Associations of Circulating Methylmalonic Acid and Vitamin B-12 Biomarkers Are Modified by Vegan Dietary Pattern in Adult and Elderly Participants of the Adventist Health Study 2 Calibration Study

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ABSTRACT

Background: Elevated plasma methylmalonic acid (MMA) is a functional biomarker of vitamin B-12 status but limited information is available on its prevalence in US vegetarians.

Objectives: The study examines the prevalence of plasma MMA ≥ 0.27 μmol/L in those consuming vegetarian diets, its associations with vitamin B-12 intake and biomarkers, and the modifying effect of vegetarian patterns on these associations.

Methods: In this cross-sectional study ($n = 785$), concentrations of MMA, vitamin B-12, holotranscobalamin (holoTC), and homocysteine (Hcy) were determined in participants of the calibration substudy of the Adventist Health Study 2 (AHS-2). Vitamin B-12 intake from food, fortified food, and supplements was assessed by six 24-h recalls. Regression models were used to estimate ORs of having high MMA as related to vitamin B-12 status biomarkers, vitamin B-12 intake, and dietary pattern.

Results: The prevalence of low vitamin B-12 status defined by serum vitamin B-12 <148 pmol/L, holoTC <35 pmol/L, MMA ≥ 0.27 and ≥ 0.37 μmol/L, or Hcy ≥ 15 μmol/L, and the OR of having high MMA did not differ by dietary pattern, possibly due to intake from fortified food and supplements. Total daily vitamin B-12 intake in the second tertile range of 4.4–14.5 μg/d reduced the likelihood of elevated MMA by 69%; and a doubling of vitamin B-12 intake was associated with a 4.3% decrease in plasma MMA. The association between log plasma MMA and biomarkers was modified by diet, with the vegan pattern showing an ~3-fold stronger association with log serum vitamin B-12 and Hcy than did the nonvegetarian pattern.

Conclusions: The prevalence of vitamin B-12 intake <2.0 μg/d was 15.2% in vegans, 10.6% in lacto-ovo-vegetarians, and 6.5% in nonvegetarians. Given the irreversible neurological consequences of vitamin B-12 inadequacy, the importance of regular supplemental vitamin B-12 intake in adult and elderly individuals is stressed. *Curr Dev Nutr* 2020;4:nzaa008.

Introduction

Owing to exclusion from the diet of meat and fish by lacto-ovo-vegetarians, and all animal products by vegans, the nutrient deficit most often associated with vegetarian eating is that of vitamin B-12. The consequences of vitamin B-12 (cobalamin) deficiency include neurologic damage and cognitive impairment which may not be fully reversible (1, 2). Of microbial origin and found in adequate quantities only in foods of animal origin, vitamin B-12 is involved in one-carbon pathways essential for methylation of DNA, histones, and other regulators of gene expression critical for normal hematologic and neurologic

function (3). Vitamin B-12 as adenosylcobalamin is a cofactor for methyl CoA-mutase which converts methylmalonyl CoA to succinyl CoA and impaired activity of the enzyme leads to elevations of methylmalonic acid (MMA) (4). Because low serum vitamin B-12 concentration is not always reflective of poor status, elevated plasma MMA is employed as a functional biomarker of deficiency (4, 5). Although mechanisms explaining the etiology of cellular and neurologic manifestations accompanying vitamin B-12 deficiency are unclear, some evidence points to impaired methylation patterns and to the inflammatory and oxidative effects of elevated MMA as factors in the neurodegeneration and renal insufficiency which develop (6, 7). Along with inadequate

vitamin B-12 intake, increased plasma MMA can arise from malabsorption and other gastrointestinal disorders, or from polymorphisms and genetic abnormalities (8).

Currently vegetarian and plant-based eating is promoted to lower risk of coronary artery disease and type 2 diabetes (9–11). In addition to an emphasis on consuming plant foods, these regimens promote restriction or avoidance of animal source foods. As a consequence, nutrient shortfalls, particularly those of vitamin B-12, must be considered. Vegetarians and persons adhering to plant-based eating may be at risk of inadequate intake unless they regularly consume vitamin B-12–fortified foods and vitamin B-12–containing supplements. This risk may be greater for elderly vegetarians owing to poor oral intake or owing to malabsorption which often arises from age-associated impairment in gastric acid secretion and gastrointestinal disturbances (12–14). Medications such as proton pump inhibitors, H2 receptor antagonists, and oral antidiabetic drugs may also contribute to malabsorption of the vitamin (15).

Studies examining vitamin B-12 status in vegetarians report high rates of depletion. In a systematic review of studies utilizing serum vitamin B-12 concentrations as a biomarker, Pawlak et al. (16) reported worldwide low vitamin B-12 status prevalence rates in adult and elderly vegetarians of ≤85%. In the European Prospective Investigation into Cancer and Nutrition Oxford cohort, Gilsing et al. (17) classified 52% of vegans and 7% of vegetarians as vitamin B-12 deficient, defined as serum vitamin B-12 <118 pmol/L. The prevalence of vitamin B-12 deficiency assessed as plasma MMA >0.27 μmol/L in the NHANES population is relatively low and increases with age, affecting 3.9% of those aged 19–39 y old, 5.1% of those 40–59 y old, and 15.9% of those >60 y old (18). Compared with nonvegetarians, more frequent elevated plasma MMA has been reported among vegetarian adults in Spain (19), preadolescent refugee girls in India (20), reproductive-age women in Nepal (21), and pregnant women in Canada (22). However, despite its importance in detecting functional vitamin B-12 abnormalities, studies using plasma MMA as a biomarker in US vegetarians have been limited to small sample sizes and to macrobiotic and vegan groups (23–25).

