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Effects of One Year of Vitamin D and Marine Omega-3 Fatty Acid Supplementation on Biomarkers of Systemic Inflammation in Older US Adults

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BACKGROUND: Observational studies suggest vitamin D and marine ω-3 fatty acid (n-3 FA) supplements are associated with lower systemic inflammation. However, past trials have been inconsistent.

METHODS: The randomized, double-blind, placebo-controlled VITamin D and OmegA-3 TriaL (VITAL) tested vitamin D (2000 IU/day) and/or n-3 FA (1 g/day) supplementation in a 2 × 2 factorial design among women ≥55 and men ≥50 years of age. We assessed changes in interleukin (IL)-6, tumor necrosis factor receptor 2 (TNFR2), and high-sensitivity C-reactive protein (hsCRP) concentrations from baseline to 1 year among participants randomized to vitamin D + n-3 FA (392), vitamin D (392), n-3 FA (392), or placebo only (385). Geometric means and percent changes were compared, adjusting for baseline factors.

RESULTS: Baseline characteristics were well balanced. In the active arms, 25-OH vitamin D rose 39% and n-3 FA rose 55% vs minimal change in placebo arms. Neither supplement reduced biomarkers at 1 year. Vitamin D resulted in 8.2% higher IL-6 (95% CI, 1.5%–15.3%; adjusted $P = 0.02$), but TNFR2 and hsCRP did not. Among 784 receiving vitamin D, hsCRP increased 35.7% (7.8%–70.9%) in those with low (<20 ng/mL) but not with higher baseline serum 25(OH) vitamin D [0.45% (−8.9% to 10.8%); P interaction = 0.02]. Among 777 randomized to n-3 FA, hsCRP declined [−10.5% (−20.4% to 0.8%)] in those with baseline low (<1.5 servings/week), but not with higher fish intake [6.4% (95% CI, −7.11% to 21.8%); P interaction = 0.06].

CONCLUSIONS: In this large sample from a population-based randomized controlled trial, neither vitamin D nor n-3 FA supplementation over 1 year decreased these biomarkers of inflammation.

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Vitamin D and marine ω-3 fatty acids (n-3 FA)[6] are widely consumed supplements advertised to prevent disease and reduce systemic inflammation. Their purported health benefits have received enormous attention in the medical and popular presses. Observational evidence of antiinflammatory and immunomodulatory associations is substantial, although measurable effects on systemic inflammation in the general adult population have not been well established *(1–10)*.

Active 1,25-dihydroxy (OH) vitamin D binds to its receptors, abundant on T and B cells, macrophages, and dendritic cells, to regulate inflammatory genes. In vitro 1,25OH vitamin D inhibits monocyte differentiation into dendritic cells and blocks T-cell stimulatory effects *(11)*. It promotes monocyte differentiation into macrophages, prevents release of inflammatory cytokines, and decreases antigen presentation. It also has been shown to downregulate interleukin (IL)-2, a T-cell growth factor, and suppresses T-cell cytokines IL-12, tumor necrosis factor-α (TNFα), and IL-6 *(11–14)*. Cross-sectional and observational studies have demonstrated lower 25-OH vitamin D concentrations among individuals with many inflammatory diseases *(15–18)*. However, some small randomized trials of supplemental vitamin D in selected

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[6] Nonstandard abbreviations: n-3 FA, ω-3 fatty acid; IL, interleukin; TNFα, tumor necrosis factor-α; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; VITAL, VITamin D and OmegA-3 TriaL; BMI, body mass index; hsCRP, high-sensitivity C-reactive protein; TNFR2, tumor necrosis factor receptor 2; SPM, specialized proresolving mediator of inflammation.

populations have shown antiinflammatory effects on biomarkers, whereas others have not *(1–6)*.

N-3 FAs also hold promise for reducing systemic inflammation. Metabolites eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) exert effects through leukotriene and prostaglandin pathways, decreasing inflammatory mediators and cytokine production. Blocking nuclear translocation of nuclear factor κB decreases gene transcription for inflammatory cytokines, adhesion molecules, and cyclooxygenase enzymes *(19–21)*. In observational studies, high fish and n-3 FA intakes have been inversely associated with risks of rheumatoid arthritis and Crohn disease *(22, 23)*. Recent small trials reported that n-3 FA supplementation of various doses and durations reduced these biomarkers in patients with heart failure, Alzheimer disease, and end-stage renal disease *(7–10, 24)*.

The VITamin D and OmegA-3 TriaL (VITAL) is a recently completed NIH-funded randomized, double-blind, placebo-controlled clinical trial of these 2 supplements for the primary prevention of cancer and cardiovascular disease among 25 871 Americans (women age ≥55 years; men age ≥50 years). Main results were published: Neither supplement was associated with a statistically significant reduction in incidence of invasive cancers nor composite cardiovascular disease events over randomized 5 years of follow-up *(25, 26)*. However, there were a few signals of potential benefit in preplanned subgroup analyses: Participants with normal body mass index (BMI < 25 mg/kg^2) randomized to active vs placebo vitamin D showed a significant reduction in invasive cancers, and African Americans had a borderline significant reduction. Cardiovascular disease events were significantly lower for active vs placebo n-3 FA among individuals who consumed <1.5 fish servings/week. Additionally, n-3 FA supplementation reduced risk of myocardial infarction, a prespecified secondary end point, with particularly strong effect among African Americans *(25)*.

IL-6, TNFα, and high-sensitivity C-reactive protein (hsCRP) are common biomarkers of systemic inflammation. IL-6 is produced by subcutaneous adipose tissue, as well as lymphocytes, monocytes, fibroblasts, and endothelial cells, and is a strong predictor of cardiovascular disease and type 2 diabetes *(27–29)*. Circulating IL-6 increases with age, BMI, and percentage body fat *(30, 31)*. TNFα is a critical proinflammatory cytokine in the pathogenesis of multiple inflammatory diseases. TNF receptor 2 (TNFR2) expression parallels TNFα and is a surrogate marker. CRP, an acute phase reactant produced in response to IL-6, is a sensitive but nonspecific biomarker of inflammation. CRP elevation within the normal range, detected by the hsCRP, reflects low-grade systemic inflammation and is an independent predictor of cardiovascular disease *(27)*. Increases in these biomarkers are associated with diseases of aging and obesity, including cardiovascular disease, heart failure, osteoporosis, diabetes mellitus, some cancers, and neurodegenerative diseases such as Alzheimer disease *(32, 33)*.

