Research Article

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Factors Associated with Multiple Biomarkers of Systemic Inflammation

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Abstract

Background: While much is known about correlates of C-reactive protein (CRP), little is known about correlates of other inflammation biomarkers. As these measures are increasingly being used in epidemiologic studies, it is important to determine what factors affect inflammation biomarker concentrations.

Methods: Using age, sex, and body mass index (BMI) adjusted linear regression, we examined 38 exposures (demographic and anthropometric measures, chronic disease history, NSAIDs, dietary factors, and supplement use) of 8 inflammation biomarkers [CRP, IL1β, IL6, IL8, TNFα, and soluble TNF receptors (sTNFR) in plasma; and prostaglandin E_2 metabolite (PGE-M) in urine] in 217 adults, ages 50 to 76 years.

Results: Increasing age was associated with higher concentrations of all biomarkers except IL1β. BMI was positively associated with CRP and sTNFR I and II. Saturated fat intake was associated with increased CRP, sTNFRII, TNFα, and IL1β, whereas eicosapentaenoic acid + docosahexaenoic acid (EPA+DHA) intake (diet or total) was associated with decreased CRP, TNFα, and IL1β. Results for sex were varied: CRP and IL6 were lower among men, whereas PGE-M and sTNFRI were higher. Higher CRP was also associated with smoking, hormone replacement therapy use, and γ-tocopherol intake; lower CRP with physical activity, and intakes of dietary vitamin C and total fiber.

Conclusions: Although the associations varied by biomarker, the factors having the greatest number of significant associations ($P \leq 0.05$) with the inflammation biomarkers were age, BMI, dietary saturated fat, and EPA+DHA omega-3 fatty acids.

Impact: Our results suggest that potential confounders in epidemiologic studies assessing associations with inflammation biomarkers vary across specific biomarkers. *Cancer Epidemiol Biomarkers Prev; 25(3); 521–31. ©2016 AACR.*

Introduction

A vast literature over the past decade links low-grade, systemic inflammation with many chronic diseases, such as type II diabetes (1), cardiovascular disease (2), and many cancers (3), although the mechanisms for these associations are not entirely clear. Against this background, many circulating biomarkers of inflammation, for example, cytokines, acute-phase proteins, chemokines, and other soluble immune factors, are commonly evaluated to determine disease risk. Given that these biomarkers are increasingly used in epidemiologic and intervention studies, it is important to establish whether certain factors are consistently associated with inflammation biomarker concentrations and how these determinants differ based on the biomarker under study. Although factors affecting circulating concentrations of some cytokines have been well characterized, for example, increasing age and higher body mass index (BMI) are associated with greater blood concentrations of C-reactive protein (CRP; refs. 4–6), less is known about correlates of other biomarkers of inflammation. In addition, much of the prior work has been carried out among individuals with chronic conditions. In this analysis, we examined the associations of 38 exposures, including demographic factors, anthropometric measures, history of chronic disease, medication use, dietary factors, and supplement use, with a panel of 8 inflammation biomarkers [CRP, prostaglandin E_2 metabolite (PGE-M), IL1β, IL6, IL8, TNFα, and the soluble TNF receptors (sTNFR) I and II] to identify similarities and differences in the associations with these biomarkers.

Materials and Methods

Study population

The VITAL biomarker study includes 220 individuals drawn from the parent VITAL cohort, a prospective study of 77,719 Western Washington residents, ages 50 to 76 years, focused on supplement use in relation to cancer incidence (7). VITAL study participants who completed the VITAL questionnaire between October 2000 and February 2001, lived in the Seattle metropolitan area, and did not have insulin-dependent diabetes were randomly selected for participation in the VITAL biomarker substudy. Of the 290 individuals contacted, 220 (76%) agreed to participate and completed the study protocol. The biomarker study consisted of a second mailed validity questionnaire and an in-home visit about 3 to 4 months after baseline, at which time an interview was conducted and fasting blood and spot urine were collected. All participants provided written informed consent and study procedures were approved by the Fred Hutchinson Cancer Research Center (FHCRC; Seattle, WA) Institutional Review Board.

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Exposures

Our analyses included assessment of demographic factors (age, sex, race/ethnicity, and education), lifestyle/anthropometric factors [BMI (kg/m^2), smoking, alcohol consumption, and physical activity], medical history (cancer, cardiovascular disease, and diabetes), as well as current medication use [regular aspirin, low-dose aspirin, non-aspirin NSAIDs, hormone replacement therapy (HRT), and cholesterol-lowering drugs]. To reduce measurement error in our self-reported BMI (kg/m^2) variable, we used the average BMI from the two questionnaires (baseline questionnaire and validity questionnaire). Similarly, for smoking, a pack-years variable was derived for each questionnaire, then averaged, and categorized as never-smoker, pack-years below the median, or pack-years above the median. Alcohol consumption was assessed by a food frequency questionnaire (FFQ) and categorized as tertiles of intake. Recreational physical activity was determined through a series of questions on minutes/day and days/week of 14 types of activities, plus intensity for walking. This information was used to categorize individuals as engaging in any moderate/vigorous activity in the prior month (no/yes). History of cancer excluded history of nonmelanoma skin cancers. History of cardiovascular disease was defined by a self-reported history of any of myocardial infarction, angina, angioplasty, coronary bypass surgery, or stroke. Use of low-dose aspirin, regular aspirin, and NSAIDs was defined as current days per week of use, categorized as 0, 1 to 3 days/week, or 4+ days/week. Use of HRT and cholesterol-lowering drugs were defined as current use (yes/no).

We also examined dietary factors [total energy, fiber, saturated fat, α-tocopherol, and γ-tocopherol intake, vitamin C, vitamin D, β-carotene, and long-chain omega-3 polyunsaturated fatty acids (PUFA)], supplement use (fiber, vitamin C, vitamin D, vitamin E, β-carotene, fish oil, and multivitamins), plus total dietary + supplemental intake (fiber, α-tocopherol, vitamin C, vitamin D, β-carotene, and long-chain omega-3 PUFA).