In healthy adults, plasma MMA concentrations are associated with age, creatinine concentrations, sex, dietary vitamin B-12 intake (26–28), and race (28). Plasma MMA concentrations are negatively associated with serum vitamin B-12 and with holotranscobalamin (holoTC), the fraction of the circulating vitamin B-12 taken up by cells, and positively with the metabolite homocysteine (Hcy). The modifying effect of vegetarian dietary patterns on these associations has not been examined. As the quantity of animal food in the diet decreases, so does natural-source vitamin B-12, leading to a greater dependence on fortified products and vitamin B-12–containing supplements which may not be sufficiently or regularly consumed. Also, vegetarian and plant-rich diets are high in folate and folate concentrations are known to affect vitamin B-12 metabolism. It is therefore important to establish whether vegetarian dietary patterns influence plasma MMA concentrations, particularly in older individuals.

To address this knowledge gap, the current study was designed to investigate clinical and dietary factors associated with likelihood of elevated MMA in adult-to-elderly participants of the Adventist Health Study 2 (AHS-2) calibration substudy. In addition to the population comprising many vegans and lacto-ovo-vegetarians with consistent dietary habits, the nonvegetarians in the population consume small to moderate amounts of dairy foods and meats (29, 30). In this study we aimed to compare the prevalence of elevated plasma MMA in individuals adhering to vegan, lacto-ovo-vegetarian, and nonvegetarian dietary patterns and the associations of dietary pattern, vitamin B-12 intake, and vitamin B-12–related biomarkers with circulating MMA. In addition we sought to examine the modifying effect of dietary pattern on the relation between vitamin B-12 intake, biomarkers, and plasma MMA.

Methods

Study population

The study was conducted on participants of the calibration subgroup selected from the larger cohort of 96,194 white and black men and women of the ongoing AHS-2 cohort. AHS-2 was designed to study associations between lifestyle and risk of cancer. Study recruitment and selection methods have been described elsewhere (31). Participants had to be ≥30 y of age for inclusion and blacks were oversampled. The calibration subgroup, also described elsewhere (31), was formed as a representative sample ($n = 1011$) selected from the parent cohort by church and then by subject-within-church. Participants attended clinics at their local church where anthropometric measurements and fasting blood specimens were collected. Sociodemographic and lifestyle variables including age; sex; race; education; physical activity; cigarette smoking; alcohol use; and use of supplements, drugs, and various medications were assessed by questionnaire. The duration of the calibration substudy was ~9–12 mo for any one subject. Of the total calibration study sample, 850 participants had a minimum of five of six 24-h recalls and biomarker data. Because impaired clearance of MMA from blood occurs owing to renal dysfunction, we excluded 29 participants with missing creatinine data and 18 participants with serum creatinine >1.47 mg/dL (>130 μmol/L) (32, 33). Also excluded were 3 participants with BMI (in kg/m^2) <16 and >60 and 16 participants whose BMI was missing, resulting in a final cohort of 785 participants. All subjects gave written informed consent. The study protocol was approved by the Institutional Review Board of Loma Linda University.

Dietary data

Total vitamin B-12 intakes from foods, fortified foods, and supplements were obtained via 24-h dietary recalls obtained by telephone and the interviews were digitally recorded. Typically a set of 3 recalls were obtained (1 Saturday, 1 Sunday, and 1 weekday) 2 mo before the clinic visits and a second set of 3 (1 Saturday, 1 Sunday, and 1 weekday) ~6 mo after the clinic visits. Dietary intakes are often rather different on Saturdays, Sundays, and on typical weekdays. Thus, within each of the 2 sets of 24-h recalls, each day was appropriately weighted and summed to produce a synthetic week (Saturday intake + Sunday intake + 5 × weekday intake), then divided by 7 to obtain the mean daily food intake estimate. Further details on adjustments and weighting of dietary recall data have been published (30). The recalls were administered unannounced by telephone by a trained dietitian using standard probes and a multiple-pass methodology. Recall data were entered into a database using the Nutrition Data System for Research version 5.0 of the Nutrition Coordinating Center, Minneapolis, MN.

As described in a previous AHS-2 publication (34), dietary patterns were determined from FFQ data according to reported intake of animal source foods. Thus, individuals defined as vegan consumed meats (including poultry and fish), dairy products, and eggs <1 time/mo; lacto-ovo-vegetarians consumed dairy products and eggs ≥1 time/mo and meats <1 time/mo; pesco-vegetarians consumed fish ≥1 time/wk and other meats <1 time/mo; semivegetarians consumed nonfish meals ≥1 time/mo and all meats ≥1 time/mo but ≤1 time/wk; and nonvegetarians consumed all meats >1 time/wk. Because of small numbers, in this analysis pesco- and semivegetarians were merged with the nonvegetarian group.

Laboratory analyses

Collection and processing of blood after an overnight fast followed standard protocols. Serum was separated from cells within 30 min of collection, placed on wet ice, and shipped or transported to the central study laboratory at Loma Linda, CA for further processing. Blood was divided into aliquots of 0.5-mL samples and immediately stored in liquid nitrogen until analysis. Serum holoTC was measured by enzyme-immunoassay kits from Axis-Shield Diagnostics Ltd and serum vitamin B-12 and Hcy by ELISA kits from Monobind Inc. according to the manufacturer's directions. For holoTC, the mean intrabatch CV was 7.2% and the interbatch CV was 10.0%; for vitamin B-12, the mean intrabatch CV was 3.5% and the interbatch CV was 7.0%; and for Hcy, the mean intrabatch CV was 2.4% and the interbatch CV was 4.3%. MMA was determined in serum by LC-tandem MS stable isotope dilution analysis (Mayo Clinic, Rochester, MN). RBC folate was assayed on washed erythrocytes at the Center for Human Nutrition Biomarker laboratories (University of California, Los Angeles). Erythrocyte folate was assayed using an immunoassay and the automated Immulite Chemiluminescence Immunoassay Analyzer (Siemens Healthcare Diagnostics). For RBC folate, the mean intrabatch CV was 5.2% and the interbatch CV was 7.4%.

