Exploring the Relationship between Dietary Supplement Use and Serum Biomarkers Among Postmenopausal Women in a Controlled Feeding Study

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Abstract

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Background: Dietary supplement use is common among older adults; however, there remains limited knowledge on the contribution of dietary supplements to the blood profile of nutrient biomarkers. Prior studies have failed to examine these biomarkers of dietary supplement intake under controlled feeding conditions.

Objective: This study aimed to determine whether users of dietary supplements had higher serum concentrations of corresponding biomarkers, and whether those using multiple sources of the nutrient in dietary supplement form had higher serum concentrations compared to nonusers and those using only one source.

Methods: Postmenopausal women from the Women's Health Initiative were enrolled in a 2-week feeding study (n = 153). Participants consumed an individualized menu. Detailed information on dietary supplement use was also collected, and participants continued to consume

these dietary supplements during the feeding period. Serum vitamin B12, lutein + zeaxanthin, and phospholipid fatty acids (PLFAs) including eicosapentaenoic acid and docosahexaenoic acid were measured at the beginning and end of the 2-week feeding period. Linear regression of dietary supplement use, along with participant characteristics, on log-transformed serum biomarker was used to evaluate the association between dietary supplement use and corresponding serum biomarker. One way ANOVA was used to determine whether there were significant differences in mean serum biomarker among groups based on number of sources of a nutrient consumed via dietary supplement.

Results: Linear regression models without participant characteristics (n = 152) showed a positive association between use of vitamin B12 containing dietary supplements, lutein + zeaxanthin containing dietary supplements, and omega-3 fatty acids and respective serum biomarker ($p < 0.0001$, $p = 0.012$, and $p < 0.0001$, respectively). Once additional covariates were included, the positive association remained for vitamin B12 ($p < 0.001$) and omega-3 fatty acids ($p < 0.0001$). Users of two sources of a nutrient from a dietary supplement had higher serum biomarkers than users of only a multivitamin and users of neither ($p < 0.0001$).

Conclusions: These results add to the existing body of research on the use of serum vitamin B12 and serum PLFAs as biomarkers of dietary supplement use in postmenopausal women. The relationship between nutrient and biomarker differs for each nutrient studied, and the unique factors impacting these associations should be considered.

Introduction

Dietary supplement use is high among older adults with 70% reporting use of at least one dietary supplement.[1] Multivitamin and mineral supplements (MVIM), vitamin B12 supplements, carotenoid-based eye supplements, and omega-3 fatty acid supplements are all commonly used with 39%, 16%, 17%, and 22% of US adults reporting use of each supplement, respectively.[1–3] Typical motivation for dietary supplement use revolves around improving overall health.[1] Further, various health benefits have been purported in relation to vitamin B12, lutein + zeaxanthin, and omega-3 fatty acid supplementation including improved cognitive functioning, improved eye health, and reduced risk of coronary heart disease.[4–14] Despite these perceived health benefits and the high frequency of dietary supplement use, there remains limited robust knowledge on the health benefits of using single supplements or multiple supplements. Biological measurements allow for objective measurement of the association between nutrient intake and disease.

Prior studies considering the impact of supplementation of vitamin B12, lutein + zeaxanthin, and omega-3 fatty acids have found increases in serum concentrations in response to supplementation.[5,8–10,12,15–18] Additionally, a dose-dependent relationship has been reported, with increasing doses leading to increased blood concentrations for all nutrients mentioned.[5,8,9,17] This relationship is attenuated for vitamin B12 in older adults due to decreased bioavailability; however, serum vitamin B12 has still been shown to increase in the setting of vitamin B12 supplementation even among older adults.[5]

When investigating potential biomarkers, it is important to consider dietary intake in addition to supplementation intake. However, studies to date have relied on diet records and food frequency questionnaires (FFQ), which are prone to random and systematic bias.[19,20] Thus, there

is a need for evaluation of these biomarkers in the setting of dietary intake data free from the bias often plaguing traditional dietary self-assessment methods. Additionally, although prior studies have investigated serum concentrations in response to singular supplement intake, it is unknown how these serum biomarkers may respond when participants are taking multiple dietary supplements at once. Further, establishment of differences in biomarkers associated with supplement use will allow for more concrete understanding of the health benefits of these supplements in future studies.

We examined the association between use of vitamin B12 supplements, lutein + zeaxanthin containing supplements, and omega-3 fatty acid supplements and serum concentrations of vitamin B12, lutein + zeaxanthin, and phospholipid fatty acids (PLFAs) including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and DHA + EPA fatty acids, respectively. We hypothesized that users of these dietary supplements would have higher serum concentrations of the corresponding biomarker. Secondly, we hypothesized that users of multiple sources of dietary supplements containing vitamin B12 or lutein + zeaxanthin would have higher serum concentrations of the corresponding biomarker.

Methods

Study Design, Population, and Setting

We used data from the Nutrition and Physical Activity Assessment Study Feeding Study (NPAAS-FS), a 2-week feeding study conducted in the Seattle area among participants of the Women's Health Initiative (WHI). WHI is a long-term national health study funded by the National Heart, Lung, and Blood Institute. The original WHI study began in 1991 with clinical trials, and an observational study all aimed at investigating major causes of death and frailty

among postmenopausal women. The WHI continues to this day through extension and ancillary studies, such as NPAAS-FS, which was created for the purpose of discovery of novel and robust biomarkers of dietary intake among the postmenopausal women enrolled in WHI. Briefly, recruitment began in April 2011 and 450 women were approached who met the following inclusion and exclusion criteria: 1) were enrolled in the WHI Extension Study; 2) had been previously enrolled in the Observational Study cohort, the nonintervention group of the Dietary Modification Trial, or the Hormone Therapy Trials; 3) had an address within King county or the greater Seattle area; 4) had full follow-up status within the WHI; 5) were ≤ 80 years old as of April 2011; 6) were free of any medical conditions that would limit successful completion of the study including diabetes, kidney disease, bladder incontinence requiring the use of special garments or medications, or routine use of oxygen.[21] The NPAAS-FS protocol was approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center, and each participant gave written informed consent.

As part of the NPAAS-FS protocol, women completed a 4-day food record (4DFR) prior to beginning the feeding study.[21] Women were instructed to record all food and beverages (on alternate days) they consumed during the 4-day period.[21] Additionally, they were asked to record all dietary supplements, including MVIM, specialty supplements including B-complex or antioxidant mixtures, and single-ingredient supplements, taken during the 4-day recording period.[21] After this recording period, all participants had an in-depth interview with a study dietitian to confirm information from the 4DFR and discuss anything possibly missed within the 4DFR.[21] Unlike typical feeding studies, the NPAAS-FS attempted to preserve each participants habitual food intake by providing individual menu plans for each woman.[21] Individual menu plans were created for each participant based on their usual food intake as provided via the 4DFR

and the in depth interview with the dietitian.[21] Participants returned unconsumed food, and a consumed menu was then created, and data entered into NDSR (version 2010, University of Minnesota) for nutrient analysis. Additionally, each woman was instructed to continue taking the dietary supplements they reported taking during the 2-week feeding period.[21] The study concluded in October 2013.[21]

Dietary Supplements

Participants self-reported all current dietary supplement use in two ways. First, this information was collected via completion of an interviewer-led standardized form prior to the start of the 2-week feeding period.[22] They were instructed to bring all usual dietary supplements to the first clinic visit where staff transcribed type, frequency and duration of use of select dietary supplements of interest to the WHI onto a standardized form.[22] Second, dietary supplement use was collected via the 4DFR where participants self-reported all dietary supplement use and gave detailed information on frequency including pills per day. Whenever possible, the labels of each dietary supplement were viewed to confirm the 4DFR report. For this study, the detailed data from the 4DFR will be used. The NDSR dietary supplement module provided specific amounts of micronutrients (when available) and carotenoids in each dietary supplement. For dietary supplements lacking specific nutrient content information, the National Institutes of Health Office of Dietary Supplement's Dietary Supplement Label Database (DSLD) was used to gather reasonable information on the content of the supplement during the years surrounding the feeding study. Each dietary supplement was matched to a supplement within the database based on year of label (2011-2013), serving size, brand, and name, as possible. For any remaining dietary supplements unable to be found within the DSLD, reasonable judgement, including an internet search of the supplement name and ingredients, was implemented to determine whether

vitamin B12, lutein + zeaxanthin, or omega-3 fatty acids may be present in any amount in the supplement. Dietary supplements from the 4DFR were then grouped into constructed variables based on type and nutrient content. The number of dietary supplements used by each participant was constructed based on self-reported dietary supplement use.

Biomarker Measurements

Fasting blood samples were drawn at baseline and at the end of the 2-week feeding period and the second blood draw was used for this analysis. Assays were completed in the Fred Hutchinson Cancer Center Public Health Sciences Biomarker Laboratory. Serum vitamin B12 concentrations were determined by radioimmunoassay.[23] Mean interbatch coefficient of variation (CV) for laboratory quality control (QC) samples was 4.1%.[21] Serum lutein + zeaxanthin was measured via high performance liquid chromatography with detection at 476 nm, with a mean interbatch CV for laboratory QC samples of <6.0%.[21,24] Serum PLFAs were measured via gas chromatography with flame ionization detection.[25] The mean interbatch CV for laboratory QC samples was <10.0% for all PLFAs.[21]

Additional Data Collection

Additional covariates included in this analysis were either gathered at enrollment in the WHI, or at enrollment in the NPAAS-FS. Race, ethnicity, and education level were self-reported for each woman at the time of enrollment in WHI. Age, smoking status, and alcohol use were assessed at enrollment in NPAAS-FS. Smoking status was assessed based on whether a participant was a current smoker and if so, how many cigarettes they smoked per day. Alcohol use was assessed via questions around frequency and servings of beer, wine, and liquor consumption, which was used to compute a variable for alcohol servings per week. If this was greater than 0, then the participant was considered an alcohol user. Weight was measured by staff

at NPAAS-FS clinic visit number one, and together with WHI baseline height, body mass index (BMI) was computed. Finally, mean daily dietary nutrient intake of vitamin B12, lutein + zeaxanthin, EPA, DHA, and omega-3 fatty acids from the consumed menu was used to represent dietary intake of key nutrients for this analysis.