Dietary factors were assessed by an FFQ embedded within the main questionnaire. This FFQ was adapted from the one used in the Women's Health Initiative and captures intake and serving size of 120 foods and beverages (7). The University of Minnesota's Nutrition Coding Center Database was used to convert FFQ data into nutrient intake. Participants were excluded from nutrient calculations if they did not complete all pages of the FFQ or if they reported biologically implausible energy intake (men reporting <800 kcal/day or >5,000 kcal/day; women reporting <600 kcal/day or >4,000 kcal/day; ref. 8). Dietary variables from each of the two FFQ completed were averaged to reduce measurement error.

Use of vitamins C, D, E, and β-carotene, was ascertained at a supplement inventory performed at the time of the biomarker study home visit. For each supplement currently taken, interviewers ascertained frequency of use and number of pills taken and vitamin or mineral dose per pill from each supplement bottle label. This information was used to calculate the average amount of each supplement taken/day as follows: [(days/week)/(7 days/week)] × dose per day, summed over the individual supplements and multivitamins containing the nutrient of interest. These supplements were categorized as no use, low use, and high use, with the threshold between low use and high use set so as to best separate intake from multivitamins and individual supplements using dosing information on popular multivitamins at the time of questionnaire. The following thresholds were used to separate high use of supplements: vitamin C (>560 mg), vitamin D (>10 mg), vitamin E (>430 mg), and β-carotene (>600 μg). Fish oil, fiber, and multivitamin supplement use were categorized as current use/no use. Appropriate weighting was used to yield dietary + supplemental intake of α-tocopherol (mg) and β-carotene (μg), while total dietary + supplemental eicosapentaenoic acid + docosahexaenoic acid (EPA+DHA) was calculated using an estimate of supplemental dose (9). The VITAL biomarker study was used as the data source for all exposures unless otherwise noted. If information on a given variable was missing in the VITAL validity questionnaire, information was pulled from the VITAL baseline questionnaire.

Outcomes and exclusions

Outcomes include the following biomarkers of inflammation: plasma CRP, IL1β, IL6, IL8, TNFα, sTNFRI and sTNFRII, and urinary PGE-M. The blood and urine samples were collected at the home interview after participants had fasted for at least 6 hours, processed within 2 hours of collection, and stored at −80°C. CRP was measured on a Roche Cobas Mira Plus chemistry analyzer using CRP Ultra Wide Range Reagent (Sekisui Diagnostics) with a high sensitivity protocol. IL1β, IL6, IL8, and TNFα were assayed using the MILLIPLEX High Sensitivity Human Cytokine Kit (Millipore) according to the manufacturer's instructions. The lowest standard of this assay was 0.182 pg/mL, and values below this threshold were entered as half of this value, 0.091 (IL1β, $n = 50$; IL6, $n = 13$; and TNFα, $n = 1$). sTNFRI and sTNFRII were measured using MILLIPLEX MAP Human Soluble Cytokine Receptor Panel Kit according to the manufacturer's instructions. Intra-assay coefficient of variations (CV) were 5.4% for CRP, 5.6% for sTNFRI, 9.1% for sTNFRII, 8.7% for IL1β, 12.8% for IL6, 14.8% for IL8, and 13.3% for TNFα. Urinary PGE-M was assayed at the Vanderbilt Eicosanoid Core Laboratory using unacidified urine. The LC/MS-MS method was used to assess PGE-M, the stable metabolite of PGE_2 (10). Urine was unavailable for 7 individuals, thus they were excluded from the PGE-M analyses. To avoid inclusion of individuals with acute illness, we excluded those values from all biomarker analyses where CRP was in the top 2% for the participants age–BMI–gender group ($n = 3$; ref. 11). After making the above-listed exclusions, 210 participants remained for analyses of PGE-M and 217 for all other biomarkers.

Statistical analysis

Given the skewed distribution of the biomarker data, all outcomes were log-transformed using the natural logarithm. Pearson correlation coefficients were used to evaluate the correlation between biomarkers. Multivariable-adjusted linear regression was used to evaluate the association between categories of each exposure and each biomarker; the exponentiated betas for each exposure are interpreted as the ratio of the biomarker for each exposure category compared with the lowest exposure category. The Wald test was used to test for a statistical difference in biomarker values for binary exposures. For tests for trend in the biomarker values across values of other exposures, the exposure was modeled as continuous for underlying continuous variables and grouped as linear for ordered categorical variables. The exception to this is that alcohol was tested for its association with each biomarker using a global P for differences among the tertiles of alcohol intake, as alcohol effects are often not linear trends. All models were adjusted for age (continuous), sex, and BMI (continuous), and all models with diet variables were additionally adjusted for energy intake. To determine the percent of variance exposures explained, R^2 values were calculated for each biomarker

including all significant factors for that particular biomarker in a single model. Stata (v 12; StataCorp) was used for all analyses. As the goal of this study was to examine consistencies between exposures and the 8 biomarkers, we discuss associations that were nominally significant ($P < 0.05$), rather than use a more stringent P value that is adjusted for multiple testing to provide readers with a framework of biomarker consistency.

Results

Participant characteristics are given in Table 1. In this inflammation biomarker panel, there was a good correlation between concentrations of TNFα and the ILs (IL1β, IL6, and IL8; r ranging from 0.66–0.80). sTNFRI and sTNFRII were only modestly correlated with one another ($r = 0.50$), while the rest of the biomarkers were either uncorrelated or the correlations were weak (Table 2).