Covariates

Selection of covariates was based on univariate analysis and published literature. Age, sex, ethnicity/race, and other demographic characteristics and health behaviors were self-reported. Educational attainment was characterized as ≤12 y of high school or less, compared with >12 y as some college and a bachelor or higher degree as college graduate. Smoking and alcohol use were characterized as "ever" for previous or current smoker or drinker or as "never" smoker or drinker. Dietary sources of vitamin B-12 were determined from food intakes reported in the 24-h recalls as previously described (35). Briefly, animal food sources included all meats, dairy foods, and eggs and fortified foods included all items fortified with vitamin B-12, mainly some ready-to-eat breakfast cereals, meat and dairy alternatives, and nutritional yeasts. Of the 60% of subjects who reported consuming vitamin B-12–containing supplements, about half obtained the vitamin from multivitamin preparations and half from individual preparations containing 50–100 μg vitamin B-12 per dose.

Use of oral diabetic medications or acid-reducers (yes, no) was obtained from the questionnaire administered at baseline. To estimate the prevalence of inadequate vitamin B-12 status, the commonly used cutoffs were applied: serum vitamin B-12 <148 pmol/L; holoTC <35 pmol/L; Hcy >15 μmol/L; and MMA ≥0.27 μmol/L and ≥0.37 μmol/L (2, 18, 26, 36). Owing to the absence of hematocrit values, folate concentrations in washed RBC are reported as nanomoles per gram of hemoglobin and the median value (less than/greater than) of 6.6 nmol/g hemoglobin (equivalent to ~1400 nmol/L folate in whole blood) was used to calculate the OR for high MMA.

Statistical methods

Tests for differences in subject characteristics, biomarker measures, and intake and sources of vitamin B-12 from foods and supplements between dietary groups were conducted using 1-factor ANOVA and the Kruskal–Wallis test for continuous variables, and χ^2 test and Fisher's exact test for categorical variables.

Dietary sources of vitamin B-12 intake were energy adjusted by the residual method. Zero intake was partitioned out from energy adjustment. Before adjustment, vitamin B-12 dietary intake was log transformed and then energy-adjusted log vitamin B-12 was back-transformed. Total vitamin B-12 intake was the sum of the energy-adjusted dietary intake and the unadjusted supplementary vitamin B-12 intake. Because the dietary recall data contained 2 synthetic weeks, the repetition allowed the estimation of within-person variance and the correction of coefficients for attenuating effects of random errors in the recalls.

To explore the predictive effect of vitamin B-12 biomarkers and intake on elevated MMA, multivariable logistic regression was conducted to model the likelihood of having MMA ≥0.27 μmol/L, which represents values more commonly found in adults with normal renal function (5). For each variable tested (vitamin B-12 intake, vegetarian dietary pattern, and individual vitamin B-12 biomarkers), we calculated ORs and 95% CIs. In model 1, we included dietary pattern and we quantified vitamin B-12 intake as tertiles of total vitamin B-12 intake (animal source + fortified food + supplements). In model 2, vitamin B-12 intake was partitioned into vitamin B-12 supplement use (yes/no) and intake from food (animal source + fortified). In model 3, we fitted both vitamin B-12 supplement use (yes/no), and dietary vitamin B-12 intake further separated into total intake from food (animal source + fortified) and intake from fortified food, meat, fish, and egg/dairy. All models were adjusted for age (continuous), sex, race (white/black), smoking (never/ever), alcohol (never/ever), medication use (yes/no), and blood creatinine concentrations (continuous).

All biomarkers with skewed distribution values were log transformed. To determine the associations between MMA and each of the vitamin B-12 biomarkers and between MMA and vitamin B-12 intake and supplement use, linear regression models were fitted with the log of MMA as the dependent variable and with each of log serum vitamin B-12, holoTC, Hcy, log RBC folate, and log total vitamin B-12 intake as the predictor. Regression assumptions including normality and linearity were assessed by visual inspection of residual plots (see **Supplemental Figure 1**). There were no severe violations of the assumptions. Finally, to determine whether dietary pattern may modify the relation between MMA and the biomarkers, we further added dietary pattern and its interaction with each biomarker into each linear regression model. (For plots see **Supplemental Figure 2**.) All models were adjusted for age (continuous), sex, race (white/black), smoking (never/ever), alcohol (never/ever), medication use (yes/no), and blood creatinine (continuous).

TABLE 1 Characteristics of participants by dietary pattern; the calibration substudy of the Adventist Health Study 2[1]