Statistical Analysis

All biomarker measurements and mean dietary nutrient intake information from the 2-week menus were skewed, and therefore they were log transformed prior to analysis. One participant had a value of 0 mg mean dietary EPA intake. To include this participant in the analysis, the smallest nonzero value for mean dietary EPA intake of 0.643 mg was divided by 2 and this value of 0.321 mg was imputed for the 0 mg mean EPA intake. Serum phospholipid DHA + EPA was calculated for each participant by taking the sum of mol/L serum DHA and mol/L serum EPA. Mean dietary DHA + EPA was calculated by taking the sum of mean dietary DHA and EPA. Missing data were checked for and participants with any missing variables were removed prior to analysis. Outliers of serum biomarkers, mean nutrient intake from consumed menus, and BMI were assessed for any possible errors in measurement. Several covariate levels were collapsed prior to analysis including education level, race and ethnicity, and total dietary supplements used. Education level of bachelor's degree, some post-graduate study, master's degree, and doctoral degree were combined, as well as vocational or training school and some college or associate degree. Race and ethnicity of American Indian or Alaska Native, Asian or Pacific Islander, Black or African American, and Hispanic/Latino were combined for analysis due to a sample size of <10. For analysis, total dietary supplement use was constructed into the following levels of dietary supplements used: 0, 1-2, 3-4, 5-6, 7-8, and >8.

First, the association between dietary supplement use and serum biomarker was assessed via linear regression analyses for each serum biomarker including serum vitamin B12, serum lutein + zeaxanthin, and serum phospholipid DHA, EPA, and DHA + EPA. Dietary supplement use was regressed on corresponding log-transformed serum biomarkers. Dietary supplement users were defined as those who used any source of vitamin B12 for models involving serum vitamin B12, users of any source of lutein + zeaxanthin for models involving serum lutein + zeaxanthin, and users of omega-3 fatty acids in the form of fish oil or flax oil for models involving serum phospholipid DHA, EPA, and DHA + EPA. Mean dietary nutrient intake, race and ethnicity, age, alcohol use, smoking status, education level, BMI, and number of supplements used may all impact participants serum biomarker concentrations and exposure status.[1,3,4,26–30] Due to this a priori determined impact, these additional covariates were then added to the models. Partial R^2 values, p-values, beta coefficients, and standard errors were used to characterize the strength of association for these nutrient-biomarker relationships, as well as to determine how well dietary supplement use explained the variance in serum biomarker concentrations.

Second, one-way ANOVA was used to assess the difference in mean serum biomarker concentrations among groups characterized by their usage of different dietary supplements. If the one-way ANOVA determined that there were statistically significant differences, post hoc analysis using a least significance difference was conducted to determine which groups had significantly different means. Mean serum vitamin B12 was compared among women who took single ingredient vitamin B12, a multivitamin (MVI) or MVIM containing vitamin B12, both, or neither. Mean serum lutein + zeaxanthin was compared among women who took eye supplements containing lutein + zeaxanthin, MVI or MVIM containing lutein + zeaxanthin, both,

or neither. Additionally, one-way ANOVA was conducted to determine if there were significant differences in mean dietary nutrient intake between users and nonusers of the dietary supplements of interest to rule out the known role of dietary intake on serum biomarker level. Serum biomarkers and mean dietary nutrient intake were log transformed for these one-way ANOVA analyses, but back-transformed values in their original units are presented for ease of interpretation.

Statistical analyses were conducted in RStudio (version 4.3.1). All tests were two-sided and α level = 0.05 was used as criteria for statistical significance.

Results

Baseline Demographic and Dietary Supplement Use Characteristics

A total of 153 postmenopausal women enrolled and completed the 2-week feeding study (**Figure 1**). Most women were white, well educated, and nonsmokers (**Table 1**). One participant had a missing value for education level, but all other data were complete. Dietary supplement use was high; only 14.4% of women reported use of no dietary supplements and 5.9% of women reported use of >8 dietary supplements (**Table 2**).

Figure 1. Flow diagram of NPAAS-FS study enrollment and inclusion in analysis.

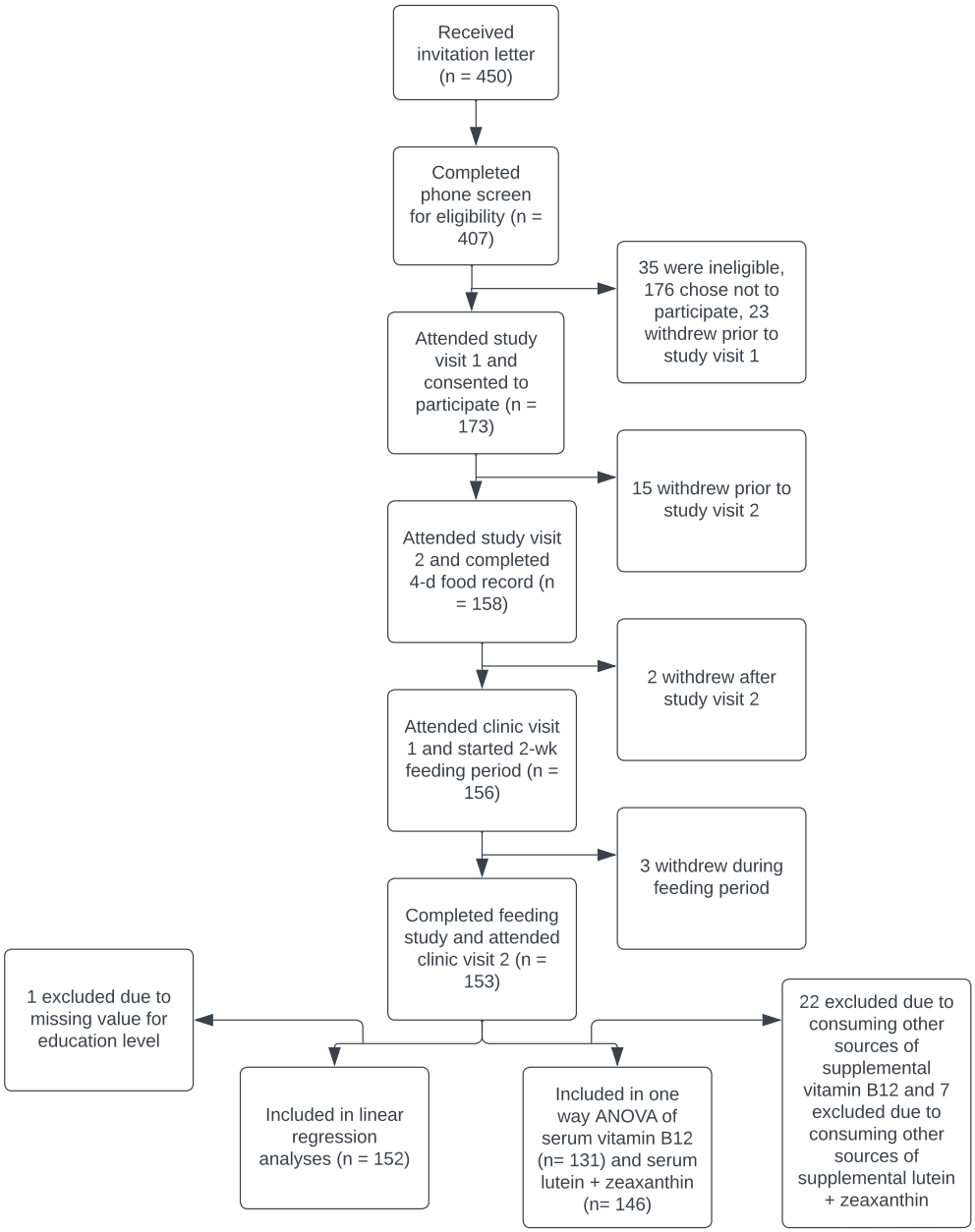


Table 1. Baseline demographic characteristics of the 153 postmenopausal women of the NPAAS-FS.[1]

Characteristics	N (%) or Mean ± SD
Age[2] (years)	
60-69	10 (6.5%)
70-79	127 (83%)
80-89	16 (10.5%)
Race and ethnicity[4]	
White, not Hispanic	144 (94.2%)
All others[5]	9 (5.8%)
Height[2] (cm)	161.6 ± 6.1[6]
Weight[2] (kg)	69.0 ± 11.7[6]
BMI[2] (kg/m^2)	
Normal (<25.0)	61 (39.9%)
Overweight (25.0-29.9)	60 (39.2%)
Obese (≥30.0)	32 (20.9%)
Education[3]	
High school/GED[7]	10 (6.5%)
Schooling after high school	16 (10.5%)
College degree or higher	126 (82.4%)
Missing	1 (0.6%)
Current smoking[2]	
Yes	3 (2.0%)
No	150 (98.0%)

[1] NPAAS-FS, Nutrition and Physical Activity Assessment Study Feeding Study.
[2] Measured at the time of enrollment in the NPAAS-FS.
[3] Collected at the time of enrollment in the Women's Health Initiative.
[4] Uses National Institutes of Health race and ethnicity categories, whereas, analyses use the originally collected race and ethnicity data upon which the NPAAS-FS was designed.
[5] "All others" included 2 participants identifying as having more than one race, 1 American Indian or Alaska Native, 1 Asian or Pacific Islander, 3 Black or African American, and 2 Hispanic participants.
[6] Mean ± SD.
[7] General Education Development.