Tables 3 and 4 give the associations of the biomarkers of inflammation with the demographic and lifestyle factors studied, for factors associated with at least one biomarker. Increasing age was associated with higher concentrations of all biomarkers (all $P < 0.02$) except IL1β. Women had higher concentrations than men for CRP ($P < 0.001$) and IL6 ($P = 0.03$), whereas men had higher concentrations for PGE-M ($P = 0.04$) and sTNFRI ($P < 0.05$). BMI was positively associated with CRP ($P < 0.001$), sTNFRI ($P < 0.05$), and sTNFII ($P = 0.005$), whereas physical activity was inversely associated with CRP ($P = 0.005$) and sTNFRII ($P = 0.04$). Saturated fat intake was associated with increased CRP ($P = 0.004$), sTNFRII ($P < 0.05$), TNFα ($P = 0.02$), and IL1β ($P = 0.004$). EPA+DHA intake from diet was associated with decreased CRP ($P < 0.001$) and TNFα ($P = 0.04$), whereas EPA+DHA from diet plus supplements was associated with decreased IL1β ($P = 0.03$). Many factors were only associated with one biomarker. In particular, alcohol intake was only associated with sTNFRII, with a highly significant inverse association ($P < 0.001$) and low-dose aspirin was only associated with decreased PGE-M ($P = 0.04$). Several factors were only associated with increased CRP (smoking, HRT use, and γ-tocopherol intake) or decreased CRP (dietary vitamins C and D and fiber from diet plus supplements; all $P < 0.05$). R^2 values for each biomarker including all significant factors were as follows: CRP, 0.44; PGE-M, 0.08; sTNFRI, 0.10; sTNFRII, 0.30; TNFα, 0.08; IL1β, 0.06; IL6, 0.04; and IL8, 0.04.

Table 1. VITAL biomarker study participant characteristics

Characteristic	*N* (%)
Age (years)	
50–<55	53 (24.4)
55–<60	53 (24.4)
60–<65	37 (17.1)
65–<70	30 (13.8)
70+	44 (20.3)
Sex	
Female	106 (48.9)
Male	111 (51.2)
Race/ethnicity	
White	205 (94.5)
Nonwhite	12 (5.53)
BMI (kg/m^2)	
<25	83 (38.3)
25–<30	97 (44.7)
30+	37 (17.1)

Discussion

To our knowledge, no other study has simultaneously evaluated the associations of a large number of exposures across a panel of circulating inflammation biomarkers. In this study, the most consistent associations with systemic inflammation were age, BMI, and intakes of saturated fat and EPA and DHA omega-3 PUFAs. More factors were associated with CRP concentrations than any other marker we evaluated. Surprisingly, increasing BMI, a strong determinant of CRP concentrations in this study and others (5, 12), was only significantly associated with higher concentrations of sTNFRI and II, but not any of the other proinflammatory cytokines commonly thought to be elevated in obesity. Also unexpected was a lack of an association between higher intakes of fiber with any of the biomarkers other than CRP.

Demographic

It is well established that CRP, the most widely studied of the biomarkers of inflammation, increases with age (4, 13–15). We found similar increases with age for the other biomarkers, except IL1β, consistent with other studies (16, 17). Women have also been shown to have consistently higher concentrations of CRP (11, 13, 14, 18, 19); however, among the other markers, only IL6 was higher among women, while PGE-M and sTNFRI appeared to be somewhat higher among men. The data on sex differences in these biomarkers of inflammation, other than CRP, are either mixed (20–23) or lacking.

Although age-associated increases in many inflammation biomarkers correlate with chronic disease (15), the mechanisms for sex differences remain inconclusive. Hormone use is thought to be a contributor as women using estrogen have higher CRP concentrations than those who do not (24–26). Indeed, in this study, women who reported current use of HRT had 74% higher CRP concentrations. Greater adiposity in women may play a role, although sex differences in CRP remain, in our study and in others, even after adjustment for HRT and BMI (24, 25, 27, 28). Rossi, and colleagues (19) found that sex differences in the relationship between CRP and adiposity were mediated by leptin. Variants in the leptin receptor have previously been shown to correlate with circulating CRP concentrations (29). Thus, it is possible that leptin is partly responsible for sex-mediated differences in CRP, but teasing apart the contributions of leptin versus adiposity will be difficult, and there are likely other, as yet unidentified factors.

Lifestyle/anthropometric

As others have noted, concentrations of many biomarkers of inflammation increase among individuals with a higher BMI (4, 12, 30–32). Obesity is now recognized as an inflammatory condition resulting largely from adipose tissue infiltration of macrophages and other immune cells and their proinflammatory activation, leading to metabolic dysfunction characterized by increased circulating free fatty acids and insulin resistance (1, 33, 34). Correspondingly, overweight and obese individuals have higher mean CRP concentrations compared with adults with BMI in the normal range (5, 12, 30, 31). Our results for CRP were in agreement with previous studies, while the other markers in our panel were not higher among overweight/obese individuals with the exception of modestly higher concentrations of the sTNFRs. It is not clear why the other inflammatory markers in our panel were

Table 2. Correlation matrix: biomarkers of inflammation

Biomarkers	CRP (mg/L) ($N = 217$)	PGE-M (ng/mg creatinine) ($N = 210$)	sTNFRI (pg/mL) ($N = 217$)	sTNFRII (pg/mL) ($N = 217$)	TNFα (pg/mL) ($N = 217$)	IL1β (pg/mL) ($N = 217$)	IL6 (pg/mL) ($N = 217$)	IL8 (pg/mL) ($N = 217$)
CRP (mg/L)	1							
PGE-M (ng/mg creatinine)	0.13 ($P = 0.06$)	1						
sTNFRI (pg/mL)	0.14 ($P = 0.04$)	0.07 ($P = 0.32$)	1					
sTNFRII (pg/mL)	0.23 ($P = 0.0006$)	0.17 ($P = 0.01$)	0.50 ($P < 0.0001$)	1				
TNFα (pg/mL)	0.08 ($P = 0.25$)	0.14 ($P = 0.04$)	0.23 ($P = 0.0006$)	0.16 ($P = 0.02$)	1			
IL1β (pg/mL)	0.04 ($P = 0.59$)	−0.01 ($P = 0.88$)	0.13 ($P = 0.07$)	−0.02 ($P = 0.74$)	0.73 ($P < 0.0001$)	1		
IL6 (pg/mL)	0.19 ($P = 0.0053$)	0.05 ($P = 0.49$)	0.24 ($P = 0.0005$)	0.12 ($P = 0.08$)	0.73 ($P < 0.0001$)	0.80 ($P < 0.0001$)	1	
IL8 (pg/mL)	0.13 ($P = 0.06$)	0.10 ($P = 0.14$)	0.17 ($P = 0.01$)	0.16 ($P = 0.02$)	0.71 ($P < 0.0001$)	0.66 ($P < 0.0001$)	0.72 ($P < 0.0001$)	1

