.09	0.12	0.004	—
Lymphocyte count				
Age	−0.33	0.11	—	0.023
Diet	0.29	0.20	0.08	0.046
Complement factor 3				
Diet	0.51	0.26	—	0.000
BMI	0.23	0.29	0.03	—

The question was raised as to whether the immune status results observed in this study are a consequence of the relatively low body weights of the vegans. Multiple regression analysis of the data did not show that BMI had an effect independent of diet. Further research is needed to elucidate the associations between diet, body weight, and immune function measures in healthy, lean individuals.

In summary, we observed that vegans had lower numbers of circulating white cells and less complement factor 3. There were no reductions in functional measures such as mitogen stimulation and natural killer cell activity. It is not possible to determine from these finding whether the immune status of vegans is compromised or enhanced compared with other groups. Future investigators might consider a longitudinal study to determine whether the vegan diet is a risk or protective factor for morbidity and common infections.

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REFERENCES

1. Hardinge MG, Stare FJ. Nutritional studies of vegetarians. 1. Nutritional, physical and laboratory studies. Am J Clin Nutr 1954;2:73–82.
2. Wokes F, Badenoch J, Sinclair HM. Human dietary deficiency of vitamin B_{12}. Am J Clin Nutr 1955;3:375–82.
3. Guggenheim K, Weiss Y, Fostick M. Composition and nutritive value of diets consumed by strict vegetarians. Br J Nutr 1962;16:467–71.
4. Ellis FR. Mumford P. The nutritional status of vegans and vegetarians. Proc Nutr Soc 1967;26:209–16.
5. Ellis FR. Montegriffo VME. Veganism, clinical findings and investigations. Am J Clin Nutr 1970;23:249–55.
6. Sanders TAB, Ellis FR, Dickerson JWT. Haematological studies on vegans. Br J Nutr 1978;40:9–15.
7. Lindenbaum J, Healton EB, Savage DG, et al. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. N Engl J Med 1988;318:1720–8.
8. Bar-sella P, Rakover Y, Ratner D. Vitamin B12 and folate levels in long-term vegans. Isr J Med Sci 1990;26:309–12.
9. Harland BF, Peterson M. Nutritional status of lacto-ovo-vegetarian Trappist monks. J Am Diet Assoc 1978;72:259–64.
10. Latta D, Liebman M. Iron and zinc status of vegetarian and non-vegetarian males. Nutr Rep Int 1984;30:141–9.
11. Dwyer JT. Nutritional consequences of vegetarianism. Annu Rev Nutr 1991;11:61–91.
12. Strachan DP, Powell KJ, Thaker A, Millard FJ, Maxwell JD. Vegetarian diet as a risk factor for tuberculosis in immigrant south London Asians. Thorax 1995;50:175–80.
13. Smith JC, Butrimovitz GP, Purdy WC. Direct measurement of zinc in plasma by atomic absorption spectroscopy. Clin Chem 1997;25:1487–91.
14. Allen RH, Stabler SP, Savage DG, Lindenbaum J. Elevation of 2-methylcitric acid I and II levels in serum, urine, and cerebrospinal fluid of patients with cobalamin deficiency. Metabolism 1993;42: 978–88.
15. Stabler SP, Lindenbaum J, Savage DG, Allen RH. Elevation of serum cystationine levels in patients with cobalamin and folate deficiency. Blood 1993;81:3404–13.
16. Kelly JP, Parker CW. Effect of arachidonic acid and other unsaturated fatty acids on mitogenesis in human lymphocytes. J Immunol 1979; 122:1556–62.
17. Pross HF, Baines MG, Rubin P, Shragge P, Patterson MS. Spontaneous human lymphocyte-mediated cytotoxicity against tumor target cell. IX. The quantitation of natural killer cell activity. J Clin Immunol 1981;1:51–63.