Table 2. Baseline supplement use characteristics of the 153 postmenopausal women of the NPAAS-FS.[1]

Variable	N (%)
Number of supplements used[2]	
0	22 (14.4%)
1-2	36 (23.5%)
3-4	45 (29.4%)
5-6	28 (18.3%)
7-8	13 (8.5%)
>8	9 (5.9%)
Vitamin B12 use[2,3]	
Single ingredient B12	6 (6.2%)
MVIM or MVI containing B12	62 (63.9%)
Multiple sources of B12[4]	26 (26.8%)
Other supplements containing B12[5]	3 (3.1%)
Lutein + zeaxanthin use[2,9]	
Eye supplement	6 (8.5%)
MVIM or MVI containing lutein + zeaxanthin	53 (74.6%)
Multiple sources of lutein + zeaxanthin[10]	8 (11.3%)
Other source of lutein + zeaxanthin[11]	4 (5.6%)
Omega-3 fatty acid use[2,6,7]	
Fish oil	57 (90.5%)
Flax oil	2 (3.2%)
Multiple sources of omega-3 fatty acids[8]	4 (6.3%)

[1] NPAAS-FS, Nutrition and Physical Activity Assessment Study Feeding Study.
[2] Measured at enrollment in the NPAAS-FS based on self-reported dietary supplement use.
[3] % among users of vitamin B12 calculated based on 97 total users.
[4] Includes 21 women who consumed 2 sources of vitamin B12 and 5 women who consumed 3 sources of vitamin B12.
[5] "Other" sources of vitamin B12 includes 8 multi-ingredient botanicals, 1 glucosamine mixture, 15 b-complexes, 2 probiotics, 1 whole food based dietary supplement, and 1 multivitamin containing omega-3 supplement.
[6] % among users of omega-3 fatty acids calculated based on 63 total users.
[7] Possible additional sources of omega-3 fatty acids include 2 eye vitamins, 1 evening primrose oil supplement, 1 multi-ingredient botanical, and 1 multivitamin containing omega-3 fatty acids, however, due to inability to determine exact nutrient content of these supplements, only users of fish or flax oil are included.
[8] Includes 4 women who consumed 2 sources of omega-3 fatty acids.
[9] % among users of lutein + zeaxanthin calculated based on 71 total users.
[10] Includes 7 women who consumed 2 sources of lutein + zeaxanthin containing supplements and 1 woman who consumed 3 sources of lutein + zeaxanthin containing supplements.
[11] "Other" source of lutein + zeaxanthin includes 2 single sources of lutein + zeaxanthin, 2 eye supplements with botanical sources of lutein, 1 multivitamin with a botanical source of lutein, 2 single sources of lutein.

Dietary Nutrient and Serum Biomarker Characteristics

Mean dietary vitamin B12, DHA, EPA, DHA + EPA, and total omega-3 fatty acid intake did not differ significantly among users and non-users of corresponding dietary supplements (**Table 3, Supplementary Tables 1, 2, 3, 4, and 5**). However, mean dietary lutein + zeaxanthin intake did differ significantly among users and nonusers of supplemental lutein + zeaxanthin (**Supplementary Table 6**). Users of supplemental lutein + zeaxanthin had statistically significant higher mean dietary intake ($p = 0.01$). Serum biomarker concentrations among the 153 participants varied largely (**Table 4**). Mean serum biomarker concentrations were 475.1 ± 432.9 pmol/L for vitamin B12, 0.53 ± 0.34 μmol/L for lutein + zeaxanthin, 193.0 ± 56.3 μmol/L for phospholipid DHA, 58.2 ± 45.9 μmol/L for phospholipid EPA, and 264.5 ± 92.5 μmol/L for phospholipid DHA + EPA. There were no missing data on serum biomarker concentrations or mean dietary nutrient intake for any participants.

Table 3. Menu nutrient intake information for the 153 postmenopausal women of the NPAAS-FS.[1]

	Vitamin B12 food intake[3] (mcg/d)	Lutein + Zeaxanthin food intake[3] (mg/d)	DHA food intake[3] (mg/d)	EPA food intake[3] (mg/d)	DHA + EPA food intake[3] (mg/d)	Omega-3 food intake[3,5] (mg/d)
All (n= 153)[2]	5.6 ± 3.2	3.20 ± 3.22	172.7 ± 118.8	107.5 ± 83.0	280.2 ± 198.5	2431.5 ± 1375.1
Vitamin B12 use[2]						
Nonuser (n= 56)	5.4 ± 2.6	2.75 ± 1.88	178.0 ± 114.6	106.8 ± 75.6	284.9 ± 186.7	2135.0 ± 816.2
Single ingredient B12 (n= 17)	6.7 ± 4.5	2.40 ± 1.47	170.4 ± 158.1	114.3 ± 112.8	284.7 ± 270.0	2042.9 ± 733.1
MVIM or MVI containing B12 (n= 81)	5.8 ± 3.7	3.12 ± 2.20	181.2 ± 121.6	116.2 ±87.7	297.4 ± 205.6	2589.0 ± 1594.9
Any source (n= 97)[4]	5.7 ± 3.5	3.46 ± 3.77	169.6 ± 121.6	107.9 ± 87.4	277.5 ± 205.9	2602.7 ± 1591.0
Lutein + zeaxanthin use[2]						
Nonuser (n= 82)	5.4 ± 3.1	3.02 ± 3.86	175.1 ± 116.6	107.2 ± 79.2	282.3 ± 193.1	2339.4 ± 1194.5
Eye supplement (n=11)	4.6 ± 2.1	2.65 ± 1.32	128.1 ± 138.9	68.1 ± 64.6	196.2 ± 192.7	2651.0 ± 1542.6
MVIM or MVI containing lutein + zeaxanthin (n= 61)	5.8 ± 3.5	3.44 ± 2.39	180.7 ± 124.4	115.2 ± 89.2	295.9 ± 209.2	2556.0 ± 1583.7
Any source (n= 71)[4]	5.7 ± 3.3	3.41 ± 2.28	170.0 ± 122.1	107.9 ± 87.9	277.8 ± 205.9	2538.0 ± 1559.9
Omega-3 fatty acid use[2]						
Nonuser (n= 90)	5.4 ± 2.5	3.02 ± 2.80	158.1 ± 106.2	97.9 ± 73.3	256.0 ± 177.1	2287.0 ± 996.0
Fish oil (n= 61)	5.8 ± 4.0	3.50 ± 3.81	196.9 ± 133.7	123.6 ± 94.7	320.5 ± 223.8	2651.9 ± 1796.2
Flax oil (n = 6)	7.5 ± 3.7	2.02 ± 0.75	163.1 ± 122.3	106.6 ± 82.4	269.7 ± 203.6	2126.0 ± 825.8
Any source (n= 63)[4]	5.8 ± 4.0	3.46 ± 3.75	193.6 ± 132.8	121.3 ± 94.2	314.9 ± 222.5	2637.2 ± 1771.6

[1] NPAAS-FS, Nutrition and Physical Activity Assessment Study Feeding Study.
[2] Mean ± SD.
[3] Represent mean nutrient consumed from consumed menu provided over the two-week feeding study.
[4] Represent users of any source of the nutrient in dietary supplement form, including users of sources listed and other sources not explicitly listed.
[5] Omega-3 fatty acid food intake includes total mean consumption of eicosapentaenoic acid, docosahexaenoic acid, and alpha-linolenic acid from consumed menu.

Table 4. Serum biomarker data by dietary supplement use for the 153 postmenopausal women of the NPAAS-FS.[1]

	Serum B12[3] (pmol/L)	Serum lutein + zeaxanthin[3] (µmol/L)	Serum phospholipid DHA[3] (µmol/L)	Serum phospholipid EPA[3] (µmol/L)	Serum phospholipid DHA + EPA[3] (µmol/L)
All (n= 153)[2]	475.1 ± 432.9	0.53 ± 0.34	193.0 ± 56.3	58.2 ± 45.9	264.5 ± 92.5
Vitamin B12 use[2]					
Nonuser (n= 56)	296.6 ± 118.0	0.53 ± 0.33	189.5 ± 53.7	64.7 ± 49.3	254.1 ± 91.8
Single ingredient B12 (n= 17)	1085.4 ± 910.4	0.33 ± 0.10	190.3 ± 57.1	71.5 ± 31.7	261.8 ± 80.6
MVIM or MVI containing B12 (n= 81)	570.8 ± 528.8	0.53 ± 0.36	196.0 ± 56.5	75.4 ± 43.9	271.3 ± 90.5
Any source (n= 97)[4]	578.2 ± 509.3	0.53 ± 0.35	195.8 ± 57.9	74.6 ± 43.7	270.4 ± 92.8
Lutein + zeaxanthin use[2]					
Nonuser (n= 82)	398.9 ± 282.6	0.46 ± 0.24	193.7 ± 56.1	65.6 ± 37.6	259.2 ± 85.4
Eye supplement (n= 11)	425.3 ± 214.9	0.91 ± 0.45	181.6 ± 51.2	91.5 ± 85.3	273.0 ± 126.4
MVIM or MVI containing lutein + zeaxanthin (n= 61)	593.5 ± 582.4	0.55 ± 0.39	197.3 ± 57.4	78.5 ± 47.3	275.8 ± 95.8
Any source (n= 71)[4]	563.2 ± 547.5	0.61 ± 0.42	193.3 ± 56.9	77.2 ± 53.6	270.5 ± 100.3
Omega-3 fatty acid use[2]					
Nonuser (n= 90)	397.6 ± 286.3	0.50 ± 0.27	174.8 ± 50.0	49.7 ± 20.4	224.5 ± 63.6
Fish oil (n= 61)	591.2 ± 573.9	0.57 ± 0.43	222.2 ± 53.9	103.5 ± 54.1	325.7 ± 96.3
Flax oil (n= 6)	541.8 ± 139.3	0.40 ± 0.18	196.1 ± 62.8	94.3 ± 52.9	290.4 ± 108.8
Any source (n= 63)[4]	585.9 ± 566.1	0.57 ± 0.24	220.2 ± 54.4	101.3 ± 54.6	321.5 ± 97.6

[1] NPAAS-FS, Nutrition and Physical Activity Assessment Study Feeding Study.
[2] Mean ± SD.
[3] Represent fasting blood samples measured at the end of the 2-week study.
[4] Represent users of any source of the nutrient in dietary supplement form, including users of sources listed and other sources not explicitly listed.