NOTE: All values log-transformed prior to correlation analysis.

not associated with BMI. In particular, IL1β has been linked with visceral obesity (35, 36) and TNFα is positively correlated with overall increasing adiposity (37). Both are produced by activated macrophages and have strong proinflammatory potential in addition to involvement in other diverse cellular functions (37–40).

Regular physical activity is fairly consistently associated with lower concentrations of many markers of systemic inflammation (15, 39–42); however, the only markers that were significantly decreased with increased physical activity in our panel were CRP and sTNFRII. One of the mechanisms by which physical activity may reduce inflammation, other than through reducing body mass which was adjusted for in our analysis (43), is protection from cardiovascular disease and diabetes, which are associated with increased inflammation. There is also some evidence that exercise elevates antioxidant defenses (43, 44).

CRP concentrations were higher among individuals who reported smoking 18 or more pack-years compared with never-smokers, with a similar but borderline significant association with PGE-M. The association between smoking and increased concentrations of inflammatory markers has long been recognized (4, 45–47). In addition to activation of NF-κB, a transcription factor and central mediator of the inflammatory response, exposure to cigarette smoke increases production of reactive oxygen species (46), and COX, a rate-limiting enzyme in the production of PGE_2 (48, 49).

Alcohol consumption was inversely associated with one biomarker, sTNFRII, thought to act as a buffer by removing TNFα from circulation but also itself is involved in apoptotic and proinflammatory signaling in response to TNFα binding (50). Concentrations typically rise in parallel to increasing TNFα concentrations (50). As plasma TNFα concentrations are generally much lower, and measurement less accurate owing to interactions with their cognate receptors, sTNFRI and II are often used as surrogate measures of TNFα concentrations (50). Data evaluating the association of alcohol intake and concentrations of these soluble receptors in healthy adults are sparse. Results of a population-based inflammation index based on literature-derived dietary intake and a panel of six inflammation markers found that alcohol intake at approximately 14 g/day was associated with decreased systemic inflammation (51), although sTNFRI and II were not included in the panel. Polyphenols such as resveratrol in red wine (52) and hops in beer (53) may contribute to an anti-inflammatory effect through inhibition of the transcription factor NF-κB (54).

Medication use

Although the use of aspirin and NSAIDs might be expected to reduce biomarkers of inflammation, clinical trials of aspirin and NSAIDs have not consistently shown reduction in the markers of inflammation (55–59). Among three NSAID exposures analyzed (use of low-dose aspirin, regular aspirin, and nonaspirin NSAIDs) and 8 biomarkers, only use of low-dose aspirin was associated with significantly lower concentrations of PGE-M. PGE-M is a stable metabolite of PGE_2 and is used as a surrogate measure of systemic PGE_2 concentrations (60). By irreversibly acetylating COX-1 and modifying the activity of COX-2, aspirin inhibits the enzymes involved in the conversion of arachidonic acid into proinflammatory prostaglandins (61). There is also evidence that aspirin is involved in inflammation resolution programs through enhanced conversion of resolvins, protectins, and other

Table 3. Demographic and lifestyle factors and their associations with CRP, PGE-M, and sTNFRs in the VITAL biomarker study[a]