	Vegan	Lacto-ovo-vegetarian	Nonvegetarian, semivegetarian, and pesco-vegetarian	P value[2]
n	76	221	488	
Age, y	64.7 ± 12.4	60.2 ± 13.9	56.9 ± 12.8	<0.001[a]
BMI, kg/m^2	24.9 ± 5.3	25.2 ± 4.8	28.7 ± 6.1	<0.001[b]
Sex, %				
Female	72.4	62.0	67.4	0.186[c]
Male	27.6	38.0	32.6	
Race, %				
White	56.6	74.7	48.6	<0.001[c]
Black	43.4	25.3	51.4	
Education, %				
High school or less	29.3	24.1	27.3	0.791[c]
Some college	33.3	34.5	35.6	
College graduate	37.3	41.4	37.1	
Alcohol, %				
Never	71.6	73.3	51.1	<0.001[c]
Ever	28.4	26.7	48.9	
Smoking, %				
Never	90.8	88.2	78.7	0.001[c]
Ever	9.2	11.8	21.3	
Physical activity, min/wk				
0	6 (8.0)	43 (20.2)	94 (20.1)	0.002[c]
>0–60	21 (28.0)	66 (31.0)	179 (38.3)	
>60	48 (64.0)	104 (48.8)	194 (41.5)	
Diabetic medication user, %	2.6	5.0	9.8	0.017[c]
Acid reducer user, %	3.9	3.6	7.2	0.133[c]
Serum vitamin B-12, pmol/L	306 [265–387]	322 [274–401]	316 [269–374]	0.489[b]
<148	6 (7.9)	13 (6.1)	20 (4.2)	0.240[c]
HoloTC, pmol/L	102 [61–136]	99 [67–129]	94 [66–131]	0.482[b]
<35	2 (2.7)	13 (6.0)	26 (5.4)	0.589[d]
Methylmalonic acid, μmol/L	0.14 [0.12–0.19]	0.14 [0.11–0.18]	0.13 [0.11–0.18]	0.084[b]
≥0.27	8 (10.8)	9 (4.2)	23 (4.8)	0.088[d]
≥0.37	3 (4.1)	4 (1.9)	7 (1.5)	0.248[d]
Homocysteine, μmol/L	12.0 [10.3–14.2]	11.1 [9.4–12.9]	11.4 [9.3–13.4]	0.046[b]
≥15	10 (14.3)	26 (13.1)	66 (15.5)	0.730[d]
RBC folate, nmol/g hemoglobin	6.70 [5.46–8.34]	6.88 [5.40–8.60]	6.20 [4.62–7.87]	0.001[b]

[1] Values are mean ± SD, n (%), or median [IQR] unless otherwise indicated. HoloTC, holotranscobalamin.
[2] P value for differences between dietary patterns: [a] 1-factor ANOVA, [b] Kruskal–Wallis test, [c] chi-square test, [d] Fisher's exact test.

Results

After the exclusion of those with fewer than five 24-h recalls, those with no biomarker values, and those whose serum creatinine was >130 μmol/L, the analytic sample consisted of 785 participants. Of these, 76 were vegan, 221 lacto-ovo-vegetarian, and 488 nonvegetarian. Ages ranged from 29 to 94 y with a mean ± SD age of 58.6 ± 13.3 y. BMI ranged from 16.6 to 54.9 with a mean ± SD BMI of 27.3 ± 6.0.

Table 1 shows selected characteristics by vegetarian status. There were significant differences in age, BMI, race, alcohol use, smoking, and use of oral diabetic medication between the dietary groups. Of the biomarkers tested, median circulating concentrations of Hcy and RBC folate differed significantly between the dietary groups. However, the prevalence of vitamin B-12 deficiency based on serum vitamin B-12 <148 pmol/L, holoTC <35 pmol/L, MMA >0.27 μmol/L, or Hcy >15 μmol/L did not differ by dietary pattern.

Table 2 shows the median and IQR daily intakes of vitamin B-12 from foods, fortified foods, and supplements by dietary pattern.

Although dietary intakes of vitamin B-12 from animal source and fortified foods differed between dietary groups, median vitamin B-12 intake from supplements and total vitamin B-12 intake were not significantly different. However, the prevalence of vitamin B-12 intakes below the Estimated Average Requirement (EAR) of 2.0 μg/d (29) was highest in the vegan and lowest in the nonvegetarian dietary groups.

Supplemental Table 1 presents results of logistic regression analysis stratified by MMA concentration. Age, race, educational level, and blood creatinine concentrations were associated with MMA ≥0.27 μmol/L, but not sex, alcohol use, smoking, or use of oral diabetic medication or acid reducers.

Table 3 presents models of predictors of elevated serum MMA by circulating concentrations of biomarkers, vitamin B-12 intake, and dietary pattern. The adjusted OR of elevated plasma MMA was significantly higher in those with holoTC <35 pmol/L, RBC folate <6.6 nmol/g hemoglobin, and Hcy ≥15 μmol/L. The association between elevated plasma MMA, dietary pattern, and dietary vitamin B-12 intake was examined in 3 models. In model 1, neither of the

TABLE 2   Intake of vitamin B-12 from animal source foods, fortified foods, and supplements by dietary pattern[1]

	Vegan	Lacto-ovo-vegetarian	Nonvegetarian, semivegetarian, and pesco-vegetarian	P value[2]
Dietary source of vitamin B-12				
Intake from food (animal source + fortified food), μg/d	3.1 [1.8–5.5]	3.4 [2.1–5.6]	3.7 [2.5–5.4]	0.127[a]
Animal source foods, μg/d	0.0 [0.0–0.1]	0.2 [0.1–0.6]	1.1 [0.5–2.1]	<0.001[a]
Meat and poultry, μg/d	0.0 [0.0–0.0]	0.0 [0.0–0.0]	0.1 [0.0–0.4]	<0.001[a]
Fish, μg/d	0.0 [0.0–0.0]	0.0 [0.0–0.0]	0.0 [0.0–0.4]	<0.001[a]
Dairy and egg, μg/d	0.0 [0.0–0.0]	0.17 [0.0–0.5]	0.4 [0.1–0.8]	<0.001[a]
Fortified food + nutritional yeast, μg/d	1.9 [1.2–3.0]	1.7 [0.6–3.6]	0.9 [0.2–2.1]	<0.001[a]
Intake from supplements (multivitamin, vitamin B-12, etc.), μg/d	1.9 [0.0–48.7]	2.6 [0.0–18.1]	1.5 [0.0–12.3]	0.117[a]
Total vitamin B-12 intake (animal source + fortified food + supplements), μg/d	9.4 [2.7–79.3]	6.6 [3.8–26.1]	7.3 [3.7–19.3]	0.638[a]
Prevalence of vitamin B-12 intake below EAR				
Intake <2.0 μg/d	10 (15.2)	19 (10.6)	26 (6.5)	0.0314[b]