Aim 1: Examining the Association Between Dietary Supplement Use and Serum Biomarker

Vitamin B12

Use of vitamin B12 supplements was positively associated with serum vitamin B12 ($p < 0.0001$). Users of vitamin B12 dietary supplements had geometric mean serum concentrations of vitamin B12 that were 74% higher than non-users (**Supplementary Table 7**). Despite any influence of the additional covariates (**Table 5**), use of vitamin B12 supplements remained positively associated with serum vitamin B12 and explained 10% of the variance of serum vitamin B12 ($p < 0.001$). Comparatively, mean dietary vitamin B12 intake explained only 4% of the variance of serum vitamin B12 ($p = 0.019$). Although being a user of >8 dietary supplements explained only 3% of the variance, it was positively associated with serum vitamin B12 ($p = 0.041$).

Table 5. Association between use of vitamin B12-containing dietary supplements and participant characteristics, and serum vitamin B12.[1]

Variable	β ± SE	P value	R^2
Intercept	2.363 ± 0.422	<0.0001	0.19
User of vitamin B12[2]	0.198 ± 0.050	<0.001	0.10
Mean vitamin B12 intake (log mcg/day)	0.202 ± 0.086	0.019	0.04
Race and ethnicity (white)[3]	0.160 ± 0.086	0.066	0.02
Age (years)	0.0005 ± 0.005	0.93	0.00006
Alcohol user[4]	-0.014 ± 0.042	0.73	0.0009
Smoker[5]	0.071 ± 0.130	0.59	0.002
Education (some schooling after high school)[6]	-0.110 ± 0.089	0.22	0.01
Education (college degree or higher)[6]	0.007 ± 0.074	0.92	0.00007
BMI (kg/m^2)	-0.005 ± 0.004	0.28	0.009
User of 1-2 supps[7]	0.037 ± 0.064	0.56	0.002
User of 3-4 supps[7]	0.016 ± 0.66	0.81	0.0004
User of 5-6 supps[7]	0.075 ± 0.079	0.35	0.006
User of 7-8 supps[7]	0.010 ± 0.092	0.29	0.008
User of >8 supps[7]	0.200 ± 0.097	0.041	0.03

[1] n = 152.
[2] Compared to nonusers of any type of vitamin B12 supplements (n = 55).
[3] Compared to all other races and ethnicities grouped into one variable due to sample sizes <10 including 1 American Indian or Alaskan Native, 1 Asian or Pacific Islander, 3 Black or African American, and 2 Hispanic participants (n= 7).
[4] Compared to non-users of alcohol (n= 41).
[5] Compared to non-smokers (n = 149).
[6] Compared to those with the highest education level of a high school diploma or GED (n = 10).
[7] Compared to those who do not use any dietary supplements (n = 22).
Model R^2 value = 0.33

Lutein + Zeaxanthin

Use of lutein + zeaxanthin containing dietary supplements was positively associated with serum lutein + zeaxanthin (**Table 6**; p = 0.012). Users of these supplements had geometric mean serum concentrations of lutein + zeaxanthin that were 24% higher than non-users. However, once all additional covariates were included (**Table 7**), being a user of supplemental lutein + zeaxanthin was no longer associated with serum lutein + zeaxanthin concentrations. Instead, dietary intake of lutein + zeaxanthin and BMI were stronger contributors (p < 0.0001). Dietary intake explained 21% of the variance of serum lutein + zeaxanthin and BMI explained 15% of the variance of serum lutein + zeaxanthin. Although only explaining 7% of the variance, individuals identifying as white were found to have lower serum lutein + zeaxanthin (p = 0.001).

Table 6. Association between use of lutein + zeaxanthin containing dietary supplements, and serum lutein + zeaxanthin.[1]

Variable	β ± SE	P value
Intercept	-0.633 ± 0.025	<0.0001
User of lutein + zeaxanthin[2]	0.093 ± 0.037	0.012

[1] n =152.
[2] Compared to nonusers of any type of lutein + zeaxanthin containing dietary supplements (n = 81).
Model R^2 value = 0.04

Table 7. Association between use of lutein + zeaxanthin containing dietary supplements and participant characteristics, and serum lutein + zeaxanthin.[1]

Variable	β ± SE	P value	R^2
Intercept	-0.407 ± 0.376	0.280	0.009
User of lutein + zeaxanthin[2]	0.013 ± 0.083	0.722	0.0009
Mean lutein + zeaxanthin intake (log mg/day)	0.358 ± 0.059	<0.0001	0.21
Race and ethnicity (white)[3]	-0.252 ± 0.076	0.001	0.07
Age (years)	0.005 ± 0.005	0.315	0.007
Alcohol user[4]	0.004 ± 0.037	0.920	0.00007
Smoker[5]	-0.129 ± 0.114	0.260	0.009
Education (some schooling after high school)[6]	0.038 ± 0.079	0.630	0.002
Education (college degree or higher)[6]	0.030 ± 0.065	0.642	0.002
BMI (kg/m^2)	-0.019 ± 0.004	<0.0001	0.15
User of 1-2 supps[7]	0.011 ± 0.054	0.844	0.0003
User of 3-4 supps[7]	0.013 ± 0.054	0.819	0.0004
User of 5-6 supps[7]	0.064 ± 0.060	0.287	0.008
User of 7-8 supps[7]	0.024 ± 0.072	0.740	0.0008
User of >8 supps[7]	0.137 ± 0.082	0.098	0.02

[1] n =152.
[2] Compared to nonusers of any type of lutein + zeaxanthin supplements (n = 81).
[3] Compared to all other races and ethnicities grouped into one variable due to sample sizes <10 including 1 American Indian or Alaskan Native, 1 Asian or Pacific Islander, 3 Black or African American, and 2 Hispanic participants (n= 7).
[4] Compared to non-users of alcohol (n= 41).
[5] Compared to non-smokers (n = 149).
[6] Compared to those with the highest education level of a high school diploma or GED (n = 10).
[7] Compared to those who do not use any dietary supplements (n = 22).
Model R^2 value = 0.38

Omega-3 Fatty Acids

In all models testing the association between use of omega-3 fatty acids and serum biomarkers, use of these supplements was positively associated with serum phospholipid DHA, serum phospholipid EPA, and serum phospholipid DHA+EPA (**Supplementary Tables 8, 9, and 10**); $p < 0.0001$).

After including all additional covariates, being a user of omega-3 fatty acid dietary supplements remained positively associated (**Tables 8-10; Supplementary Tables 11-17**; $p < 0.0001$). Users of omega-3 fatty acids had geometric mean serum concentrations of phospholipid DHA that were 24-31% higher than non-users, geometric mean serum concentrations of phospholipid EPA that were 84-96% higher than non-users, and geometric mean serum concentrations of serum phospholipid DHA+EPA that were 38-46% higher than non-users when all other covariates were held constant. When considering the role of mean dietary intake of DHA, EPA, DHA + EPA, and total omega-3 fatty acids, mean dietary intake of omega-3 fatty acids was not associated with serum phospholipid DHA, serum phospholipid EPA, or serum phospholipid DHA + EPA. For serum phospholipid DHA, mean dietary DHA and DHA + EPA explained 30% and 31% of the variance, respectively, compared to omega-3 fatty acid supplement use, which explained only 12% and 11% of the variance. Similarly, mean dietary EPA, DHA, and DHA + EPA explained 31%, 31%, and 32% of the variance of serum phospholipid DHA + EPA, respectively, compared to omega-3 fatty acid supplement use which explained 21%, 20%, and 21% of the variance. On the other hand, mean dietary EPA and DHA + EPA explained 21% and 20% of the variance of serum phospholipid EPA, respectively, compared to omega-3 fatty acid use, which explained 27% and 26% of the variance.

Table 8. Association between use of omega-3 fatty acids and participant characteristics, and serum phospholipid DHA.[1]

Variable	β ± SE	P value	R^2
Intercept	2.174 ± 0.357	<0.0001	0.21
User of omega-3 fatty acids[2]	0.117 ± 0.027	<0.0001	0.12
Mean total omega-3 fatty acid dietary intake (log mg/day)	-0.012 ± 0.064	0.85	0.0003
Race and ethnicity (white)[3]	0.008 ± 0.051	0.87	0.0002
Age (years)	0.0001 ± 0.003	0.97	0.000008
Alcohol user[4]	0.005 ± 0.025	0.85	0.0003
Smoker[5]	-0.047 ± 0.077	0.54	0.003
Education (some schooling after high school)[6]	0.132 ± 0.053	0.02	0.04
Education (college degree or higher)[6]	0.082 ± 0.044	0.06	0.03
BMI (kg/m^2)	-0.00006 ± 0.003	0.98	0.000004
User of 1-2 supps[7]	-0.011 ± 0.036	0.76	0.0007
User of 3-4 supps[7]	-0.016 ± 0.037	0.66	0.001
User of 5-6 supps[7]	0.008 ± 0.041	0.84	0.0003
User of 7-8 supps[7]	-0.057 ± 0.050	0.26	0.009
User of >8 supps[7]	-0.026 ± 0.056	0.64	0.001

[1] n =152.
[2] Compared to nonusers of any type of omega-3 fatty acid supplements (n = 90).
[3] Compared to all other races and ethnicities grouped into one variable due to sample sizes <10 including 1 American Indian or Alaskan Native, 1 Asian or Pacific Islander, 3 Black or African American, and 2 Hispanic participants (n= 7).
[4] Compared to non-users of alcohol (n= 41).
[5] Compared to non-smokers (n = 149).
[6] Compared to those with the highest education level of a high school diploma or GED (n = 10).
[7] Compared to those who do not use any dietary supplements (n = 22).
Model R^2 value = 0.19

Table 9. Association between use of omega-3 fatty acids and participant characteristics, and serum phospholipid EPA.[1]