		CRP ($N = 217$)		PGE-M ($N = 210$)		sTNFRI ($N = 217$)		sTNFRII ($N = 217$)	
	***N* (%)**	**Ratio[b]**	**95% CI**	**Ratio[b]**	**95% CI**	**Ratio[b]**	**95% CI**	**Ratio[b]**	**95% CI**
R^2 values[c]		**0.44**		**0.08**		**0.1**		**0.3**	
Demographic									
Age (years)									
50–<55	53 (24.4)	1	Ref	1	Ref	1	Ref	1	Ref
55–<60	53 (24.4)	1.26	0.85–1.87	1	0.78–1.27	1.05	0.93–1.19	1.01	0.91–1.12
60–<65	37 (17.1)	1.45	0.94–2.23	1.03	0.79–1.35	1.1	0.95–1.26	1.06	0.94–1.19
65–<70	30 (13.8)	1.51	0.95–2.41	1.17	0.88–1.57	1.12	0.96–1.3	1.12	0.99–1.26
70+	44 (20.3)	1.58	1.04–2.41	1.37	1.06–1.78	1.3	1.14–1.49	1.41	1.26–1.58
$P_{continuous}$			0.02		0.009		<0.001		<0.001
Sex									
Female	106 (48.9)	1	Ref	1	Ref	1	Ref	1	Ref
Male	111 (51.2)	0.45	0.34–0.59	1.2	1.01–1.42	1.09	1–1.2	1.04	0.97–1.12
$P_{difference}$			<0.001		0.04		<0.05		0.29
Lifestyle/anthropometric									
BMI (kg/m^2)									
<25	83 (38.3)	1	Ref	1	Ref	1	Ref	1	Ref
25–<30	97 (44.7)	1.56	1.14–2.13	0.98	0.81–1.19	1.06	0.96–1.18	1.12	1.03–1.22
30+	37 (17.1)	3.62	2.41–5.44	1.21	0.94–1.56	1.14	1–1.3	1.17	1.05–1.3
$P_{continuous}$			<0.001		0.41		<0.05		0.005
Current physical activity (moderate/vigorous)									
None	119 (55.4)	1	Ref	1	Ref	1	Ref	1	Ref
Any	96 (44.7)	0.67	0.51–0.89	1.01	0.85–1.2	0.98	0.89–1.07	0.92	0.86–1
$P_{difference}$			0.005		0.88		0.61		0.04
Smoking (pack-years)									
Never-smokers	109 (50.2)	1	Ref	1	Ref	1	Ref	1	Ref
Below median (<18)	55 (25.4)	1.13	0.81–1.58	1.01	0.82–1.24	1.09	0.97–1.21	1.09	1–1.19
Above median (18+)	53 (24.4)	1.52	1.08–2.12	1.25	1.01–1.53	0.99	0.89–1.11	0.98	0.89–1.07
P_{trend}			0.02		0.05		0.9		0.87
Alcohol (grams/week)									
Low tertile (<3)	72 (33.3)	1	Ref	1	Ref	1	Ref	1	Ref
Mid tertile (3–<56)	72 (33.3)	1.08	0.77–1.51	0.94	0.76–1.16	1.07	0.96–1.19	0.92	0.84–1
High tertile (56+)	72 (33.3)	0.92	0.65–1.3	0.95	0.76–1.17	0.99	0.89–1.11	0.84	0.77–0.92
Global *P*			0.64		0.80		0.32		<0.006
Current medication use									
Low-dose aspirin									
No	155 (73.1)	1	Ref	1	Ref	1	Ref	1	Ref
Low (1–3 days/week)	20 (9.43)	0.9	0.55–1.46	0.99	0.74–1.33	0.96	0.82–1.13	1.01	0.89–1.15
High (4+ days/week)	37 (17.5)	1.11	0.75–1.62	0.76	0.6–0.97	1	0.88–1.13	0.99	0.89–1.09
P_{trend}			0.72		0.04		0.87		0.84
HRT (women only)									
No	46 (45.1)	1	Ref	1	Ref	1	Ref	1	Ref
Yes	56 (54.9)	1.74	1.18–2.58	0.91	0.72–1.13	0.97	0.84–1.13	0.9	0.8–1.02
$P_{difference}$			0.006		0.38		0.71		0.1
Medical history									
History of CVD									
No	187 (86.2)	1	Ref	1	Ref	1	Ref	1	Ref
Yes	30 (13.8)	0.62	0.41–0.95	1.02	0.79–1.32	0.99	0.86–1.13	1.01	0.9–1.13
$P_{difference}$			0.03		0.90		0.85		0.89
History of cancer									
No	179 (82.5)	1	Ref	1	Ref	1	Ref	1	Ref
Yes	38 (17.5)	1.29	0.90–1.85	1.23	0.98–1.54	1.12	1–1.26	1.12	1.02–1.24
$P_{difference}$			0.16		0.08		0.05		0.02
Dietary intake									
Energy (kcal/day)									
Q1 (<1,373)	55 (25.7)	1	Ref	1	Ref	1	Ref	1	Ref
Q2 (1,373–<1,769)	51 (23.8)	1.76	1.18–2.62	0.95	0.74–1.22	1.07	0.94–1.21	1.01	0.91–1.13
Q3 (1,769–<2,220)	54 (25.2)	1.41	0.92–2.15	0.85	0.65–1.11	0.95	0.83–1.09	0.94	0.84–1.06
Q4 (2,220+)	54 (25.2)	1.11	0.72–1.72	0.81	0.62–1.07	0.91	0.79–1.05	0.9	0.8–1.02
$P_{continuous}$			0.95		0.25		0.07		0.03
Saturated fat (g/day)									
Q1 (<14.5)	54 (25.2)	1	Ref	1	Ref	1	Ref	1	Ref
Q2 (14.5–<20)	52 (24.3)	1.96	1.31–2.94	1.09	0.85–1.41	1.1	0.97–1.26	1.07	0.96–1.19
Q3 (20–<27.5)	54 (25.2)	1.75	1.14–2.68	1.09	0.84–1.43	1.16	1.01–1.34	1.13	1.01–1.27
Q4 (27.5+)	54 (25.2)	2.61	1.45–4.71	1.38	0.95–2	1.2	0.99–1.46	1.13	0.97–1.33
$P_{continuous}$			0.004		0.42		0.25		<0.05

(Continued on the following page)

Table 3. Demographic and lifestyle factors and their associations with CRP, PGE-M, and sTNFRs in the VITAL biomarker study[a] (Cont'd)