We investigated effects of vitamin D_3 and n-3 FA supplementation for 1 year upon biomarkers of systemic inflammation within a sample of VITAL participants. We hypothesized that one or both supplements would significantly reduce IL-6, TNFR2, and/or hsCRP. We also sought to examine prespecified effects, by age, sex, race, BMI, smoking, baseline fish intake, baseline biomarker concentration, baseline 25-OH vitamin D concentration (or ω-3 index), and the other randomized agent. Additionally, we conducted exploratory analyses incorporating the potential effect of statin use on hsCRP over time.

Materials and Methods

STUDY POPULATION

VITAL is a randomized, double-blind, placebo-controlled trial, designed to test effects of vitamin D_3 [2000 IU (50 μg)/day] and marine n-3 FAs (Omacor® 1 g/day; 460 mg of EPA and 380 mg of DHA) upon incidence of cancer and cardiovascular disease *(25, 26)*. The trial consented, enrolled, and followed 25 871 randomized participants over a median of 5.3 years *(34)*. Briefly, nationwide recruitment of men age ≥50 years and women age ≥55 years began in March 2011. More than 20% African American enrollment was obtained. VITAL used a placebo run-in, and participants were randomized within 5-year age-groups if they (*a*) demonstrated high adherence; (*b*) were willing to continue; (*c*) reported no new ineligibility; and (*d*) were willing to limit supplemental vitamin D intake to ≤800 IU/day and to forego n-3 FA supplements. In this 2 × 2 factorial trial, 25% of participants were randomized to each arm *(34)*. The n-3 FA supplement did not contain vitamin D. Excellent results were obtained in nutrient stability tests at a range of temperatures and humidity at 3 to 24 months.

The original aim of this ancillary study was to test biomarker concentrations in approximately 2000 participants across the 4 trial arms at baseline and follow-up. We identified 1561 trial participants with sufficient blood sample for biomarker assays at baseline and 1-year follow-up, balanced by sex, and matched on blood draw season. VITAL and the biomarkers substudy were registered at clinicaltrials.gov (NCT01169259; NCT01351805). The Partners' Human Research Committee approved these studies. Before randomization, participants completed a questionnaire assessing demographic, clinical, and lifestyle factors, including age, sex, race, years of education, household income, height and weight, physical activity, smoking, alcohol intake, and medical history, including cholesterol-lowering medications *(2)*. They

completed a modified food frequency questionnaire assessing vitamin D and n-3 FA-containing foods and supplements. Side effects were elicited for monitoring by the Data and Safety Monitoring Board. At 1 year, >95% of participants returned follow-up questionnaires, and approximately 90% were taking more than two-thirds of study pills (definition of compliance).

During run-in, participants willing to provide a blood sample were sent a kit with consent form, supplies, and instructions to have blood drawn into two 10-mL EDTA vials, one 6-mL sodium heparin vial, and one 6-mL serum vial, and a gel-filled freezer pack. Participants were asked to provide a fasting blood sample, record times of venipuncture and last meal, and send samples in freezer packs in ≤24 h. On receipt, samples were centrifuged to separate plasma, red blood cells, and buffy coat, aliquoted into 2-mL Nunc vials, and stored in −80 °C freezers. The process ensured that samples were frozen <30 to 36 h after venipuncture. One-year blood samples were similarly collected (all baseline and 1-year samples collected during winter/early spring), shipped, and stored.

LABORATORY ANALYSES

Total serum 25(OH)D concentrations were assayed by Quest Diagnostics using LC-MS/MS. Plasma samples were assayed for plasma phospholipid DHA, EPA, and *ω*-3 index (EPA plus DHA) as a percentage of total fatty acids by Quest Diagnostics by LC-MS/MS *(35)*. We selected 1561 individuals, approximately 50% each sex, with available and sufficient paired baseline and 1-year samples. These were thawed, aliquoted, sent to the Boston Children's Hospital Immunology Core (Nadar Rifai, PhD, Director), and run in 14 batches. Baseline and 1-year blood samples were assayed in tandem, in random order, and with masking of randomization status. IL-6 was assayed by an ultrasensitive quantitative sandwich enzyme immunoassay from R&D Systems. TNFR2 was assayed by a quantitative sandwich ELISA assay from R&D Systems. HsCRP was assayed by latex-enhanced immunonephelometric assay on a BNII analyzer (Dade Behring). CVs from 10% blinded split quality control samples from VITAL included across batches were good: 10.5% lnIL-6, 5.1% lnTNFR2, and 8.8% lnhsCRP. The within-run SDs were 0.71 lnIL-6, 0.33 lnTNFR2, and 1.10 lnhsCRP.

STATISTICAL ANALYSES

We assessed participant characteristics across treatment groups using *t*-tests for continuous variables and χ^2 tests for categorical variables. Box plots and histograms were used to examine biomarker distributions. Biomarker concentrations were right-skewed, and results were natural log-transformed (ln) to improve normality. Geometric means of biomarker concentrations with 95% CI were calculated for baseline and 1-year biomarkers by treatment group. Linear repeated measures models calculated percent change in means for each biomarker from baseline to 1 year and the overall effect of randomized treatment (net difference in logs expressed as a ratio of ratios, converted to overall percent change) with 95% confidence limits. Primary models were unadjusted; secondary models included age, sex, race (white, African American, other/unknown), and the other randomized treatment to adjust for potential imbalance.

We assessed for effect modification, calculating percent changes in biomarkers stratified by prespecified baseline factors, including age, sex, race, BMI (≤ vs >25 kg/m^2), smoking, fish intake (< vs ≥1.5 servings/week), serum 25(OH) vitamin D concentration (<20 vs ≥20 ng/mL), plasma *ω*-3 index (median <2.60 vs >2.60), biomarker concentration (< vs ≥ mean), and other randomized treatment. We assessed interactions between randomized treatment assignment and baseline variables of interest by including interaction terms in linear biomarker models. In an exploratory sensitivity analysis, to examine the extent to which statin use affected hsCRP, we imputed a constant statin treatment effects on hsCRP of constant 20% and 30% reductions *(36)*. Statistical significance was interpreted from 95% CIs excluding zero and 2-tailed *P* values <0.05. Strict adjustments for multiple comparisons were not performed, but results were interpreted cautiously considering the strengths of the underlying biologic mechanisms and a priori hypotheses. SAS version 9.4 was used for all analyses.