Variable	β ± SE	P value	R^2
Intercept	0.731 ± 0.557	0.19	0.01
User of omega-3 fatty acids[2]	0.292 ± 0.041	<0.0001	0.27
Mean total omega-3 fatty acid dietary intake (log mg/day)	0.143 ± 0.100	0.16	0.01
Race and ethnicity (white)[3]	0.115 ± 0.079	0.15	0.02
Age (years)	0.003 ± 0.005	0.54	0.003
Alcohol user[4]	0.045 ± 0.039	0.25	0.010
Smoker[5]	-0.090 ± 0.120	0.46	0.004
Education (some schooling after high school)[6]	0.121 ± 0.083	0.15	0.02
Education (college degree or higher)[6]	0.076 ± 0.068	0.26	0.009
BMI (kg/m^2)	0.001 ± 0.004	0.75	0.0007
User of 1-2 supps[7]	-0.038 ± 0.056	0.50	0.003
User of 3-4 supps[7]	-0.033 ± 0.058	0.57	0.002
User of 5-6 supps[7]	-0.011 ± 0.064	0.87	0.0002
User of 7-8 supps[7]	-0.082 ± 0.078	0.29	0.008
User of >8 supps[7]	-0.023 ± 0.087	0.79	0.0005

[1] n =152.
[2] Compared to nonusers of any type of omega-3 fatty acid supplements (n = 90).
[3] Compared to all other races and ethnicities grouped into one variable due to sample sizes <10 including 1 American Indian or Alaskan Native, 1 Asian or Pacific Islander, 3 Black or African American, and 2 Hispanic participants (n= 7).
[4] Compared to non-users of alcohol (n= 41).
[5] Compared to non-smokers (n = 149).
[6] Compared to those with the highest education level of a high school diploma or GED (n = 10).
[7] Compared to those who do not use any dietary supplements (n = 22).
Model R^2 value = 0.39

Table 10. Association between use of omega-3 fatty acids and participant characteristics, and serum phospholipid DHA+EPA.[1]

Variable	β ± SE	P value	R^2
Intercept	2.080 ± 0.376	<0.0001	0.18
User of omega-3 fatty acids[2]	0.165 ± 0.028	<0.0001	0.20
Mean total omega-3 fatty acid dietary intake (log mg/day)	0.020 ± 0.067	0.77	0.0006
Race and ethnicity (white)[3]	0.030 ± 0.054	0.58	0.002
Age (years)	0.001 ± 0.003	0.76	0.0007
Alcohol user[4]	0.016 ± 0.027	0.55	0.003
Smoker[5]	-0.058 ± 0.081	0.47	0.004
Education (some schooling after high school)[6]	0.132 ± 0.056	0.02	0.04
Education (college degree or higher)[6]	0.080 ± 0.046	0.09	0.02
BMI (kg/m^2)	0.00008 ± 0.003	0.98	0.000006
User of 1-2 supps[7]	-0.018 ± 0.038	0.63	0.002
User of 3-4 supps[7]	-0.019 ± 0.039	0.62	0.002
User of 5-6 supps[7]	0.003 ± 0.043	0.94	0.00005
User of 7-8 supps[7]	-0.067 ± 0.053	0.21	0.01
User of >8 supps[7]	-0.023 ± 0.059	0.70	0.001

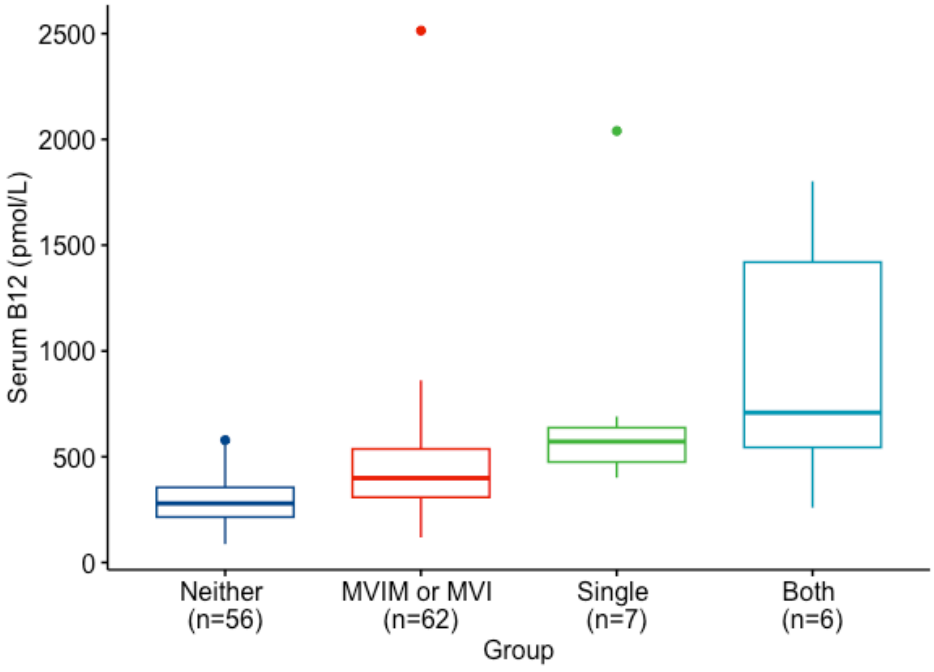
[1] n =152.
[2] Compared to nonusers of any type of omega-3 fatty acid supplements (n = 90).
[3] Compared to all other races and ethnicities grouped into one variable due to sample sizes <10 including 1 American Indian or Alaskan Native, 1 Asian or Pacific Islander, 3 Black or African American, and 2 Hispanic participants (n= 7).
[4] Compared to non-users of alcohol (n= 41).
[5] Compared to non-smokers (n = 149).
[6] Compared to those with the highest education level of a high school diploma or GED (n = 10).
[7] Compared to those who do not use any dietary supplements (n = 22).
Model R^2 value = 0.29

Aim 2: Assessing Number of Sources and Impact on Serum Biomarkers

Vitamin B12

Women consuming both a MVIM or MVI and single ingredient vitamin B12 had significantly different mean serum vitamin B12 concentrations compared to women only consuming a MVIM or MVI containing vitamin B12 and women consuming neither (1031.7 pmol/L vs 553.6 pmol/L vs 370.7 pmol/L; $p < 0.0001$) (**Figure 2**) (**Supplementary Table 18**). Women consuming only a single ingredient vitamin B12 did not have significantly different serum vitamin B12 compared to women consuming both sources (861.5 pmol/L).

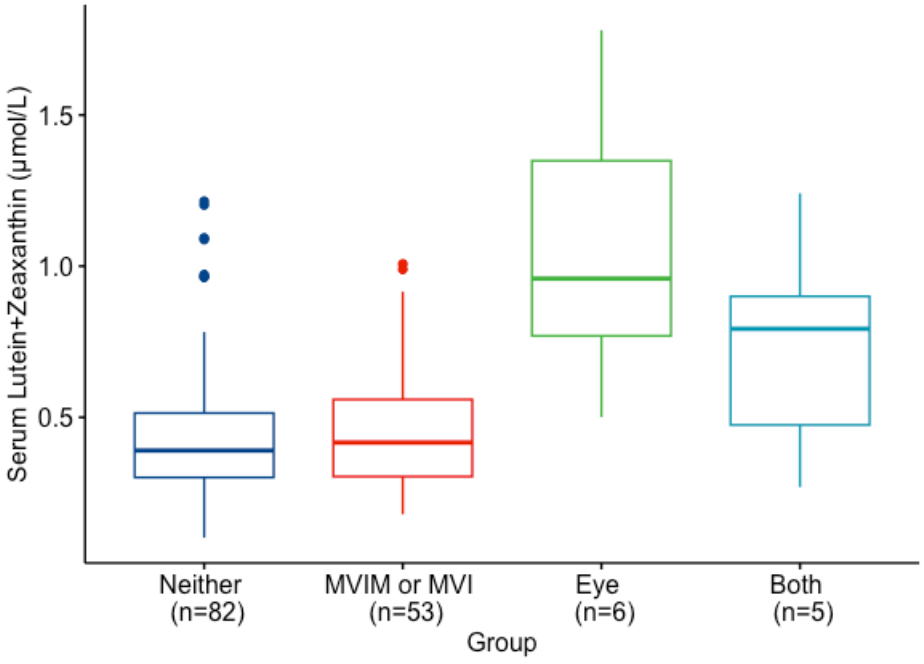
Figure 2. Boxplots of serum vitamin B12 concentration by type of supplementary vitamin B12 use.



Lutein + Zeaxanthin

Women consuming eye supplements and both eye supplements and a MVIM or MVI containing lutein + zeaxanthin did not have significantly different mean serum lutein + zeaxanthin (0.98 µmol/L vs 0.65 µmol/L) (**Figure 3**) (**Supplementary Table 19**). However, serum lutein + zeaxanthin concentrations among these two groups did differ significantly from women consuming only a MVIM or MVI containing lutein + zeaxanthin (0.42 µmol/L) and women consuming neither an eye supplement nor a MVIM or MVI containing lutein + zeaxanthin (0.41 µmol/L).

Figure 3. Boxplots of serum lutein + zeaxanthin concentration by type of supplementary lutein + zeaxanthin use.



Discussion

In this secondary analysis of a feeding trial conducted in postmenopausal women in Seattle, WA, we demonstrated that use of supplemental vitamin B12 and omega-3 fatty acids explains more of the variance of serum vitamin B12 concentrations and serum phospholipid EPA concentrations, respectively, compared to dietary intake of these nutrients or other demographic characteristics. Participants using at least one dietary supplement containing vitamin B12, lutein + zeaxanthin, or omega-3 fatty acids had higher serum concentrations of the corresponding serum biomarker compared to nonusers (see Table 6 and Supplementary Tables 7-10). Once additional covariates were added to the models, these associations remained for vitamin B12 and omega-3 fatty acids; however, lutein + zeaxanthin use was no longer significantly associated with serum lutein + zeaxanthin concentrations (see Tables 5, 7-10, and Supplementary Tables 11-17). Secondly, we found users of multiple sources of dietary supplements containing vitamin B12 or lutein + zeaxanthin had higher mean serum concentrations of the corresponding biomarker compared to nonusers or users of only a MVIM or MVI (See Figures 2 and 3). The differences observed in the association between each nutrient and biomarker suggest that it is important to examine unique factors impacting each nutrient separately.