[1]Values are median [IQR] or n (%) unless otherwise indicated. All dietary sources of vitamin B-12 are energy adjusted using the residual method. EAR, Estimated Average Requirement.
[2]P value for differences between dietary patterns: [a]Kruskal–Wallis test, [b]chi-square test.

dietary patterns showed a significant association with elevated MMA although the vegan pattern approached statistical significance. The OR for elevated MMA was lowest for total vitamin B-12 intake in the second tertile comprising an intake of 4.42–14.5 μg/d. In model 2, when vitamin B-12 intake was partitioned into supplement use (yes, no) and food intake, only the third tertile (>4.52 μg/d) approached statistical significance. In model 3, further separating vitamin B-12 consumption from supplement use (yes, no) and from animal source foods, fortified foods, meat and poultry, fish, and dairy and eggs did not show any significant associations for any of the food groups.

Table 4 presents results from regression models on the associations of individual vitamin B-12 biomarkers and total vitamin B-12 intake with circulating MMA concentrations. Log serum vitamin B-12, holoTC, log RBC folate, log total vitamin B-12 intake, and supplement use (yes) all had significant inverse associations, whereas plasma Hcy had a significant positive association, with log plasma MMA. Supplemental Figure 1 shows plots of the associations.

Table 5 shows findings of models examining effect modification by dietary pattern on the associations between circulating MMA and biomarkers. The association between log plasma MMA and log serum vitamin B-12 was significantly modified by dietary pattern ($P = 0.0157$ for interaction), with the vegan pattern showing the strongest negative association compared with the nonvegetarian pattern ($P = 0.0051$). There was no difference in slope between the lacto-ovo-vegetarian and nonvegetarian patterns. The association between log plasma MMA and plasma Hcy was significantly modified by dietary pattern ($P = 0.0019$ for interaction). The vegan dietary pattern had a stronger positive association between log plasma MMA and plasma Hcy than did the nonvegetarian pattern ($P = 0.0010$) and there was no difference in slope between the lacto-ovo-vegetarian and nonvegetarian patterns. Also, the association between dietary pattern and log RBC folate was modified by diet ($P = 0.0002$ for interaction), with the vegan pattern having a stronger negative association than the nonvegetarian pattern ($P < 0.0001$), with no difference in slope between the lacto-ovo-vegetarian and nonvegetarian patterns. Supplemental Figure 2 shows plots for these associations.

Discussion

This study presents the prevalence of high plasma MMA and related factors in adult and elderly individuals with no or relatively low-to-moderate intake of animal source foods residing in the United States and Canada. In this cross-sectional study of 785 non-Hispanic white and black participants randomly selected from Adventist churches, 10% were defined as vegan, 28% as lacto-ovo-vegetarian, and the rest as nonvegetarian, with a higher occurrence of vegans and lacto-ovo-vegetarians in whites and never-users of alcohol or tobacco, and a lower occurrence in those who used an oral diabetic medication. The prevalence of high plasma MMA ≥ 0.27 μmol/L and ≥ 0.37 μmol/L did not differ by dietary pattern and was comparable with rates in middle-aged and older individuals reported by NHANES (18). Also, the prevalence of vitamin B-12 deficiency assessed by any of the biomarkers did not differ by dietary pattern, in contrast to dietary vitamin B-12 intake derived from multiple 24-h recalls which showed a higher prevalence of intakes below the EAR (<2.0 μg/d) in the vegan (15.2%) and lacto-ovo-vegetarian dietary patterns (10.6%) than in the nonvegetarian (6.5%) dietary pattern.

Our findings support previous observations that elevated plasma MMA increases with age and is less common in those with black ethnicity (18, 26, 33). Because the sensitivity of the biomarkers MMA and Hcy lessens in older individuals owing to impaired renal function (26, 37), individuals with serum creatinine ≥ 130 μmol/L, a surrogate marker for kidney disease, were excluded from this study and regression analyses were adjusted for creatinine. Unlike some reports (38, 39) and possibly due to the small proportion of individuals reporting usage, high plasma MMA was not associated with use of either oral diabetic medications or acid reducers in our cohort. Consistent with previous findings, plasma MMA was negatively associated with serum vitamin B-12, dietary vitamin B-12 intake from food and supplements (26, 27, 33, 40), and holoTC (27, 40), and positively with plasma Hcy (27). The OR for high plasma MMA was significantly related to holoTC, Hcy, and RBC folate with the strongest OR of 9.87 shown for holoTC. This may be explained by the fact that serum holoTC is the biologically active form

TABLE 3 ORs of elevated circulating MMA $\geq$0.27 μmol/L (number of cases = 32) by specific concentrations of vitamin B-12 biomarkers, dietary patterns, and amounts of vitamin B-12 intake[1]