18. Cook JD, Finch CA. Assessing iron status of a population. Am J Clin Nutr 1979;32:2115–9.
19. Lindenbaum J, Savage DG, Stabler SP, Allen RH. Diagnosis of cobalamin deficiency. II. Relative sensitivities of serum cobalamin, methylmalonic acid and total homocysteine concentration. Am J Hematol 1990;34:99–107.
20. Abdulla M, Andersson, I, Asp N-G, et al. Nutrient intake and health status of vegans. Chemical analyses of diets using the duplicate portion sampling technique. Am J Clin Nutr 1981;34:2464–77.
21. Roshanai F, Sanders TAB. Assessment of fatty acid intakes in vegans and omnivores. Hum Nutr Appl Nutr 1984;38A:345–54.
22. Calkins BM, Whittaker DJ, Nair PP, Rider AA, Turjman N. Diet, nutrition intake, and metabolism in populations at high and low risk for colon cancer. Nutrient intake. Am J Clin Nutr 1984;40(suppl): 896S–905S.
23. Draper A, Lewis J, Malhotra N, Wheeler E. The energy and nutrient intakes of different types of vegetarian: a case for supplements? Br J Nutr 1993;69:3–19.
24. Alexander D, Ball MJ, Mann J. Nutrient intake and haematological status of vegetarians and age-sex matched omnivores. Eur J Clin Nutr 1994;48:538–46.
25. Janelle KC, Barr SI. Nutrient intakes and eating behavior scores of vegetarian and nonvegetarian women. J Am Diet Asoc 1995; 95: 180–186, 189.
26. Hazell T. Relating food composition data to iron availability from plant foods. Eur J Clin Nutr 1988;42:509–17.
27. Hallberg L, Brune M, Rossander-Hulthen L. Is there a physiological role of vitamin C in iron absorption? Ann N Y Acad Sci 1987;498:324–32.
28. Anderson BM, Gibson RS, Sabry JH. The iron and zinc status of long-term vegetarian women. Am J Clin Nutr 1981;34:1042–8.
29. Reddy S, Sanders TAB. Haematological studies on pre-menopausal Indian and Caucasian vegetarians compared with Caucasian omnivores. Br J Nutr 1990;64:331–8.
30. Salonen JT, Nyyssönen K, Korpela H, Tuomilehto J, Seppänen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in Eastern Finnish men. Circulation 1992;86:803–11.
31. Freeland-Graves JH, Bodzy PW, Eppright MA. Zinc status of vegetarians. J Am Diet Assoc 1980;77:655–61.
32. Freeland-Graves J. Mineral adequacy of vegetarian diets. Am J Clin Nutr 1988;48(suppl):859S–62S.
33. Stabler SP, Allen RH, Savage DG. Lindenbaum J. Clinical spectrum and diagnosis of cobalamin deficiency. Blood 1990;76:871–81.

34. Savage DG, Lindenbaum J, Stabler SP, Allen RH. Sensitivity of serum methylmalonic acid and total homocysteine determinations for diagnosing cobalamin and folate deficiencies. Am J Med 1994;96:239–46.
35. Miller DR, Specker BL, Ho ML, Norman EJ. Vitamin B-12 status in a macrobiotic community. Am J Clin Nutr 1991;53:524–9.
36. Gibson RS. Principles of nutritional assessment. New York: Oxford University Press, 1990.
37. Chandra RK. Serum complement and immunoglobulin in malnutrition. Arch Dis Child 1975;50:225–9.
38. Haller L, Zubler RH, Lambert PH. Plasma levels of complement components and complement haemolytic activity in protein-energy malnutrition. Clin Exp Immunol 1978;34:248–52.
39. Kelley DS, Daudu PA, Branch LB, Johnson HL, Taylor PC, Mackey B. Energy restriction decreases number of circulating natural killer cells and serum levels of immunoglobulins in overweight women. Eur J Clin Nutr 1994;48:9–18.
40. Barone J, Hebert JR, Reddy MM. Dietary fat and natural-killer-cell activity. Am J Clin Nutr 1989;50:861–7.