Dose of vitamin B12 supplementation appeared to be more important in explaining serum vitamin B12 concentrations compared to number of vitamin B12 supplements used. In our study, users of both single ingredient vitamin B12 and a MVIM did not have statistically significant higher mean serum vitamin B12 compared to users of only a single ingredient vitamin B12. A systematic review and meta-analysis found the dose-response effect is reduced starting at intake of ~100 mcg/d of vitamin B12.[31] Single ingredient vitamin B12 supplements consumed by participants in this study contained a mean of 1260.2 ±1182.3 mcg of vitamin B12 (range of 500

to 5,000 mcg), whereas MVIM or MVI contained a mean of 30.0 ± 46.1 mcg of vitamin B12 (range of 4 to 350 mcg). So, both the reduction in dose-response at intakes above 100 mcg/d, and the fact that the difference in intake between these two groups is only ±2.4% may explain our inability to distinguish additional doses in the serum concentration. Interestingly, the highest dose of vitamin B12 intake studied was 1000 mcg/d, and the limited research into the response of serum concentrations at intakes above this reveals a significant gap in the literature considering the high doses of vitamin B12 regularly consumed.

In interpreting our carotenoid data, use of lutein + zeaxanthin containing dietary supplements was not significantly associated with serum concentrations; however, several covariates were. First, dietary lutein + zeaxanthin was positively associated with serum lutein + zeaxanthin. Participants using lutein + zeaxanthin containing dietary supplements had significantly higher dietary lutein + zeaxanthin intake making it difficult to parse out the role of diet on serum concentrations from the possible role of supplement use on serum concentrations. Further, lutein + zeaxanthin containing dietary supplements have been shown to have low bioavailability and absorption when consumed on their own, possibly limiting their impact on serum lutein + zeaxanthin concentrations.[32] Secondly, higher BMI was associated with lower serum lutein + zeaxanthin concentrations. Prior studies have found similar results indicating an inverse relationship between BMI and serum lutein + zeaxanthin.[33–35] There are several possible explanations for this observed relationship. One explanation is that there is competition between uptake of lutein + zeaxanthin by the adipose and the macular pigment leading to decreased serum concentrations.[33,34] Additionally, there is a positive association between BMI and oxidative stress. As carotenoids play a role in mitigating oxidative stress, it is anticipated that serum concentrations will be lower in those with higher BMI.[33,34] Thirdly, individuals identifying as

white were found to have lower serum lutein + zeaxanthin. Similar results were found in a study examining the association of various factors, including race and ethnicity, and serum lutein + zeaxanthin among a representative sample of US adults.[27] Investigators found that the mean dietary intake of lutein + zeaxanthin in non-Hispanic Black individuals was over twice that of non-Hispanic white individuals.[27] Even after adjusting for dietary intake of lutein + zeaxanthin, non-Hispanic white individuals still had lower serum lutein + zeaxanthin.[27] Therefore, dietary and environmental factors that may differ by race and ethnicity should be considered including common foods consumed, methods of preparation, and combinations of foods eaten together that may impact absorption of these carotenoids and therefore play a role in serum concentrations.

Source of omega-3 fatty acid supplement played a big role in determining serum biomarker concentration. Mean dietary omega-3 fatty acid intake (sum of dietary ALA + DHA+ EPA) was not associated with serum phospholipid DHA, EPA, or DHA + EPA, whereas for all other dietary nutrient intake covariates (e.g., B12, lutein + zeaxanthin, EPA, DHA, DHA+EPA), there was a positive association with the serum biomarker. This suggests that dietary ALA has very little impact on serum DHA or EPA.[36] A recent systematic review found that conversion of ALA to DHA from foods or dietary supplements has been inefficient.[36] Although conversion of ALA to EPA was more efficient, prior studies demonstrated a need to consume high doses of ALA (e.g., >2 g/d) to see significant increases in EPA. Further, many other factors such as high intake of omega-6 fatty acids may limit conversion of ALA to EPA.[36] Understanding the metabolism of these omega-3 fatty acids can give context to prior studies considering associations between serum fatty acid concentrations and health. Prior studies have demonstrated that lower serum concentrations of EPA and DHA are associated with reduced memory, and that increased serum concentration of EPA and DHA leads to reduced risk of cardiovascular disease events.[37–39] Most

omega-3 users in our study consumed fish oil (see Table 2), high in EPA and DHA, which may explain the positive association we observed. Other omega-3 fatty acid supplements containing ALA, such as flax oil, may have limited ability to impact serum concentrations of EPA and DHA, so there may be a need to better understand the utility of ALA supplements.

There are several strengths of this study. Dietary intake was assessed while participants were in a feeding study, reducing the bias associated with self-reported methods of collecting dietary intake data. Further, the feeding study design, which used individualized menus for each participant reflecting their usual intake, allowed for better determination of the association between intake and biomarker by limiting any changes in biomarker concentrations that may be slow to equilibrate over the 2-week period. Additionally, although dietary supplement intake was self-reported, detailed information on specific supplement use was collected via the 4DFR, and the labels of current dietary supplements were collected and reviewed.

Despite efforts to minimize limitations, several remain. First, the homogeneity of this study population limits conclusions for the general population. Second, although this was a feeding study and rigorous protocols were put in place, the women were still free living and may have taken in foods or supplements that were not accounted for. Specifically, no information on any possible dietary supplements taken via a non-oral route was collected (e.g., vitamin B12 injections). Labeling of nutrient content in dietary supplements can also be unreliable and inaccurate, and despite viewing labels of each woman's self-reported dietary supplements, there may be discrepancies between what was reported to be contained within each dietary supplement, and what each dietary supplement actually contained.[40] Additionally, despite efforts to identify all nutrients contained within each dietary supplement, some dietary supplement formulations could not be identified. Lastly, given the half-lives of the serum concentrations

investigated: 6 days for B12, 1.97 days for EPA and DHA, 5.6 days and 5 days for lutein and zeaxanthin, respectively,[41–44] serum concentrations at the end of the study period represent the known nutrient intake from food, and the self-reported dietary supplements taken during the study period, but intake of nutrients from before the study may also be partially represented in these serum biomarker concentrations. Nonetheless, the strength in the NPAAS-FS design in maintaining typical intake for each participant helped to minimize changes in the association between intake and biomarker.[21] Additionally, correlation of pre and post serum biomarker values for our biomarkers of interest were all >0.75, further supporting the feeding study design.[21]

In summary, our results indicate there is a positive association between use of vitamin B12 dietary supplements, and omega-3 fatty acid dietary supplements, and corresponding serum biomarker concentrations. However, it is important to consider total dose of vitamin B12 intake, and type of omega-3 fatty acid supplementation, fish oil vs flax oil, and the impact this may have on serum concentrations. In contrast, although a positive association was found in the model with lutein + zeaxanthin use alone, once additional covariates were added the association was no longer significant. As described, many factors involved in absorption and metabolism of lutein + zeaxanthin could play a role in this observation including BMI, race and ethnicity, and bioavailability of current dietary supplements. Future work should consider additional feeding studies examining these biomarkers of dietary supplement intake among more heterogenous populations to strengthen the generalizability of these results. Additionally, future work should focus on serum concentration response to vitamin B12 doses >1000 mcg and continue expanding the evidence base around how different omega-3 fatty acid sources impact serum concentration considering the wide range of dietary supplements on the market. Lastly, future research could

consider a predictive analysis among a larger sample size looking at the ability to predict who is a user of a specific dietary supplement and who is not based on their serum biomarker. These results add to the existing body of research on biomarkers of nutrient intake and strengthen the use of serum vitamin B12 and serum PLFAs as valid measures of dietary supplement intake when considering diet-disease relationships among postmenopausal women.

Supplementary Data

Table S1. One way ANOVA of mean dietary vitamin B12 by supplemental vitamin B12 use.

Table S2. One way ANOVA of mean dietary lutein + zeaxanthin by supplemental lutein + zeaxanthin use.

Table S3. One way ANOVA of mean dietary DHA by supplemental omega-3 fatty acid use.

Table S4. One way ANOVA of mean dietary EPA by supplemental omega-3 fatty acid use.

Table S5. One way ANOVA of mean dietary DHA + EPA by supplemental omega-3 fatty acid use.

Table S6. One way ANOVA of mean dietary omega-3 by supplemental omega-3 fatty acid use.

Table S7. Association between use of vitamin B12 containing dietary supplements, and serum vitamin B12.

Table S8. Association between use of omega-3 fatty acids, and serum phospholipid DHA.

Table S9. Association between use of omega-3 fatty acids, and serum phospholipid EPA.

Table S10. Association between use of omega-3 fatty acids, and serum phospholipid DHA+EPA.

Table S11. Association between use of omega-3 fatty acids and participant characteristics, and serum phospholipid DHA.

Table S12. Association between use of omega-3 fatty acids and participant characteristics, and serum phospholipid DHA.

Table S13. Association between use of omega-3 fatty acids and participant characteristics, and serum phospholipid EPA.

Table S14. Association between use of omega-3 fatty acids and participant characteristics, and serum phospholipid EPA.

Table S15. Association between use of omega-3 fatty acids and participant characteristics, and serum phospholipid DHA+EPA.

Table S16. Association between use of omega-3 fatty acids and participant characteristics, and serum phospholipid DHA+EPA.

Table S17. Association between use of omega-3 fatty acids and participant characteristics, and serum phospholipid DHA+EPA.