Results

The baseline characteristics of the 1561 VITAL participants with matched baseline and 1-year biomarker results in this substudy are shown in Table 1 by randomized group. Participants were well balanced in baseline characteristics across the trial's 4 arms and a representative sample of the overall VITAL trial. (Minor differences are shown in Table 1 in the online Data Supplement.) There was a borderline significantly higher proportion of those in receiving active vs placebo vitamin D with a high school education or less (15.1% vs 11.7%; $P = 0.05$) and self-reported mean household income of <$50 000 annually (36.4% vs 30.6%; $P = 0.02$). Dietary intakes of vitamin D and n-3 FA-containing foods and supplements were evenly balanced, except for slightly, although significantly, higher vitamin D-fortified food intake among those randomized to active vitamin D (mean daily servings, 0.62 vs 0.54; $P = 0.03$).

At 1 year, mean serum 25(OH)D concentration increased by 39.1% in those randomized to vitamin D, and the mean n-3 FA concentration rose by 55.1% among those randomized to n-3 FA, whereas there were minimal changes of both in the placebo groups, consistent with

Table 1. Baseline characteristics of biomarkers of systemic inflammation subcohort (n = 1561) by randomized treatment group.

	Active vitamin D and active n-3 FA (n = 392)	Active vitamin D and placebo n-3 FA (n = 392)	Placebo vitamin D and active n-3 FA (n = 392)	Placebo vitamin D and placebo n-3 FA (n = 385)	*P*[a]	*P*[b]
Age, mean years (SD)	65.41 (7.24)	65.71 (7.72)	65.87 (7.29)	65.75 (7.08)	0.50	0.80
Female, n (%)	202 (51.53)	213 (54.34)	195 (49.74)	198 (51.43)	0.35	0.37
Race/ethnicity, n (%)						
Non-Hispanic white	277 (70.66)	266 (67.86)	279 (71.17)	272 (70.65)	0.22	0.43
African American	68 (17.35)	75 (19.13)	67 (17.09)	66 (17.14)		
Other	42 (10.71)	42 (10.71)	32 (8.16)	38 (9.87)		
Geographic residence, n (%)						
Northeast	89 (22.70)	89 (22.70)	87 (22.19)	85 (22.08)	0.74	0.35
Midwest	68 (17.35)	67 (17.09)	65 (16.58)	74 (19.22)		
West	89 (22.70)	71 (18.11)	82 (20.92)	79 (20.78)		
Southeast	146 (37.24)	165 (42.09)	158 (40.31)	146 (37.92)		
Hours fasting, mean (SD)	8.93 (4.98)	9.00 (5.01)	9.33 (5.62)	8.65 (4.94)	0.90	0.25
Drawn in winter or spring, n (%)	390 (99.49)	389 (99.23)	388 (98.98)	383 (99.48)	0.75	0.77
Highest level of education, n (%)						
High school or below	65 (16.58)	53 (13.52)	43 (10.97)	48 (12.47)	**0.05**[c]	0.65
>High school	327 (83.42)	339 (86.48)	349 (89.03)	337 (87.53)		
Self-reported household annual income <$50 000, n (%)	132 (33.67)	131 (33.42)	107 (27.30)	115 (29.87)	**0.02**	0.51
Physical activity, total MET[d]-h/week, median (interquartile range)	16.17 (4.00–32.55)	16.92 (4.25–34.66)	16.20 (5.44–33.03)	16.22 (4.42–35.48)	0.49	0.52
Smoking, n (%)						
Current	22 (5.61)	23 (5.87)	25 (6.38)	22 (5.71)	0.15	0.58
Past	145 (36.83)	148 (37.76)	170 (43.37)	152 (39.48)		
Never	221 (56.38)	214 (54.59)	194 (49.49)	207 (53.77)		

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Table 1. Baseline characteristics of biomarkers of systemic inflammation subcohort (n = 1561) by randomized treatment group. *(Continued from page 1511)*

	Active vitamin D and active n-3 FA (n = 392)	Active vitamin D and placebo n-3 FA (n = 392)	Placebo vitamin D and active n-3 FA (n = 392)	Placebo vitamin D and placebo n-3 FA (n = 385)	*P*[a]	*P*[b]
Alcohol intake, n (%)						
Never	137 (34.95)	141 (35.97)	130 (33.16)	119 (30.91)	0.22	0.93
<1/week	16 (4.08)	25 (6.38)	26 (6.63)	29 (7.53)		
1–6/week	138 (35.20)	133 (33.93)	135 (34.44)	138 (35.84)		
Daily	90 (22.96)	88 (22.45)	97 (24.97)	94 (24.42)		
BMI, kg/m^2, mean (SD)	28.73 (6.05)	28.34 (5.92)	28.07 (5.70)	28.24 (5.92)	0.21	0.72
Diabetes mellitus, n (%)	64 (16.33)	62 (15.82)	56 (14.29)	55 (14.29)	0.32	0.88
Current use of cholesterol lowering medication, n (%)	162 (41.33)	163 (41.58)	166 (42.35)	157 (40.78)	0.35	0.67
Current use of supplemental Vitamin D (≤800 IU/day), n (%)	176 (44.90)	168 (42.86)	185 (47.19)	176 (45.71)	0.30	0.48
Milk, servings/day, mean (SD)	0.68 (0.89)	0.65 (0.75)	0.76 (0.91)	0.70 (0.99)	0.15	0.38
Other vitamin D-fortified foods, servings/day, mean (SD)	0.66 (0.94)	0.57 (0.63)	0.54 (0.65)	0.54 (0.57)	**0.03**	0.18
Total fish and seafood, median <1.5 servings/week, n (%)	213 (54.34)	217 (55.36)	209 (53.32)	197 (51.17)	0.23	0.87

[a] Comparison of 2 active vitamin D groups with 2 nonactive vitamin D groups.
[b] Comparison of 2 active n-3 FA groups with 2 nonactive n-3 FA groups.
[c] Values in bold indicate statistical significance, $P < 0.05$.
[d] MET, metabolic equivalent.
Missing data: race/ethnicity, 37; fasting time, 16; income, 113; physical activity, 9; smoking, 18; alcohol, 25; diabetes, 12; BMI, 48; milk, 37; other vitamin D, fish intake, 25.

Table 2. Biomarkers of systemic inflammation at baseline and 1 year, by vitamin D randomization.