Variable	OR (95% CI)	*P* value
Biomarker		
Serum vitamin B-12, pmol/L		
<148	2.68 (0.83, 7.43)	0.0730
$\geq$148	1.00	
HoloTC, pmol/L		
<35	9.87 (3.22, 28.64)	<0.0001
$\geq$35	1.00	
Homocysteine, μmol/L		
<15	1.00	
$\geq$15	5.59 (2.47, 12.71)	<0.0001
RBC folate, nmol/g hemoglobin		
<6.6	1.00	
$\geq$6.6	0.42 (0.19, 0.91)	0.0314
Dietary pattern and vitamin B-12 intake (energy adjusted)		
Model 1[2,3]		
Dietary pattern		
Nonvegetarian, semivegetarian, and pesco-vegetarian	1.00	
Lacto-ovo-vegetarian	0.65 (0.23, 1.68)	0.3916
Vegan	2.49 (0.90, 6.53)	0.0678
Total vitamin B-12 intake (animal source + fortified food + supplements)[5]		
Tertile 1 (<4.42 μg/d)	1.00	
Tertile 2 (4.42–14.5 μg/d)	0.31 (0.10, 0.84)	0.0291
Tertile 3 (>14.5 μg/d)	0.59 (0.25, 1.34)	0.2107
Model 2[2,4]		
Vitamin B-12 supplement use		
No	1.00	
Yes	0.61 (0.29, 1.28)	0.1884
Vitamin B-12 intake from food (animal source + fortified food)[5]		
Tertile 1 (<2.66 μg/d)	1.00	
Tertile 2 (2.26–4.52 μg/d)	0.96 (0.42, 2.16)	0.9109
Tertile 3 (>4.52 μg/d)	0.35 (0.11, 0.96)	0.0545
Model 3[5,6]		
Vitamin B-12 supplement use		
No	1.00	
Yes	0.62 (0.29, 1.32)	0.2083
Vitamin B-12 intake (animal source food)[5]		
Tertile 1 (<0.29 μg/d)	1.00	
Tertile 2 (0.29–1.19 μg/d)	0.67 (0.18, 2.52)	0.5524
Tertile 3 (>1.19 μg/d)	0.30 (0.05, 1.74)	0.1844
Vitamin B-12 intake (fortified food)[5]		
Tertile 1 (<0.60 μg/d)	1.00	
Tertile 2 (0.60–2.03 μg/d)	1.18 (0.46, 3.08)	0.7240
Tertile 3 (>2.03 μg/d)	0.80 (0.30, 2.18)	0.6673
Vitamin B-12 (meat and poultry)[5]		
Never	1.00	
>0 μg/d	1.35 (0.49, 3.63)	0.8853
Vitamin B-12 intake (fish)		
None	1.00	
>0 μg/d	0.38 (0.08, 1.26)	0.1499
Vitamin B-12 intake (dairy and egg)[5]		
Tertile 1 (<0.10 μg/d)	1.00	
Tertile 2 (0.10–0.51 μg/d)	1.09 (0.33, 3.20)	0.8842
Tertile 3 (>0.51 μg/d)	1.61 (0.36, 7.38)	0.5367

[1]Separate logistic regression was conducted to model the odds of having elevated MMA (>0.27 μmol/L). For each variable (dichotomized concentration of serum vitamin B-12, holoTC, homocysteine, and RBC folate, vitamin B-12 intake, and dietary pattern), an OR was estimated. HoloTC, holotranscobalamin; MMA, methylmalonic acid.
[2]Models were adjusted for age (continuous), sex, race (white/black), smoking (never/ever), alcohol (never/ever), medication use (yes/no), and blood creatinine (continuous).
[3]Model 1: total vitamin B-12 intake from animal source food plus fortified food plus vitamin B-12 supplements was the only predictor variable.
[4]Model 2: vitamin B-12 intake divided into dietary supplement intake (yes/no) and dietary intake (animal source plus fortified food) quantified as tertiles was the predictor variable.
[5]Zero dietary vitamin B-12 intake was partitioned out of energy adjustment. Vitamin B-12 intake was log transformed before adjustment and energy-adjusted dietary log vitamin B-12 was back-transformed. Total vitamin B-12 intake is the sum of energy-adjusted dietary intake and unadjusted supplementary intake.
[6]Model 3: dietary vitamin B-12 intake further divided into intakes from animal source foods, fortified foods, meat, fish, and egg/dairy was fitted as the predictor variable.

TABLE 4 β-Coefficients and SEs from regression models on the associations of individual vitamin B-12 biomarkers, vitamin B-12 intake, and circulating MMA concentrations[1]

	β estimate	SE	P value
Model 1			
Log serum vitamin B-12, μmol/L	−0.1796	0.0258	<0.0001
HoloTC, pmol/L	−0.0019	0.0003	<0.0001
Hcy, μmol/L	0.0241	0.0038	<0.0001
Log RBC folate	−0.1397	0.0366	0.0015
Log total vitamin B-12 intake (animal source food + fortified food + supplements), μg/d	−0.0630	0.0153	<0.0001
Model 2			
Log total vitamin B-12 intake (animal source food + fortified food + supplements), μg/d	−0.0733	0.0282	0.0097
Supplement use: yes	−0.0626	0.0152	<0.0001

[1]Separate linear regression models were fitted with log of MMA (μmol/L) as the dependent variable and each variable as a predictor. Models were adjusted for age (continuous), sex, race (white/black), smoking (never/ever), alcohol (never/ever), medication use (yes/no), and blood creatinine (continuous). Hcy, homocysteine; HoloTC, holotranscobalamin; MMA, methylmalonic acid.

of the vitamin, directly involved in binding vitamin B-12 and facilitating its receptor-mediated transport into cells where it is converted to the coenzyme adenosyl-CoA for conversion of methylmalonyl-CoA to succinyl-CoA (4). However, unlike findings from the post–folic acid fortification NHANES population, the OR for high plasma MMA was not significantly higher in those with serum vitamin B-12 <148 pmol/L, or in those using supplements (33). This may be attributed to the inclusion of older adults and elderly in the current study, and it has been observed that the association between dietary vitamin B-12 intake and vitamin B-12 biomarkers weakens with age owing to increasing malabsorption disorders and other age-related influences contributing to reduced bioavailability of the vitamin, especially from animal products, in the elderly (15, 41). Also, the likelihood of high MMA was not related to dietary pattern. The lack of association with dietary pattern may be accounted for by the fact that the mean total daily vitamin B-12 intake derived from food, fortified food, and supplements did not differ by dietary pattern in this cohort.