Table S18. One way ANOVA of serum vitamin B12 by type of supplementary vitamin B12 use.

Table S19. One way ANOVA of serum lutein + zeaxanthin by type of supplementary lutein + zeaxanthin use.

Table S1. One way ANOVA of mean dietary vitamin B12 by supplemental vitamin B12 use.[1]

Supplement Use	Mean Dietary Vitamin B12 – mcg/d (Mean ± SE)
Nonusers of supplemental vitamin B12 (n=56)	4.78 ± 0.14
Users of supplemental vitamin B12 (n=97)	4.98 ± 0.11
	P-value = 0.62

[1] n = 153.

Table S2. One way ANOVA of mean dietary lutein + zeaxanthin by supplemental lutein + zeaxanthin use.[1]

Supplement Use	Mean Dietary Lutein + Zeaxanthin – mg/d (Mean ± SE)
Nonusers of supplemental lutein + zeaxanthin (n=82)	2.20 ± 0.07
Users of supplemental lutein + zeaxanthin (n=71)	2.86 ± 0.10
	P-value = 0.01

[1] n = 153.

Table S3. One way ANOVA of mean dietary DHA by supplemental omega-3 fatty acid use.[1]

Supplement Use	Mean Dietary DHA – mg/d (Mean ± SE)
Nonusers of supplemental omega-3 fatty acids (n=90)	104.09 ± 5.03
Users of supplemental omega-3 fatty acids (n=63)	140.57 ± 8.13
	P-value = 0.09

[1] n = 153.

Table S4. One way ANOVA of mean dietary EPA by supplemental omega-3 fatty acid use.[1]

Supplement Use	Mean EPA – mg/d (Mean ± SE)
Nonusers of supplemental omega-3 fatty acids (n=90)	51.69 ± 3.48
Users of supplemental omega-3 fatty acids (n=63)	71.01 ± 5.71
	P-value = 0.19

[1] n = 153.

Table S5. One way ANOVA of mean dietary DHA + EPA by supplemental omega-3 fatty acid use.[1]

Supplement Use	Mean DHA + EPA – mg/d (Mean ± SE)
Nonusers of supplemental omega-3 fatty acids (n=90)	160.52 ± 8.45
Users of supplemental omega-3 fatty acids (n=63)	217.72 ± 13.70
	P-value = 0.11

[1] n = 153.

Table S6. One way ANOVA of mean dietary omega-3 by supplemental omega-3 fatty acid use.[1]

Supplement Use	Mean Omega-3 Fatty Acid– mg/d (Mean ± SE)
Nonusers of supplemental omega-3 fatty acids (n=90)	2136.18 ± 40.55
Users of supplemental omega-3 fatty acids (n=63)	2294.55 ± 52.07
	P-value = 0.30

[1] n = 153.

Table S7. Association between use of vitamin B12 containing dietary supplements, and serum vitamin B12.[1]

Variable	β ± SE	P value
Intercept	2.568 ± 0.030	<0.0001
User of vitamin B12[2]	0.241 ± 0.038	<0.0001

[1] n = 152.
[2] Compared to nonusers of any type of vitamin B12 supplements (n = 55).
Model R^2 value = 0.21

Table S8. Association between use of omega-3 fatty acids, and serum phospholipid DHA.[1]

Variable	β ± SE	P value
Intercept	2.224 ± 0.013	<0.0001
User of omega-3 fatty acids[2]	0.105 ± 0.021	<0.0001

[1] n =152.
[2] Compared to nonusers of any type of omega-3 fatty acids supplements (n = 90)
Model R^2 value = 0.14

Table S9. Association between use of omega-3 fatty acids, and serum phospholipid EPA.[1]

Variable	β ± SE	P value
Intercept	1.661 ± 0.021	<0.0001
User of omega-3 fatty acids[2]	0.288 ± 0.033	<0.0001

[1] n = 152.
[2] Compared to nonusers of any type of omega-3 fatty acids supplements (n = 90).
R^2 value = 0.34

Table S10. Association between use of omega-3 fatty acids, and serum phospholipid DHA+EPA.[1]

Variable	β ± SE	P value
Intercept	2.333 ± 0.014	<0.0001
User of omega-3 fatty acids[2]	0.154 ± 0.022	<0.0001

[1] n = 152.
[2] Compared to nonusers of any type of omega-3 fatty acids supplements (n = 90).
R^2 value = 0.25

Table S11. Association between use of omega-3 fatty acids and participant characteristics, and serum phospholipid DHA.[1]

Variable	β ± SE	P value	R^2
Intercept	1.830 ± 0.213	<0.0001	0.21
User of omega-3 fatty acids[2]	0.092 ± 0.022	<0.0001	0.11
Mean DHA dietary intake (log mg/day)	0.154 ± 0.020	<0.0001	0.30
Race and ethnicity (white)[3]	-0.047 ± 0.043	0.27	0.009
Age (years)	0.001 ± 0.003	0.69	0.001
Alcohol user[4]	0.009 ± 0.020	0.65	0.002
Smoker[5]	-0.067 ± 0.064	0.30	0.008
Education (some schooling after high school)[6]	0.059 ± 0.045	0.20	0.012
Education (college degree or higher)[6]	0.044 ± 0.037	0.23	0.011
BMI (kg/m^2)	0.0004 ± 0.002	0.86	0.0002
User of 1-2 supps[7]	-0.013 ± 0.030	0.66	0.001
User of 3-4 supps[7]	-0.020 ± 0.031	0.51	0.003
User of 5-6 supps[7]	0.012 ± 0.034	0.72	0.0009
User of 7-8 supps[7]	-0.028 ± 0.042	0.50	0.003
User of >8 supps[7]	-0.016 ± 0.046	0.72	0.0009

[1] n =152.
[2] Compared to nonusers of any type of omega-3 fatty acid supplements (n = 90).
[3] Compared to all other races and ethnicities grouped into one variable due to sample sizes <10 including 1 American Indian or Alaskan Native, 1 Asian or Pacific Islander, 3 Black or African American, and 2 Hispanic participants (n= 7).
[4] Compared to non-users of alcohol (n= 41).
[5] Compared to non-smokers (n = 149).
[6] Compared to those with the highest education level of a high school diploma or GED (n = 10).
[7] Compared to those who do not use any dietary supplements (n = 22).
Model R^2 value = 0.44

Table S12. Association between use of omega-3 fatty acids and participant characteristics, and serum phospholipid DHA.[1]

Variable	β ± SE	P value	R^2
Intercept	1.818 ± 0.212	<0.0001	0.35
User of omega-3 fatty acids[2]	0.094 ± 0.022	<0.0001	0.11
Mean DHA + EPA dietary intake (log mg/day)	0.143 ± 0.018	<0.0001	0.31
Race and ethnicity (white)[3]	-0.049 ± 0.043	0.26	0.0009
Age (years)	0.001 ± 0.003	0.57	0.001
Alcohol user[4]	0.010 ± 0.020	0.64	0.002
Smoker[5]	-0.063 ± 0.064	0.32	0.007
Education (some schooling after high school)[6]	0.059 ± 0.045	0.20	0.01
Education (college degree or higher)[6]	0.044 ± 0.037	0.23	0.01
BMI (kg/m^2)	0.0007 ± 0.002	0.75	0.0007
User of 1-2 supps[7]	-0.010 ± 0.029	0.73	0.0009
User of 3-4 supps[7]	-0.021 ± 0.031	0.50	0.003
User of 5-6 supps[7]	0.013 ± 0.033	0.70	0.001
User of 7-8 supps[7]	-0.026 ± 0.042	0.53	0.003
User of >8 supps[7]	-0.013 ± 0.046	0.78	0.0006

[1] n =152.
[2] Compared to nonusers of any type of omega-3 fatty acid supplements (n = 90).
[3] Compared to all other races and ethnicities grouped into one variable due to sample sizes <10 including 1 American Indian or Alaskan Native, 1 Asian or Pacific Islander, 3 Black or African American, and 2 Hispanic participants (n= 7).
[4] Compared to non-users of alcohol (n= 41).
[5] Compared to non-smokers (n = 149).
[6] Compared to those with the highest education level of a high school diploma or GED (n = 10).
[7] Compared to those who do not use any dietary supplements (n = 22).
Model R^2 value = 0.44

Table S13. Association between use of omega-3 fatty acids and participant characteristics, and serum phospholipid EPA.[1]

Variable	β ± SE	P value	R^2
Intercept	1.053 ± 0.354	0.004	0.061
User of omega-3 fatty acids[2]	0.266 ± 0.037	<0.0001	0.27
Mean EPA dietary intake (log mg/day)	0.144 ± 0.024	<0.0001	0.21
Race and ethnicity (white)[3]	0.047 ± 0.072	0.51	0.003
Age (years)	0.002 ± 0.004	0.61	0.002
Alcohol user[4]	0.039 ± 0.034	0.26	0.009
Smoker[5]	-0.090 ± 0.107	0.40	0.005
Education (some schooling after high school)[6]	0.055 ± 0.076	0.47	0.004
Education (college degree or higher)[6]	0.032 ± 0.062	0.60	0.002
BMI (kg/m^2)	0.004 ± 0.004	0.29	0.008
User of 1-2 supps[7]	-0.023 ± 0.050	0.65	0.001
User of 3-4 supps[7]	-0.031 ± 0.052	0.55	0.003
User of 5-6 supps[7]	0.020 ± 0.056	0.72	0.0009
User of 7-8 supps[7]	-0.028 ± 0.071	0.70	0.001
User of >8 supps[7]	0.034 ± 0.077	0.66	0.001

[1] n =152.
[2] Compared to nonusers of any type of omega-3 fatty acid supplements (n = 90).
[3] Compared to all other races and ethnicities grouped into one variable due to sample sizes <10 including 1 American Indian or Alaskan Native, 1 Asian or Pacific Islander, 3 Black or African American, and 2 Hispanic participants (n= 7).
[4] Compared to non-users of alcohol (n= 41).
[5] Compared to non-smokers (n = 149).
[6] Compared to those with the highest education level of a high school diploma or GED (n = 10).
[7] Compared to those who do not use any dietary supplements (n = 22).
Model R^2 value = 0.51