	Active vitamin D, n = 784	Placebo vitamin D, n = 777	Overall percent change active/ placebo vitamin D (95% CI)	*P* unadjusted model[a]	*P* adjusted model[b]
Baseline ln (IL-6) pg/mL, geometric mean (95% CI)	1.71 (1.65–1.78)	1.68 (1.61–1.74)			
Year 1 ln (IL-6) pg/mL, geometric mean (95% CI)	1.80 (1.73–1.87)	1.63 (1.57–1.69)			
Percent change from baseline (95% CI)	5.08% (0.46–9.91)	−2.88% (−7.16 to 1.60)	8.19% (1.52–15.31)	**0.02**[c]	**0.02**
Baseline ln (TNFR2) pg/mL, geometric mean (95% CI)	2546.5 (2508.6–2584.9)	2525.4 (2482.7–2568.9)			
Year 1 ln (TNFR2) pg/mL, geometric mean (95% CI)	2604.7 (2565.2–2644.7)	2567.9 (2523.9–2612.7)			
Percent change from baseline (95% CI)	2.32% (1.13–3.53)	1.68% (0.49–2.89)	0.63% (−1.03 to 2.31)	0.46	0.57
Baseline ln (hsCRP) mg/L, geometric mean (95% CI)	1.48 (1.40–1.57)	1.51 (1.43–1.60)			
Year 1 ln (hsCRP) mg/L, geometric mean (95% CI)	1.62 (1.53–1.71)	1.54 (1.46–1.63)			
Percent change from baseline (95% CI)	9.46% (2.93–16.39)	2.18% (−3.94 to 8.68)	7.12% (−1.81 to 16.87)	0.12	0.16

[a] Linear models comparing natural logarithm of biomarker concentration in active vs placebo vitamin D groups.
[b] Linear models comparing natural logarithm of biomarker concentration in active vs placebo vitamin D groups, adjusted for age, sex, race, and n-3 FA randomized treatment group.
[c] Values in bold indicate statistical significance, $P < 0.05$.

high compliance. Significant increases from baseline to 1 year in both serum 25-OH vitamin D concentrations and ω-3 FA indices among those in the active vs placebo arms of the trial are shown in Table 2 of the online Data Supplement. The number of hours fasting (time since last meal) at blood draw was comparable for all groups at baseline compared with follow-up blood draw (data not shown, all $P > 0.05$). Cholesterol-lowering medication use was also well balanced in follow-up ($P = 0.79$ across n-3 FA arms and $P = 0.93$ across vitamin D arms). Almost all follow-up blood samples were similarly drawn in winter/spring season of the year (>97.9% throughout; all $P > 0.05$). Adverse effects were rare and previously reported for the entire VITAL trial *(25, 26)*.

The 3 biomarkers of systemic inflammation in the active vs placebo vitamin D arms at baseline, year 1, and percent changes from baseline to year 1 are shown in Table 2. At year 1, the IL-6 concentration had risen 5.08% from baseline among those randomized to active vitamin D, whereas it fell (by 2.88%) among those randomized to placebo; the overall increase was significantly greater among those in the active than in the placebo vitamin D group [overall effect, +8.19% (95% CI, 1.52%–15.31%); adjusted $P = 0.03$]. None of the other percent changes in biomarker concentrations over the year was significantly different comparing active vs placebo vitamin D groups. Table 3 similarly displays the geometric means and percent changes for the 3 biomarkers among those randomized to active vs placebo n-3 FA. For all 3 biomarkers, overall percent changes over the year did not differ between active and placebo n-3 FA groups.

For all 3 biomarkers, stratified percent changes from baseline to year 1 in placebo and active vitamin D groups, percent changes, and tests of interactions between the strata are shown in Table 4. No significant interactions were observed for IL-6 between the vitamin D randomized group and sex, BMI, race, smoking, baseline IL-6, or vitamin D concentration. For vitamin D's effect upon TNFR2, we observed a statistical interaction with race: The overall effect on TNFR2 concentration was 1.69% (95% CI, −0.26% to 3.68%) among whites, −1.50% (95% CI, −5.43% to 2.60%) among African Americans, and −4.61% (95% CI, −9.58% to 0.63%) among those of other or unknown race (adjusted *P* interaction 0.03 for effect among those of white vs other or unknown race; Table 4). Among those with baseline vitamin D <20 ng/mL, active vitamin D was associated with a 35.71% (95% CI, 7.78%–70.89%) net *increase* in hsCRP from baseline to year 1, while among those with baseline 25(OH) vitamin D concentration >20 ng/mL, there was no change [0.49% (95% CI, −8.89% to 10.83%); adjusted *P* interaction 0.02; Table 4]. A directionally similar nonsignificant increase of 20.5% in IL-6 was also noted among participants with low baseline 25(OH)D concentration.

Table 3. Biomarkers of systemic inflammation at baseline and 1 year, by n-3 FA randomization.

	Active n-3 FA, n = 784	Placebo n-3 FA, n = 777	Overall percent change active/ placebo n-3 FA (95% CI)	*P* unadjusted model[a]	*P* adjusted model[b]
Baseline ln (IL-6) pg/mL, geometric mean (95% CI)	1.69 (1.63–1.75)	1.70 (1.63–1.77)			
Year 1 ln (IL-6) pg/mL, geometric mean (95% CI)	1.70 (1.64–1.77)	1.72 (1.66–1.79)			
Percent change from baseline (95% CI)	0.67% (−3.76 to 5.31)	1.41% (−3.07 to 6.10)	−0.73% (−6.87 to 5.81)	0.82	0.97
Baseline ln (TNFR2) pg/mL, geometric mean (95% CI)	2571.3 (2528.1–2615.2)	2500.9 (2463.6–2538.8)			
Year 1 ln (TNFR2) pg/mL, geometric mean (95% CI)	2605.3 (2561.4–2649.9)	2567.3 (2527.7–2607.6)			
Percent change from baseline (95% CI)	1.35% (0.17–2.55)	2.66% (1.46–3.87)	−1.27% (−2.89 to 0.39)	0.13	0.13
Baseline ln (hsCRP) mg/L, geometric mean (95% CI)	1.57 (1.49–1.66)	1.43 (1.35–1.51)			
Year 1 ln (hsCRP) mg/L, geometric mean (95% CI)	1.63 (1.54–1.72)	1.53 (1.45–1.62)			
Percent change from baseline (95% CI)	3.70% (−2.49 to 10.27)	7.90% (1.44–14.77)	−3.89% (−11.92 to 4.86)	0.37	0.44

[a] Linear models comparing natural logarithm of biomarker concentration in active vs placebo n-3 FA groups.
[b] Linear models comparing natural logarithm of biomarker concentration in active n-3 FA vs placebo n-3 FA groups, adjusted for age, sex, race, and vitamin D randomized treatment group.