In our previous study on the same cohort (35), we found plasma vitamin B-12 concentrations to be correlated to vitamin B-12 intake from supplements, fortified foods, and fortified milk alternatives. In the current study, only total daily vitamin B-12 intake from all sources in the second tertile (4.4–14.5 μg/d) reduced the likelihood of elevated MMA, a reduction of ~69%. That higher vitamin B-12 doses do not affect MMA may be explained by the reduced efficiency of vitamin B-12 absorption at high doses (5). On the other hand, although the current RDA for vitamin B-12 in adults and elderly is 2.4 μg/d (42), studies suggest higher amounts may be needed for functional adequacy. In a cross-sectional study of postmenopausal women, Bor et al. (43) found that a daily vitamin B-12 consumption of 6 μg is necessary to correct all vitamin B-12–related biomarkers including MMA; and in a Dutch elderly population van Wijngaarden et al. (27) estimated that saturation of biomarkers occurs with daily dietary intakes >5 μg vitamin B-12.

A possible explanation as to why intake from any single vitamin B-12–containing animal source food category was not predictive in reducing risk of elevated MMA may be the minimal intake of these food items in our cohort. In this analysis, the median daily dietary intake of vitamin B-12 from all animal products was 0.2 μg/d and 1.1 μg/d in the lacto-ovo-vegetarian and nonvegetarian patterns, respectively. These amounts represent low intakes of dairy products and eggs by lacto-ovo-vegetarians and of all animal source foods by nonvegetarians.

TABLE 5 Effect of dietary pattern on the associations between log plasma MMA and log serum vitamin B-12, plasma Hcy, and log RBC folate[1]

	Slope (SE)	P value	P-interaction
Model 1[2]			
Vegan	−0.429 (0.090)	<0.0001	0.0157
Lacto-ovo-vegetarian	−0.153 (0.046)	0.0019	
Nonvegetarian, semivegetarian, and pesco-vegetarian	−0.160 (0.032)	<0.0001	
Model 2[3]			
Vegan	0.071 (0.014)	<0.0001	0.0019
Lacto-ovo-vegetarian	0.018 (0.006)	0.0010	
Nonvegetarian, semivegetarian, and pesco-vegetarian	0.023 (0.005)	<0.0001	
Model 3[4]			
Vegan	−0.547 (0.110)	<0.0001	0.0002
Lacto-ovo-vegetarian	−0.196 (0.014)	0.0036	
Nonvegetarian, semivegetarian, and pesco-vegetarian	−0.063 (0.043)	0.0727	

[1]Models were adjusted for age (continuous), sex, race (white/black), smoking (never/ever), alcohol (never/ever), medication use (yes/no), and blood creatinine (continuous). MMA, methylmalonic acid.
[2]Model 1: effect of the interaction between dietary pattern, log plasma MMA (μmol/L), and log serum vitamin B-12 (pmol/L).
[3]Model 2: effect of the interaction between dietary pattern, log plasma MMA (μmol/L), and plasma Hcy (μmol/L).
[4]Model 3: effect of the interaction between dietary pattern, log plasma MMA (μmol/L), and log RBC folate (nmol/g hemoglobin).

This observation is substantiated by FFQ data obtained from the parent AHS-2 cohort (34) which estimated median consumption of dairy in lacto-ovo-vegetarians and of meat in nonvegetarians to be 120 g/d and 59 g/d, respectively, quantities considerably smaller than those consumed by the general population (44).

Our analysis of the association between total vitamin B-12 intake, biomarkers, and circulating MMA showed significant results but small effect sizes. For example, for every 10% increase in serum vitamin B-12, MMA decreased by 1.6%. The association between log total vitamin B-12 intake and log plasma MMA generated a β (-0.06) which is weaker than the β (-0.11) calculated by a meta-analysis of observational studies in the adult and elderly population (45). A doubling of vitamin B-12 intake decreases MMA in plasma by 4.3%, and a doubling of serum vitamin B-12 decreases MMA by 12%, and those who use vitamin B-12–containing supplements have an ~6% lower MMA than those who do not use supplements. As shown by Bailey et al. (46), the relation between serum vitamin B-12 and plasma MMA is complex, varies with age, and is influenced by extremes of the dietary and plasma vitamin B-12 distributions.

In the current study RBC folate concentrations above the median were associated with an ~58% reduced likelihood of elevated MMA. Some research suggests that high serum folic acid is related to more pronounced metabolic evidence of vitamin B-12 deficiency (36) and a worsening deficiency in those with low vitamin B-12 status (47). Our findings, based on the reduced form of folate in RBCs rather than on serum concentrations of folic acid, are consistent with NHANES data (18), which also reported a negative association between plasma MMA concentrations and RBC folate. It is likely that many who used vitamin B-12–containing supplements also consumed folic acid, which improved their status of both nutrients. The metabolism of vitamin B-12 and folate is closely intertwined in one-carbon metabolism, but the biological mechanism via which folate appears to reduce the risk of elevated MMA has not been fully explained.