Table S14. Association between use of omega-3 fatty acids and participant characteristics, and serum phospholipid EPA.[1]

Variable	β ± SE	P value	R^2
Intercept	0.910 ± 0.359	0.01	0.04
User of omega-3 fatty acids[2]	0.264 ± 0.038	<0.0001	0.26
Mean DHA + EPA dietary intake (log mg/day)	0.180 ± 0.031	<0.0001	0.20
Race and ethnicity (white)[3]	0.041 ± 0.073	0.57	0.002
Age (years)	0.003 ± 0.004	0.54	0.003
Alcohol user[4]	0.036 ± 0.034	0.30	0.008
Smoker[5]	-0.098 ± 0.108	0.36	0.006
Education (some schooling after high school)[6]	0.045 ± 0.076	0.56	0.003
Education (college degree or higher)[6]	0.031 ± 0.062	0.61	0.002
BMI (kg/m^2)	0.003 ± 0.004	0.45	0.004
User of 1-2 supps[7]	-0.029 ± 0.050	0.56	0.002
User of 3-4 supps[7]	-0.033 ± 0.052	0.52	0.003
User of 5-6 supps[7]	0.013 ± 0.057	0.81	0.0004
User of 7-8 supps[7]	-0.039 ± 0.071	0.58	0.002
User of >8 supps[7]	0.020 ± 0.077	0.79	0.0005

[1] n =152.
[2] Compared to nonusers of any type of omega-3 fatty acid supplements (n = 90).
[3] Compared to all other races and ethnicities grouped into one variable due to sample sizes <10 including 1 American Indian or Alaskan Native, 1 Asian or Pacific Islander, 3 Black or African American, and 2 Hispanic participants (n= 7).
[4] Compared to non-users of alcohol (n= 41).
[5] Compared to non-smokers (n = 149).
[6] Compared to those with the highest education level of a high school diploma or GED (n = 10).
[7] Compared to those who do not use any dietary supplements (n = 22).
Model R^2 value = 0.50

Table S15. Association between use of omega-3 fatty acids and participant characteristics, and serum phospholipid DHA+EPA.[1]

Variable	β ± SE	P value	R^2
Intercept	1.958 ± 0.221	<0.0001	0.36
User of omega-3 fatty acids[2]	0.143 ± 0.023	<0.0001	0.21
Mean EPA dietary intake (log mg/day)	0.119 ± 0.015	<0.0001	0.31
Race and ethnicity (white)[3]	-0.025 ± 0.045	0.58	0.002
Age (years)	0.001 ± 0.003	0.64	0.002
Alcohol user[4]	0.020 ± 0.021	0.35	0.006
Smoker[5]	-0.066 ± 0.067	0.32	0.007
Education (some schooling after high school)[6]	0.067 ± 0.047	0.16	0.015
Education (college degree or higher)[6]	0.041 ± 0.038	0.29	0.008
BMI (kg/m^2)	0.002 ± 0.002	0.41	0.005
User of 1-2 supps[7]	-0.011 ± 0.031	0.74	0.0008
User of 3-4 supps[7]	-0.021 ± 0.032	0.51	0.003
User of 5-6 supps[7]	0.018 ± 0.035	0.61	0.002
User of 7-8 supps[7]	-0.025 ± 0.044	0.57	0.002
User of >8 supps[7]	0.008 ± 0.048	0.88	0.0002

[1] n =152.
[2] Compared to nonusers of any type of omega-3 fatty acid supplements (n = 90).
[3] Compared to all other races and ethnicities grouped into one variable due to sample sizes <10 including 1 American Indian or Alaskan Native, 1 Asian or Pacific Islander, 3 Black or African American, and 2 Hispanic participants (n= 7).
[4] Compared to non-users of alcohol (n= 41).
[5] Compared to non-smokers (n = 149).
[6] Compared to those with the highest education level of a high school diploma or GED (n = 10).
[7] Compared to those who do not use any dietary supplements (n = 22).
Model R^2 value = 0.52

Table S16. Association between use of omega-3 fatty acids and participant characteristics, and serum phospholipid DHA+EPA.[1]

Variable	β ± SE	P value	R^2
Intercept	1.833 ± 0.222	<0.0001	0.33
User of omega-3 fatty acids[2]	0.141 ± 0.023	<0.0001	0.21
Mean DHA + EPA dietary intake (log mg/day)	0.152 ± 0.019	<0.0001	0.32
Race and ethnicity (white)[3]	-0.032 ± 0.045	0.49	0.004
Age (years)	0.002 ± 0.003	0.54	0.003
Alcohol user[4]	0.018 ± 0.021	0.40	0.005
Smoker[5]	-0.073 ± 0.067	0.28	0.009
Education (some schooling after high school)[6]	0.057 ± 0.047	0.23	0.01
Education (college degree or higher)[6]	0.040 ± 0.038	0.30	0.008
BMI (kg/m^2)	0.001 ± 0.002	0.66	0.001
User of 1-2 supps[7]	-0.016 ± 0.031	0.61	0.002
User of 3-4 supps[7]	-0.023 ± 0.032	0.47	0.004
User of 5-6 supps[7]	0.013 ± 0.035	0.72	0.0009
User of 7-8 supps[7]	-0.034 ± 0.044	0.44	0.004
User of >8 supps[7]	-0.003 ± 0.048	0.95	0.00003

[1] n =152.
[2] Compared to nonusers of any type of omega-3 fatty acid supplements (n = 90).
[3] Compared to all other races and ethnicities grouped into one variable due to sample sizes <10 including 1 American Indian or Alaskan Native, 1 Asian or Pacific Islander, 3 Black or African American, and 2 Hispanic participants (n= 7).
[4] Compared to non-users of alcohol (n= 41).
[5] Compared to non-smokers (n = 149).
[6] Compared to those with the highest education level of a high school diploma or GED (n = 10).
[7] Compared to those who do not use any dietary supplements (n = 22).
Model R^2 value = 0.52

Table S17. Association between use of omega-3 fatty acids and participant characteristics, and serum phospholipid DHA+EPA.[1]

Variable	β ± SE	P value	R^2
Intercept	1.846 ± 0.224	<0.0001	0.33
User of omega-3 fatty acids[2]	0.139 ± 0.023	<0.0001	0.20
Mean DHA dietary intake (log mg/day)	0.163 ± 0.021	<0.0001	0.31
Race and ethnicity (white)[3]	-0.030 ± 0.045	0.51	0.003
Age (years)	0.002 ± 0.003	0.54	0.003
Alcohol user[4]	0.018 ± 0.021	0.41	0.005
Smoker[5]	-0.076 ± 0.067	0.26	0.009
Education (some schooling after high school)[6]	0.058 ± 0.048	0.23	0.01
Education (college degree or higher)[6]	0.041 ± 0.039	0.30	0.008
BMI (kg/m^2)	0.0007 ± 0.002	0.77	0.0006
User of 1-2 supps[7]	-0.019 ± 0.031	0.55	0.003
User of 3-4 supps[7]	-0.023 ± 0.032	0.48	0.004
User of 5-6 supps[7]	0.011 ± 0.035	0.75	0.0007
User of 7-8 supps[7]	-0.036 ± 0.044	0.42	0.005
User of >8 supps[7]	-0.007 ± 0.048	0.89	0.0002

[1] n =152.
[2] Compared to nonusers of any type of omega-3 fatty acid supplements (n = 90).
[3] Compared to all other races and ethnicities grouped into one variable due to sample sizes <10 including 1 American Indian or Alaskan Native, 1 Asian or Pacific Islander, 3 Black or African American, and 2 Hispanic participants (n= 7).
[4] Compared to non-users of alcohol (n= 41).
[5] Compared to non-smokers (n = 149).
[6] Compared to those with the highest education level of a high school diploma or GED (n = 10).
[7] Compared to those who do not use any dietary supplements (n = 22).
Model R^2 value = 0.51

Table S18. One way ANOVA of serum vitamin B12 by type of supplementary vitamin B12 use.[1]

Supplement Use	Serum B12 – pmol/L (Mean ± SE)
Nonusers of MVIM or MVI + B12 (n=56)	370.7 ± 10.0
Users of MVIM or MVI containing B12 (n=62)	553.6 ± 14.2
Users of single ingredient B12 (n=7)	861.5 ± 65.8
Users of MVIM or MVI + single ingredient B12 (n=6)	1031.7 ± 85.1
	P-value = <0.0001

[1]N = 131. Includes only women who consumed single ingredient vitamin B12, a multivitamin w/ or w/o minerals containing vitamin B12, or neither. Women consuming other sources of vitamin B12 including multi-ingredient botanicals, glucosamine mixture, b-complexes, probiotics, whole food based dietary supplement, or multivitamins containing omega-3 fatty acids are excluded.

Table S19. One way ANOVA of serum lutein + zeaxanthin by type of supplementary lutein + zeaxanthin use.[1]

Supplement Use	Serum Lutein + Zeaxanthin – µmol/L (Mean ± SE)
Nonusers of MVIM or MVI containing lutein + zeaxanthin + eye supplements (n=82)	0.41 ± 0.005
Users of MVIM or MVI containing lutein + zeaxanthin (n=53)	0.42 ± 0.006
Users of MVIM or MVI containing lutein + zeaxanthin + eye supplements (n=5)	0.65 ± 0.033
Users of eye supplements (n=6)	0.98 ± 0.045
	P-value = <0.0001

[1]N = 146. Includes only women who consumed a multivitamin w/ or w/o minerals that contained lutein + zeaxanthin, an eye supplement containing lutein + zeaxanthin or neither. Women consuming other sources of lutein + zeaxanthin including single sources of lutein and zeaxanthin, eye supplements with botanical sources of lutein, multivitamin with a botanical source of lutein, and single sources of lutein were excluded.

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