Investigating these results, we examined whether changes in statin use in the active vs placebo vitamin D groups could have influenced the observed changes in hsCRP. There were slight differences in statin uptake and discontinuation in the active vs placebo vitamin D over 1 year with 4.0% vs 6.1% starting ($P = 0.06$) and 5.1% vs 3.5% stopping ($P = 0.14$). Imputing a treatment effect had limited effect overall. The overall net effect for the active vs placebo vitamin D groups was a 6.1% increase in hsCRP ($P = 0.18$) assuming the more extreme 30% reduction with statin use. In the subgroup with baseline 25(OH) vitamin D levels <20 ng/mL, the difference was more extreme, with 3.8% vs 9.0% starting ($P = 0.09$) and 6.8% vs 1.8% stopping ($P = 0.06$), although this was based on a small number in this subset (n = 244). Imputing a statin effect again had limited impact on overall estimates in this subgroup, with a net difference of 33.1% ($P = 0.01$) assuming a 20% reduction and 32.0% ($P = 0.02$) for a 30% reduction.

Biomarker changes comparing placebo with active n-3 FA assignment groups within strata are shown in Table 5. No significant differences in the overall effects of n-3 FA on the 1-year changes in IL-6 or TNFR2 were observed across all strata investigated. However, the overall effect of active n-3 FA upon the 1-year change in hsCRP differed by baseline fish intake. Among individuals who had <1.5 servings of fish per week at baseline, active n-3 FA was associated with a decline in hsCRP of 10.45% (95% CI, −20.44% to 0.79%), whereas an increase of 6.36% (95% CI, −7.11% to 21.78%) was seen among those with greater baseline fish intake (adjusted *P* interaction 0.06; Table 5). These results were virtually unchanged after accounting for changes in statin use. In models testing for interactions using the continuous baseline variables (age at randomization in years and BMI in mg/kg^2), no significant interactions were detected for any of the biomarker changes with either vitamin D or n-3 FA randomized group (all $P > 0.05$).

Discussion

In this substudy of 1561 participants of a large randomized trial of vitamin D and n-3 FA in older Americans, we found no evidence that either of these widely used over-the-counter nutritional supplements reduce biomarkers of systemic inflammation over 1 year. Unexpectedly, those randomized to active vitamin D compared with placebo experienced an overall 8% increase in IL-6 concentration, consistent with a nonsignificant 6.9% increase in hsCRP. In stratified analyses, we observed a large increase in hsCRP (35.7%) and nonsignificant increase of 20.5% in IL-6 among those randomized to vitamin D with low vitamin D (<20 ng/mL) at baseline, but no effect among those with higher baseline vitamin D. These results are contrary to our a priori hypothesis and are difficult to explain on a biologic basis. We found no evidence that vitamin D supplementation reduced any of these markers of potentially harmful systemic inflammation. A borderline effect of active n-3 FA upon biomarkers of systemic inflammation was observed

Table 4. Stratified analyses: changes in biomarkers of inflammation from baseline to 1 year with active vs placebo vitamin D.

Baseline factor	Category	Active vitamin D n	Active vitamin D Percent change[a] from baseline (95% CI)	Placebo vitamin D n	Placebo vitamin D Percent change[a] from baseline (95% CI)	Overall percent change active/placebo vitamin D[b] (95% CI)	*P* interaction[c]
Change in IL-6							
Sex	Men	368	7.32% (0.49–14.62)	384	−2.42% (−8.56 to 4.13)	9.99% (0.27–20.65)	0.59
	Women	415	3.35% (−2.96 to 10.07)	393	−2.64% (−8.76 to 3.89)	6.15% (−3.03 to 16.21)	
BMI, mg/kg^2	≤25	218	9.66% (−0.48 to 20.85)	246	−1.95% (−10.50 to 7.40)	11.85% (−2.09 to 27.77)	0.53
	>25	549	3.34% (−1.81 to 8.77)	512	−2.93% (−7.99 to 2.40)	6.46% (−1.13 to 14.64)	
Race	White	543	4.91% (−0.73 to 10.87)	551	−3.14% (−8.31 to 2.32)	8.31% (0.19–17.08)	0.70
	African American	143	2.67% (−7.09 to 13.46)	133	−2.11% (−11.75 to 8.58)	4.89% (−9.18 to 21.12)	
	Other/unknown	83	11.75% (−2.00 to 27.44)	70	1.52% (−12.01 to 17.13)	10.08% (−9.35 to 33.67)	0.88
Smoking	Past/never	727	4.93% (0.09–10.00)	723	−3.43% (−7.91 to 1.28)	8.65% (1.61–16.18)	0.46
	Current	45	2.55% (15.01–23.73)	47	4.66% (−13.26 to 26.28)	−2.02% (−24.88 to 27.78)	
Fish intake, servings/week	<1.5	430	2.80% (−3.37 to 9.37)	406	−3.16% (−9.16 to 24)	6.16% (−2.89 to 16.04)	0.63
	≥1.5	339	7.19% (0.09–14.79)	363	−2.24% (−8.55 to 4.51)	9.64% (−0.37 to 20.64)	
ln (IL-6), pg/mL	< mean (0.53)	438	21.79% (16.19–27.66)	434	18.38% (12.91–24.12)	2.88% (−3.77 to 9.98)	0.09
	≥ mean (0.53)	345	−12.54% (−19.13 to −5.43)	343	−24.08% (−29.87 to −17.80)	15.19% (3.04–28.78)	
25(OH)D, ng/mL	< 20	134	11.02% (−1.18 to 24.63)	112	−7.87% (−18.80 to 4.53)	20.50% (1.48–43.08)	0.14
	≥20	635	3.82% (−1.28 to 9.17)	641	−1.03% (−5.90 to 4.10)	4.89% (−2.32 to 12.64)	
n-3 FA group	Placebo	392	7.86% (1.11–15.07)	385	−5.06% (−11.07 to 1.35)	13.61% (3.63–24.55)	0.12
	Active	391	2.59% (−3.77 to 9.36)	392	0.07% (−6.18 to 6.75)	2.51% (−6.39 to 12.27)	
Change in TNFR2							
Sex	Men	368	2.54% (0.80–4.31)	384	1.10% (−0.60 to 2.82)	1.43% (−0.98 to 3.90)	0.29
	Women	415	2.07% (0.44–3.72)	392	2.47% (0.78–4.19)	−0.40% (−2.67 to 1.93)	
BMI, mg/kg^2	≤25	218	2.30% (0.12–4.53)	245	1.63% (−0.41 to 3.71)	0.67% (−2.27 to 3.69)	0.95
	>25	549	2.21% (0.78–3.67)	512	1.66% (0.17–3.18)	0.54% (−1.49 to 2.62)	
Race	White	543	2.56% (1.16–3.98)	550	0.86% (−0.51 to 2.24)	1.69% (−0.26 to 3.68)	0.17
	African American	143	2.69% (−0.18 to 5.63)	133	4.25% (1.24–7.35)	−1.50% (−5.43 to 2.60)	
	Other/unknown	83	−0.22% (−3.77 to 3.45)	70	4.60% (0.56–8.81)	−4.61% (−9.58 to 0.63)	**0.03**[d]
Smoking	Past/never	727	2.09% (0.86–3.33)	722	1.58% (0.35–2.83)	0.50% (−1.22 to 2.24)	0.83
	Current	45	4.93% (−0.01 to 10.11)	47	3.64% (−1.24 to 8.75)	1.25% (−5.42 to 8.39)	