In determining the impact of the various dietary patterns on the relations between dietary vitamin B-12 intake and biomarkers, we found significant effect modification by all 3 dietary patterns on the associations between log plasma MMA and log serum vitamin B-12, Hcy, and log RBC folate, except for RBC folate which was not modified by the nonvegetarian pattern. For all biomarkers, the magnitude of the interaction was strongest with the vegan dietary pattern. Plasma vitamin B-12, RBC folate, and plasma Hcy exhibited an almost 3-fold stronger association with plasma MMA in the vegan dietary pattern than in the other patterns. The biological mechanisms underlying these interactions are unclear. Vitamin B-12 and folate act together in Hcy metabolism and methylation reactions (3) but little is known about the impact of other nutrients and dietary components on this interaction. It has been suggested that nutrient-dense animal food products may provide richer amounts of B vitamins and factors which may have a role in vitamin B-12 function (48). More research is crucial to determining the interactive influence of the whole diet and dietary patterns on vitamin B-12 metabolism and its functional ramifications. It is also important to note that although the mean total daily vitamin B-12 intake did not differ by dietary pattern, the vegan pattern showed the highest prevalence of intakes <2.0 μg/d. This suggests that fortified foods and supplements are insufficiently or infrequently consumed by a number of individuals adhering to the vegan dietary pattern.

An expert panel concluded that the assessment of vitamin B-12 status should include measurement of ≥ 1 direct indicator such as serum vitamin B-12 and 1 functional marker, preferably plasma MMA (49). The strong association shown in this study between serum vitamin B-12 and the more costly plasma MMA, especially in vegans, confirms the importance of serum vitamin B-12 analysis and dietary recalls and histories, as strategies for assessing and monitoring vitamin B-12 status in vegans and other vulnerable groups with marginal vitamin B-12 intakes.

To the best of our knowledge, the present study is the first to report dietary and biomarker predictors of elevated MMA in individuals with a range of animal food consumption in the United States. The included number of vegans and lacto-ovo-vegetarians provides good estimates of factors related to high circulating MMA in individuals adhering to these dietary patterns. Although the baseline lifestyle questionnaire in this study did not inquire about the length of time participants had adhered to vegetarian diets, subsequent research has shown that the AHS-2 cohort demonstrates reliable and consistent long-term patterns of meat, fish, dairy, and egg intakes (50). Although dietary recalls have inherent limitations related to memory, portion estimation, and completeness, some of these concerns were minimized by the probing protocols and multiple-pass methodology utilized in our digital-based methods. To enhance the reliability of the recalls, dietary intake data were obtained from 2 sets of 3 nonconsecutive 24-h recalls conducted over a time period which closely coincided with that of acquiring the biospecimens. In addition, vitamin B-12 intakes from food, fortified foods, and supplements were corrected for within-person variations and validated accordingly (30).

The study also has limitations and its cross-sectional nature limits conclusions. The bioavailability of vitamin B-12 from various foods and supplements was not accounted for in this analysis. Although individuals with elevated serum creatinine were excluded from the study and the regression models were adjusted for serum creatinine, the creatinine cutoff used may underestimate impaired renal function and the more reliable biomarker cystatin C was not available (51). At the time biochemical tests were conducted, standard reference specimens were not available for serum vitamin B-12 and holoTC quantification so values are appropriate only for within-study comparisons. Also, rare influences on MMA metabolism such as antibodies to intrinsic factor, genetic abnormalities, and polymorphisms were not assessed (8, 52). In addition, the dietary groups in this health-aware cohort may not be representative of all vegetarians with respect to overall dietary intake and lifestyle practices.

Our results indicate that a number of adults and elderly do not achieve dietary vitamin B-12 adequacy. However, it does not inform on dose and frequency of either fortified food intake or supplement use. Owing to the lower bioavailability of food-bound vitamin B-12 in older individuals, the recommendation for adults over the age of 50 y is to meet the RDA for vitamin B-12 of 2.4 μg/d by consuming synthetic vitamin B-12 in vitamin B-12–fortified foods and vitamin B-12 supplements beyond that which is obtained from animal foods (42). The current emphasis on the benefits of eating more plant foods and reducing meat and dairy foods will invariably be accompanied by lower vitamin B-12 intakes among many. Vitamin B-12–fortified foods consist mostly of ready-to-eat cereals and meat and milk alternatives which may not be habitually consumed or preferred by all vegetarians or by those interested in plant-based diets. At present there are no consensus

guidelines for the amount and frequency of either fortified food intake or supplemental vitamin B-12 use for those adhering to vegan, lacto-ovo-vegetarian, or plant-based diets. Fortified foods may not provide adequate vitamin B-12 unless consumed in amounts $\geq$3 servings/d. Suggested daily doses of vitamin B-12 for improving status in those with marginal deficiency range from 50 μg (53) for vegetarians to 1000 μg (54) for the elderly. Given the growing interest in plant-based eating, future studies must seek to determine quantities of supplemental vitamin B-12 and other nutrients required by those who adhere to vegan diets and diets with limited animal source foods.

In conclusion, we have demonstrated a higher prevalence of inadequate vitamin B-12 intake in those adhering to vegan and lacto-ovo-vegetarian dietary patterns compared with nonvegetarian dietary patterns. The likelihood of elevated plasma MMA was reduced by 69% in those whose total dietary vitamin B-12 intake was in the 4.42–14.5 μg/d range. Also, the association between the biomarkers was modified by dietary pattern, with the vegan pattern showing an almost 3-fold stronger association between serum vitamin B-12 and Hcy and plasma MMA. Early detection of vitamin B-12 deficiency is important because the associated neurological symptoms may not be reversible. Our results indicate that serum vitamin B-12 and Hcy are important biomarkers of vitamin B-12 status, particularly in vegans. Inadequate vitamin B-12 intake and status continue to be common in adult and elderly individuals and in those who avoid animal source foods. As the popularity of plant-rich and vegetarian lifestyles increases, the importance of dietary and clinical monitoring of vitamin B-12 status and the encouragement of vitamin B-12 supplementation cannot be overemphasized.

Acknowledgments

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