		CRP ($N = 217$)		PGE-M ($N = 210$)		sTNFRI ($N = 217$)		sTNFRII ($N = 217$)	
	N (%)	Ratio[b]	95% CI	Ratio[b]	95% CI	Ratio[b]	95% CI	Ratio[b]	95% CI
R^2 values[c]		**0.44**		**0.08**		**0.1**		**0.3**	
γ-Tocopherol (mg/day)									
Q1 (<9.5)	55 (25.7)	1	Ref	1	Ref	1	Ref	1	Ref
Q2 (9.5–<13)	52 (24.3)	1.56	1.02–2.37	1.03	0.79–1.34	0.94	0.82–1.08	1.02	0.91–1.14
Q3 (13–<17.1)	54 (25.2)	1.47	0.93–2.34	0.98	0.74–1.31	0.94	0.81–1.09	0.95	0.84–1.07
Q4 (17.1+)	53 (24.8)	2.11	1.15–3.88	1.05	0.72–1.53	0.97	0.8–1.18	1.01	0.86–1.19
$P_{continuous}$			0.04		0.40		0.33		0.33
Vitamin C (mg/day)									
Q1 (<73)	55 (25.7)	1	Ref	1	Ref	1	Ref	1	Ref
Q2 (73–<113)	54 (25.2)	1.42	0.96–2.09	1.03	0.81–1.32	1.04	0.92–1.18	0.99	0.89–1.1
Q3 (113–<162)	51 (23.8)	0.99	0.65–1.51	0.93	0.72–1.21	0.93	0.81–1.07	0.96	0.86–1.08
Q4 (162+)	54 (25.2)	0.8	0.53–1.22	0.9	0.69–1.17	1	0.87–1.14	0.93	0.83–1.04
$P_{continuous}$			0.01		0.62		0.67		0.08
Vitamin D (mcg/day)									
Q1 (<3.5)	54 (25.2)	1	Ref	1	Ref	1	Ref	1	Ref
Q2 (3.5–<5.1)	53 (24.8)	0.88	0.6–1.29	0.66	0.52–0.84	1.06	0.93–1.21	0.92	0.83–1.02
Q3 (5.1–<7.3)	53 (24.8)	0.75	0.50–1.13	0.68	0.53–0.88	1.05	0.92–1.21	0.99	0.89–1.11
Q4 (7.3+)	54 (25.2)	0.33	0.2–0.55	0.77	0.57–1.05	0.98	0.83–1.15	0.93	0.81–1.06
$P_{continuous}$			<0.001		0.28		0.55		0.82
EPA+DHA (g/day)									
Q1 (<0.1)	55 (25.7)	1	Ref	1	Ref	1	Ref	1	Ref
Q2 (0.1–<0.17)	53 (24.8)	0.76	0.52–1.13	1.01	0.79–1.29	0.94	0.82–1.06	0.9	0.81–1
Q3 (0.17–<0.28)	52 (24.3)	0.81	0.55–1.21	0.91	0.71–1.16	0.97	0.85–1.1	0.97	0.88–1.08
Q4 (0.28+)	54 (25.2)	0.48	0.32–0.72	0.88	0.68–1.13	0.96	0.84–1.1	0.92	0.83–1.02
$P_{continuous}$			<0.001		0.5		0.33		0.08
Dietary + supplemental intake									
Fiber (g/day)									
Q1 (<13.8)	53 (24.9)	1	Ref	1	Ref	1	Ref	1	Ref
Q2 (13.8–<19.1)	53 (24.9)	1.05	0.71–1.57	1.28	1.01–1.63	0.93	0.81–1.06	0.98	0.88–1.09
Q3 (19.1–<25.3)	53 (24.9)	0.78	0.51–1.21	1.03	0.8–1.34	0.91	0.79–1.05	0.96	0.86–1.08
Q4 (25.3+)	54 (25.4)	0.55	0.35–0.89	0.79	0.59–1.05	0.91	0.78–1.06	0.98	0.86–1.11
$P_{continuous}$			0.04		0.22		0.46		0.42
EPA+DHA (g/day)									
Q1 (<0.1)	55 (25.7)	1	Ref	1	Ref	1	Ref	1	Ref
Q2 (0.1–<0.19)	52 (24.3)	0.96	0.65–1.43	1.03	0.8–1.32	0.94	0.82–1.07	0.93	0.83–1.03
Q3 (0.19–<0.32)	53 (24.8)	0.9	0.61–1.34	0.93	0.72–1.19	0.96	0.85–1.1	0.94	0.85–1.05
Q4 (0.32+)	54 (25.2)	0.56	0.38–0.84	0.88	0.68–1.13	0.92	0.81–1.05	0.93	0.83–1.04
$P_{continuous}$			0.07		0.91		0.94		0.88

Abbreviations: CI, confidence interval; CVD, cardiovascular disease.

[a]The above table includes factors associated with at least one biomarker. Factors not associated with any of the biomarkers of inflammation at the $\alpha = 0.05$ level are not presented, including: race/ethnicity, regular aspirin use, non-aspirin NSAID use, use of cholesterol-lowering drugs, multivitamin use, history of diabetes, education, dietary fiber intake, dietary α-tocopherol intake, dietary β-carotene intake, supplemental fiber use, supplemental vitamin E use, supplemental vitamin C use, supplemental vitamin D use, supplemental β-carotene use, supplemental fish oil use, dietary + supplemental α-tocopherol consumption, dietary + supplemental vitamin C consumption, dietary + supplemental vitamin D consumption, and dietary + supplemental β-carotene consumption.

[b]eβ in a model in which the dependent variable is ln (biomarker). All models were adjusted for age (continuous), sex, and BMI (continuous), except the models for age as categories include age (categorical), sex, and BMI (continuous) and the models for BMI as categories include age (continuous), sex, and BMI (categorical). All dietary and dietary + supplement exposures were additionally adjusted for energy intake.

[c]Includes all individually significant factors ($P < 0.05$) for the biomarker in a single model.

proresolution mediators derived from long-chain omega-3 fatty acids (62). These mediators serve to halt the production and trafficking of proinflammatory factors (63). While the effects of aspirin on PGE_2 have been well documented (64), few studies have directly evaluated the association of aspirin use and PGE-M concentrations in urine, with inconsistent results (65, 66).

Medical history

Contrary to the majority of the literature showing higher CRP concentrations with cardiovascular disease (67), in our analysis, a history of cardiovascular disease was associated with lower CRP. It is important to note that our evaluation was based on previous disease history. In response to disease diagnosis, many individuals make diet and lifestyle changes and begin pharmaceutical interventions. In our data, the association did not attenuate with adjustment for use of statins or aspirin. Many studies show a positive association between higher circulating inflammation concentrations and cancer incidence (3, 68, 69). The only biomarkers in our study that were associated with a history of cancer were the soluble TNF receptors, and the concentrations were only modestly higher in those with a history of cancer compared to those without. As with cardiovascular disease, it is likely that cancer survivors make lifestyle changes after diagnosis, and higher systemic inflammation will only be captured close to the time of ongoing cancer pathology.