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Table 4. Stratified analyses: changes in biomarkers of inflammation from baseline to 1 year with active vs placebo vitamin D. *(Continued from page 1515)*

Baseline factor	Category	Active vitamin D n	Active vitamin D Percent change[a] from baseline (95% CI)	Placebo vitamin D n	Placebo vitamin D Percent change[a] from baseline (95% CI)	Overall percent change active/placebo vitamin D[b] (95% CI)	*P* interaction[c]
Fish intake, servings/week	<1.5	430	1.69% (0.05–3.35)	406	1.55% (−0.14 to 3.27)	0.14% (−2.17 to 2.50)	0.34
	≥1.5	339	3.49% (1.77–5.23)	362	1.69% (0.05–3.36)	1.76% (−0.58 to 4.16)	
ln (TNFR2), pg/mL	<mean (7.84)	415	4.96% (3.44–6.50)	421	4.43% (2.92–5.96)	0.51% (−1.55 to 2.60)	0.95
	≥mean (7.84)	368	−0.69% (−2.50 to 1.15)	355	−1.29% (−3.13 to 0.59)	0.61% (−2.00 to 3.29)	
25(OH)D, ng/mL	< 20	134	4.60% (1.33–7.97)	112	3.56% (0.05–7.19)	1.00% (−3.62 to 5.84)	0.77
	≥20	635	1.80% (0.52–3.10)	640	1.56% (0.28–2.87)	0.24% (−1.55 to 2.05)	
n-3 FA group	Placebo	392	1.49% (−0.19 to 3.20)	385	1.28% (−0.42 to 3.01)	0.21% (−2.15 to 2.62)	0.74
	Active	391	3.10% (1.42–4.81)	391	2.31% (0.62–4.02)	0.77% (−1.55 to 3.16)	
Change in hsCRP							
Sex	Men	368	12.05% (2.07–23.02)	384	4.79% (−4.44 to 14.92)	6.93% (−6.22 to 21.92)	0.95
	Women	415	6.79% (−1.79 to 16.11)	392	0.46% (−7.86 to 9.53)	6.30% (−5.76 to 19.89)	
BMI, mg/kg^2	≤25	218	20.04% (4.31–38.15)	245	12.58% (−1.38 to 28.51)	6.63% (−12.09 to 29.34)	0.83
	>25	549	5.92% (−1.01 to 13.33)	512	−2.95% (−9.59 to 4.17)	9.14% (−1.04 to 20.37)	
Race	White	543	12.16% (3.92–21.05)	551	0.49% (−6.84 to 8.39)	11.62% (0.24–24.28)	0.18
	African American	143	3.64% (−8.03 to 16.78)	132	6.78% (−5.70 to 20.91)	−2.94% (−18.31 to 15.32)	
	Other/unknown	83	0.62% (−18.22 to 23.81)	70	11.81% (−10.79 to 40.13)	−10.00% (−33.76 to 22.28)	0.19
Smoking	Past/never	727	9.05% (2.21–16.33)	722	2.55% (−3.92 to 9.47)	6.33% (−3.00 to 16.56)	0.77
	Current	45	5.27% (−18.63 to 36.19)	47	4.75% (−19.03 to 35.52)	0.50% (−30.18 to 44.65)	
Fish intake, servings/ week	<1.5	430	12.33% (3.46–21.96)	405	0.44% (−7.75 to 9.36)	11.83% (−0.64 to 25.88)	0.37
	≥1.5	339	6.58% (−3.28 to 17.44)	363	3.44% (−5.89 to 13.7)	3.03% (−10.03 to 17.98)	
ln (hsCRP), pg/mL	< mean (0.40)	413	39.24% (29.25–50.00)	411	31.36% (21.86–41.59)	6.00% (−4.63 to 17.81)	0.88
	≥ mean (0.40)	370	−16.73% (−24.29 to −8.41)	365	−22.50% (−29.63 to −14.64)	7.44% (−6.18 to 23.04)	
25(OH)D, ng/mL	<20	134	21.64% (4.06–42.20)	112	−10.37% (−24.34 to 6.19)	35.71% (7.78–70.89)	**0.02**[d]
	≥20	635	6.65% (−0.47 to 14.28)	641	6.13% (−0.99 to 13.77)	0.49% (−8.89 to 10.83)	
n-3 FA group	Placebo	392	5.20% (−3.33 to 14.47)	384	2.80% (−5.62 to 11.96)	2.34% (−9.25 to 15.4)	0.38
	Active	391	13.46% (3.49–24.39)	392	2.35% (−6.74 to 12.32)	10.85% (−2.74 to 26.34)	

[a] Change in geometric mean of ln biomarker.
[b] Comparison of the percent changes in ln biomarker from baseline to year 1.
[c] Multiplicative interaction between strata based on linear models of ln biomarker from baseline to year 1 with *P* for interaction *between* strata, adjusted for age, race, sex, n-3 FA randomized treatment group. Tests for interaction by race for African American vs white, and other/unknown race vs white.
[d] Values in bold indicate statistical significance, $P < 0.05$.

Table 5. Stratified analyses: change in biomarkers of inflammation from baseline to 1 year with active vs placebo n-3 FA.

Baseline factor	Category	Active N-3 FA n	Active N-3 FA Percent change[a] from baseline (95% CI)	Placebo N-3 FA n	Placebo N-3 FA Percent change[a] from baseline (95% CI)	Overall percent change active/placebo n-3 FA[b] (95% CI)	*P* interaction[c]
Change in IL-6							
Sex	Men	386	0.15% (−6.13 to 6.84)	366	4.56% (−2.16 to 11.74)	−4.22% (−12.70 to 5.08)	0.22
	Women	397	2.36% (−4.05 to 9.18)	411	−1.45% (−7.51 to 5.01)	3.86% (−5.13 to 13.70)	
BMI, mg/kg^2	≤25	228	3.73% (−5.66 to 14.05)	236	2.95% (−6.24 to 13.05)	0.95% (−11.82 to 15.11)	0.87
	>25	535	0.06% (−5.03 to 5.42)	526	0.55% (−4.60 to 5.97)	−0.49% (−7.59 to 7.16)	
Race	White	556	0.63% (−4.73 to 6.29)	538	0.92% (−4.54 to 6.69)	−0.29% (−7.77 to 7.80)	0.79
	African American	135	−0.94% (−10.62 to 9.80)	141	1.58% (−8.15 to 12.34)	−2.47% (−15.55 to 12.63)	
	Other/unknown	73	10.61% (−3.87 to 27.26)	80	3.72% (−9.29 to 18.59)	6.64% (−12.17 to 29.47)	0.63
Smoking	Past/never	729	0.75% (−3.91 to 5.64)	721	0.64% (−4.04 to 5.54)	0.11% (−6.39 to 7.06)	0.90
	Current	47	2.79% (−14.66 to 23.82)	45	4.45% (−13.65 to 26.34)	−1.58% (−24.58 to 28.43)	
Fish intake, servings/week	<1.5 serving/week	422	−2.41% (−8.34 to 3.91)	414	2.24% (−4.02 to 8.91)	−4.54% (−12.67 to 4.35)	0.17
	≥1.5 serving/week	351	4.54% (−2.30 to 11.87)	351	−0.01% (−6.56 to 7.00)	4.55% (−5.00 to 15.06)	
ln (IL-6), pg/mL	<mean (0.53)	440	18.13% (12.71 to 23.81)	432	22.11% (16.45 to 28.05)	−3.26% (−9.51 to 3.42)	0.32
	≥ mean (0.53)	343	−17.05% (−23.38 to −10.19)	345	−19.77% (−25.86 to −13.18)	3.40% (−7.55 to 15.65)	
Omega-3 index	<median (2.60)	384	4.93% (−1.95 to 12.29)	344	5.13% (−2.14 to 12.94)	−0.19% (−9.57 to 10.16)	0.69
	≥ median (2.60)	361	0.35% (−5.87 to 6.98)	403	−2.40% (−8.11 to 3.66)	2.82% (−5.83 to 12.26)	
Vitamin D group	Placebo	392	7.86% (1.27–14.89)	385	2.59% (−3.71 to 9.29)	5.14% (−3.85 to 14.97)	0.12
	Active	391	−5.06% (−11.12 to 1.42)	392	0.07% (−6.33 to 6.92)	−5.13% (−13.60 to 4.16)	
Change in TNFR2							
Sex	Men	386	1.09% (−0.59 to 2.80)	366	2.57% (0.82–4.35)	−1.44% (−3.78 to 0.96)	0.85
	Women	396	1.67% (0.01–3.37)	411	2.83% (1.18–4.51)	−1.12% (−3.38 to 1.19)	
BMI, mg/kg^2	≤25	227	0.25% (−1.83 to 2.37)	236	3.61% (1.50–5.77)	−3.25% (−6.05 to −0.36)	0.11
	>25	535	1.75% (0.29–3.23)	526	2.16% (0.69–3.66)	−0.41% (−2.42 to 1.65)	
Race	White	555	0.88% (−0.48 to 2.26)	538	2.56% (1.15–3.98)	−1.63% (−3.52 to 0.29)	0.58
	African American	135	3.24% (0.27–6.29)	141	3.63% (0.72–6.63)	−0.38% (−4.36 to 3.76)	
	Other/unknown	73	1.86% (−2.03 to 5.91)	80	2.11% (−1.63 to 5.98)	−0.24% (−5.47 to 5.29)	0.63
Smoking	Past/never	728	1.18% (−0.04 to 2.41)	721	2.50% (1.26–3.76)	−1.29% (−2.97 to 0.42)	0.38
	Current	47	5.21% (0.32–10.34)	45	3.32% (−1.59 to 8.47)	1.84% (−4.87 to 9.01)	
Fish intake	<1.5 serving/week	422	0.53% (−1.11 to 2.19)	414	2.75% (1.06–4.46)	−2.16% (−4.41 to 0.15)	0.26
	≥1.5 serving/week	350	2.42% (0.74–4.13)	351	2.71% (1.03–4.41)	−0.28% (−2.58 to 2.08)	

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Table 5. Stratified analyses: change in biomarkers of inflammation from baseline to 1 year with active vs placebo n-3 FA. *(Continued from page 1517)*

		Active N-3 FA		Placebo N-3 FA			
Baseline factor	Category	n	Percent change[a] from baseline (95% CI)	n	Percent change[a] from baseline (95% CI)	Overall percent change active/placebo n-3 FA[b] (95% CI)	*P* interaction[c]
ln (TNFR2), pg/mL	< mean (7.84)	399	3.91% (2.37–5.47)	437	5.41% (3.92–6.92)	−1.42% (−3.43 to 0.63)	0.60
	≥ mean (7.84)	383	−1.23% (−3.00 to 0.57)	340	−0.70% (−2.59 to 1.21)	−0.53% (−3.11 to 2.13)	
Omega-3 index	< median (2.60)	384	2.03% (0.31–3.78)	344	2.72% (0.89–4.58)	−0.67% (−3.10 to 1.81)	0.56
	≥2.60	360	1.13% (−0.58 to 2.87)	403	2.86% (1.22–4.53)	−1.68% (−3.96 to 0.65)	
Vitamin D group	Placebo	391	1.49% (−0.14 to 3.15)	385	3.10% (1.43–4.79)	−1.56% (−3.80 to 0.73)	0.74
	Active	391	1.28% (−0.43 to 3.02)	392	2.31% (0.58–4.07)	−1.00% (−3.36 to 1.41)	
Change in hsCRP							
Sex	Men	386	4.49% (−4.66 to 14.51)	366	12.51% (2.42 to 23.60)	−7.13% (−18.55 to 5.89)	0.43
	Women	397	3.53% (−4.98 to 12.81)	410	3.81% (−4.59 to 12.96)	0.27% (−11.58 to 12.49)	
BMI, mg/kg^2	≤25	228	14.74% (0.04–31.6)	235	17.30% (2.43–34.33)	−2.18% (−19.33 to 18.62)	0.81
	>25	535	−0.82% (−7.44 to 6.26)	526	4.09% (−2.90 to 11.58)	−4.72% (−13.61 to 5.09)	
Race	White	556	4.54% (−3.06 to 12.74)	538	7.77% (−0.19 to 16.37)	−3.00% (−12.90 to 8.03)	0.59
	African American	135	6.47% (−5.84 to 20.40)	140	3.86% (−7.95 to 17.18)	2.52% (−13.70 to 21.79)	
	Other/unknown	73	−4.27% (23.20–19.33)	80	15.48% (−6.44 to 42.53)	−17.10% (−38.87 to 12.44)	0.34
Smoking	Past/never	729	2.93% (−3.53 to 9.81)	720	8.74% (1.89–16.06)	−5.35% (−13.65 to 3.75)	0.08
	Current	47	20.49% (−6.59 to 55.41)	45	−9.05% (−29.89 to 17.99)	32.47% (−7.95 to 90.65)	
Fish intake, servings/week	<1.5 serving/week	422	0.75% (−7.30 to 9.50)	413	12.51% (3.45–22.37)	−10.45% (−20.44 to 0.79)	0.06
	≥1.5 serving/week	351	8.24% (−1.64 to 19.12)	351	1.77% (−7.52 to 12.00)	6.36% (−7.11 to 21.78)	
ln (hsCRP), mg/L	< mean (0.40)	410	29.47% (20.13–39.53)	414	41.26% (31.12–52.19)	−8.35% (−17.53 to 1.86)	0.18
	≥ mean (0.40)	373	−18.35% (−25.77 to −10.19)	362	−20.91% (−28.20 to −12.89)	3.24% (−9.86 to 18.24)	
Omega-3 index	< median (2.60)	384	4.70% (−4.35 to 14.60)	344	8.45% (−1.43 to 19.31)	−3.46% (−15.35 to 10.1)	0.73
	≥ median (2.60)	361	5.47% (−3.75 to 15.57)	402	5.77% (−2.98 to 15.31)	0.28% (−12.07 to 13.09)	
Vitamin D group	Placebo	392	5.20% (−3.43 to 14.60)	384	13.46% (4.12–23.64)	−7.28% (−17.87 to 4.67)	0.38
	Active	391	2.80% (−6.20 to 12.66)	392	2.35% (−6.64 to 12.21)	0.43% (−11.79 to 14.35)	