Dietary intake

A growing body of evidence suggests that diet and dietary patterns can influence concentrations of biomarkers of systemic inflammation (51, 70). Of the dietary factors evaluated, a

Table 4. Demographic and lifestyle factors and their associations with TNFα, IL1β, IL6, and IL8 in the VITAL biomarker study[a]

		TNFα ($N = 217$)		IL1β ($N = 217$)		IL6 ($N = 217$)		IL8 ($N = 217$)	
	***N* (%)**	**Ratio[b]**	**95% CI**	**Ratio[b]**	**95% CI**	**Ratio[b]**	**95% CI**	**Ratio[b]**	**95% CI**
R^2 values[c]		**0.08**		**0.06**		**0.04**		**0.04**	
Demographic									
Age (years)									
50–<55	53 (24.4)	1	Ref	1	Ref	1	Ref	1	Ref
55–<60	53 (24.4)	1.29	0.92–1.81	1.07	0.59–1.93	1.45	0.81–2.61	1.29	0.98–1.68
60–<65	37 (17.1)	1.26	0.87–1.82	1	0.52–1.91	1.6	0.84–3.05	1.22	0.91–1.64
65–<70	30 (13.8)	1.37	0.92–2.03	1.09	0.54–2.18	1.57	0.79–3.14	1.29	0.94–1.77
70+	44 (20.3)	1.59	1.11–2.27	1.26	0.67–2.36	2.1	1.12–3.93	1.46	1.1–1.94
$P_{continuous}$			0.01		0.44		0.02		0.01
Sex									
Female	106 (48.9)	1	Ref	1	Ref	1	Ref	1	Ref
Male	111 (51.2)	0.91	0.72–1.15	0.8	0.53–1.21	0.63	0.42–0.95	0.91	0.75–1.1
$P_{difference}$			0.42		0.29		0.03		0.33
Lifestyle/anthropometric									
BMI (kg/m^2)									
<25	83 (38.3)	1	Ref	1	Ref	1	Ref	1	Ref
25–<30	97 (44.7)	0.79	0.61–1.03	0.66	0.42–1.05	1.02	0.64–1.63	0.94	0.76–1.16
30+	37 (17.1)	1.07	0.76–1.5	0.78	0.43–1.42	1.39	0.76–2.54	0.92	0.7–1.21
$P_{continuous}$			0.63		0.74		0.16		0.58
Current physical activity (moderate/vigorous)									
None	119 (55.4)	1	Ref	1	Ref	1	Ref	1	Ref
Any	96 (44.7)	0.84	0.66–1.07	0.8	0.53–1.22	0.83	0.54–1.25	0.94	0.78–1.14
$P_{difference}$			0.15		0.31		0.37		0.52
Smoking (pack-years)									
Never-smokers	109 (50.2)	1	Ref	1	Ref	1	Ref	1	Ref
Below median (<18)	55 (25.4)	1.29	0.97–1.71	1.12	0.67–1.85	1.27	0.77–2.1	1.03	0.82–1.3
Above median (18+)	53 (24.4)	1.17	0.88–1.56	1.19	0.71–1.97	1.34	0.81–2.22	1.08	0.86–1.37
P_{trend}			0.19		0.49		0.22		0.49
Alcohol (grams/week)									
Low tertile (<3)	72 (33.3)	1	Ref	1	Ref	1	Ref	1	Ref
Mid tertile (3–<56)	72 (33.3)	1.18	0.88–1.57	1.53	0.93–2.53	1.31	0.79–2.17	1.07	0.85–1.34
High tertile (56+)	72 (33.3)	0.93	0.69–1.25	1.09	0.65–1.81	1.02	0.61–1.71	0.87	0.69–1.1
Global *P*			0.25		0.20		0.49		0.21
Current medication use									
Low-dose aspirin									
No	155 (73.1)	1	Ref	1	Ref	1	Ref	1	Ref
Low (1–3 days/week)	20 (9.43)	1.18	0.78–1.78	1.4	0.68–2.87	1.18	0.57–2.44	1.05	0.76–1.44
High (4+ days/week)	37 (17.5)	1.28	0.92–1.77	1.71	0.97–3.01	1.29	0.73–2.29	1.11	0.87–1.43
P_{trend}			0.12		0.05		0.35		0.39
HRT (women only)									
No	46 (45.1)	1	Ref	1	Ref	1	Ref	1	Ref
Yes	56 (54.9)	1.08	0.76–1.53	1.18	0.62–2.26	1.17	0.65–2.1	1.09	0.83–1.43
$P_{difference}$			0.67		0.60		0.60		0.55
Medical history									
History of CVD									
No	187 (86.2)	1	Ref	1	Ref	1	Ref	1	Ref
Yes	30 (13.8)	1.17	0.81–1.68	1.3	0.69–2.45	1.25	0.67–2.36	1.3	0.98–1.73
$P_{difference}$			0.4		0.41		0.48		0.07
History of cancer									
No	179 (82.5)	1	Ref	1	Ref	1	Ref	1	Ref
Yes	38 (17.5)	0.89	0.65–1.21	0.72	0.42–1.23	0.82	0.48–1.4	1.3	0.98–1.73
$P_{difference}$			0.44		0.23		0.46		0.44
Dietary intake									
Energy (kcal/day)									
Q1 (<1,373)	55 (25.7)	1	Ref	1	Ref	1	Ref	1	Ref
Q2 (1,373–<1,769)	51 (23.8)	1.11	0.78–1.57	1.35	0.74–2.47	1.12	0.61–2.06	1.16	0.88–1.53
Q3 (1,769–<2,220)	54 (25.2)	0.97	0.67–1.4	1.13	0.59–2.14	1.08	0.57–2.06	0.93	0.70–1.25
Q4 (2,220+)	54 (25.2)	0.97	0.67–1.42	1.21	0.62–2.34	1.08	0.55–2.1	1.09	0.81–1.47
$P_{continuous}$			0.66		0.87		0.61		0.95
Saturated fat (g/day)									
Q1 (<14.5)	54 (25.2)	1	Ref	1	Ref	1	Ref	1	Ref
Q2 (14.5–<20)	52 (24.3)	0.86	0.6–1.21	0.69	0.38–1.26	0.76	0.41–1.4	0.8	0.61–1.06
Q3 (20–<27.5)	54 (25.2)	1.24	0.86–1.8	1.9	1.01–3.57	1.51	0.8–2.89	0.98	0.73–1.33
Q4 (27.5+)	54 (25.2)	1.66	1–2.75	2.66	1.11–6.34	2.05	0.84–5	0.9	0.59–1.35
$P_{continuous}$			0.02		0.004		0.07		0.82