[a] Change in geometric mean of ln biomarker.

[b] Comparison of the percent changes in ln biomarker from baseline to year 1.

[c] Multiplicative interaction between strata based on linear models of ln biomarker from baseline to year 1 with *P* for interaction *between* strata, adjusted for age, race, sex, vitamin D randomized treatment group. Tests for interaction by race for African American vs white, and other/unknown race vs white.

among those with low fish intake at baseline (a −10.45% decrease vs 6.36% increase in hsCRP among those with higher intake at baseline). This finding is interesting given the protective effect of active n-3 FA upon myocardial infarction risk observed among those with low fish intake at baseline in the parent VITAL trial *(25)*.

The current results conflict with past studies, mainly cross-sectional and case-control, which have reported inverse associations between vitamin D and n-3 FA levels and biomarkers of systemic inflammation in a range of diseases. Some, but not all, past randomized controlled trials of these supplements in specific populations, such as diabetes, hemodialysis, polycystic ovary syndrome, and heart failure, have shown reductions in hsCRP with vitamin D and marine ω-3 supplementation *(1–7, 37)*. A meta-analysis of vitamin D supplementation trials in type 2 diabetic individuals reported an overall reduction of hsCRP by 0.34 μg/mL among those randomized to ≤4000 IU/day *(5)*. Our study, which had a 15% diabetes prevalence, tested a 2000 IU/day dose of vitamin D, similar to that in the diabetes trials. However, only one of the past diabetes trials tested treatment for an entire year.

Although past trials were small, used varying doses and durations of n-3 FAs, and several were null, meta-analysis of past n-3 FA supplementation studies has suggested that marine n-3 FAs' effects upon reducing systemic inflammation might be greater in those with chronic inflammation, among the overweight, and with longer use *(7, 37)*. There is currently enormous interest in n-3 FAs' abilities to generate specialized proresolving mediators of inflammation (SPMs), which may have profound and widespread beneficial effects in both acute and chronic inflammation *(38)*. It is possible that the circulating plasma biomarkers of systemic inflammation measured here do not reflect the body's ability to produce SPMs, as inflammation stimulating SPM generation may take place in tissues and SPMs are likely generated for short periods in times of acute inflammation. There is also great interest in the ability of EPA metabolites such as icosapent ethyl in reducing cardiovascular disease risk among patients with high baseline cardiovascular disease risk, as has been recently demonstrated among those with high triglyceride levels and diabetes or other cardiovascular disease risk factors in the REDUCE-IT trial *(39)*. It is not clear whether those observed reductions in cardiovascular disease risk were mediated by reductions in systemic inflammation detectable by the current biomarkers, or attributable to mechanisms such as SPM generation or changes in triglycerides and other cholesterol subsets.

Strengths of the current study include the blinded randomized controlled trial design, a large proportion of African American enrollees, high rates of adherence with study medications, and balanced baseline covariates of the participants. Participants continued to take the blinded supplements for the year of this substudy, with these measurements at baseline and 1 year, contrasting with many past short-term studies of 6 to 24 weeks. Participants also included smokers and nonsmokers, with a range of BMI and baseline fish intake, ensuring adequately sized subgroups for analyses. The results address real-world over-the-counter use of these supplements by older community-dwelling Americans from a variety of sociodemographic backgrounds, with common comorbidities increasing the risk of systemic inflammation, such as smoking, obesity, and diabetes.

The current study has potential limitations. Many over-the-counter vitamin D and n-3 FA supplements are available to the public, and this trial tested one specific preparation and dose of each. The vitamin D_3 dose was chosen as 2000 IU/day to reduce the likelihood of adverse events; these results do not address higher dose or intermittent vitamin D use. Similarly, the Omacor preparation studied may differ from other doses and preparations of n-3 FA supplements and may not be as effective at inducing proresolution lipid mediators as others. Blood samples were shipped and stored, not assayed immediately, possibly affecting biomarker concentrations, although this should have affected all trial arms similarly. Serum 25(OH)D concentrations vary by season and geography, but baseline and follow-up samples were matched on season, and participants' geographic distribution across the 4 subsample arms was balanced. Although cholesterol-lowering medication use was evenly balanced at baseline and 1 year, we had limited information about other medications, such as corticosteroids, which potentially could have influenced biomarker concentrations.

In this study of participants in the VITAL trial, neither vitamin D 2000 IU/day nor Omacor n-3 FA supplements (1 g of EPA/DHA per day) reduced systemic inflammation biomarkers over 1 year. Thus, it is unlikely that these supplements, taken widely in the general population, have major antiinflammatory effects. These results address important questions concerning these supplements' potential effects on inflammatory pathways.

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