(Continued on the following page)

Table 4. Demographic and lifestyle factors and their associations with TNFα, IL1β, IL6, and IL8 in the VITAL biomarker study[a] (Cont'd)

		TNFα ($N = 217$)		IL1β ($N = 217$)		IL6 ($N = 217$)		IL8 ($N = 217$)	
	N (%)	Ratio[b]	95% CI	Ratio[b]	95% CI	Ratio[b]	95% CI	Ratio[b]	95% CI
R^2 values[c]		0.08		0.06		0.04		0.04	
γ-Tocopherol (mg/day)									
Q1 (<9.5)	55 (25.7)	1	Ref	1	Ref	1	Ref	1	Ref
Q2 (9.5–<13)	52 (24.3)	0.85	0.59–1.22	0.8	0.43–1.51	0.93	0.49–1.76	0.86	0.65–1.15
Q3 (13–<17.1)	54 (25.2)	0.97	0.65–1.45	1.22	0.61–2.45	1.05	0.52–2.12	0.95	0.69–1.3
Q4 (17.1+)	53 (24.8)	0.86	0.51–1.46	1.02	0.41–2.54	1.03	0.41–2.58	1.06	0.7–1.61
$P_{continuous}$			0.38		0.44		0.97		0.22
Vitamin C (mg/day)									
Q1 (<73)	55 (25.7)	1	Ref	1	Ref	1	Ref	1	Ref
Q2 (73–<113)	54 (25.2)	0.95	0.68–1.32	1.23	0.68–2.2	1.17	0.65–2.1	0.91	0.7–1.19
Q3 (113–<162)	51 (23.8)	0.87	0.6–1.24	1.44	0.77–2.71	1.13	0.6–2.13	1.02	0.77–1.36
Q4 (162+)	54 (25.2)	1.02	0.71–1.47	1.27	0.67–2.39	1.54	0.81–2.9	1.14	0.85–1.52
$P_{continuous}$			0.36		0.64		0.87		0.97
Vitamin D (mcg/day)									
Q1 (<3.5)	54 (25.2)	1	Ref	1	Ref	1	Ref	1	Ref
Q2 (3.5–<5.1)	53 (24.8)	0.97	0.69–1.37	1.19	0.66–2.16	1.3	0.72–2.36	0.87	0.67–1.15
Q3 (5.1–<7.3)	53 (24.8)	0.94	0.65–1.35	1.13	0.6–2.15	1.11	0.59–2.11	0.91	0.68–1.21
Q4 (7.3+)	54 (25.2)	0.81	0.52–1.25	0.78	0.36–1.68	0.71	0.33–1.52	0.84	0.59–1.19
$P_{continuous}$			0.59		0.79		0.60		0.78
EPA+DHA (g/day)									
Q1 (<0.1)	55 (25.7)	1	Ref	1	Ref	1	Ref	1	Ref
Q2 (0.1–<0.17)	53 (24.8)	0.85	0.61–1.19	0.86	0.47–1.55	0.77	0.42–1.39	0.86	0.66–1.13
Q3 (0.17–<0.28)	52 (24.3)	0.75	0.53–1.06	0.68	0.37–1.24	0.71	0.39–1.31	0.81	0.62–1.07
Q4 (0.28+)	54 (25.2)	0.75	0.53–1.07	0.83	0.45–1.53	0.81	0.44–1.5	0.85	0.64–1.13
$P_{continuous}$			0.04		0.39		0.35		0.36
Dietary + supplemental intake									
Fiber (g/day)									
Q1 (<13.8)	53 (24.9)	1	Ref	1	Ref	1	Ref	1	Ref
Q2 (13.8–<19.1)	53 (24.9)	1.1	0.77–1.55	1.58	0.87–2.88	1.38	0.75–2.52	1.33	1.02–1.73
Q3 (19.1–<25.3)	53 (24.9)	1.11	0.76–1.62	1.3	0.68–2.48	1.09	0.56–2.09	1.27	0.95–1.68
Q4 (25.3+)	54 (25.4)	0.97	0.65–1.47	1.09	0.53–2.21	1.09	0.53–2.22	1.4	1.03–1.92
$P_{continuous}$			0.24		0.18		0.40		0.39
EPA+DHA (g/day)									
Q1 (<0.1)	55 (25.7)	1	Ref	1	Ref	1	Ref	1	Ref
Q2 (0.1–<0.19)	52 (24.3)	0.84	0.60–1.18	0.87	0.48–1.58	0.67	0.37–1.22	0.81	0.62–1.07
Q3 (0.19–<0.32)	53 (24.8)	0.68	0.48–0.96	0.68	0.37–1.24	0.64	0.35–1.17	0.79	0.6–1.04
Q4 (0.32+)	54 (25.2)	0.71	0.50–1	0.63	0.34–1.15	0.57	0.31–1.05	0.73	0.55–0.96
$P_{continuous}$			0.12		0.03		0.12		0.18

Abbreviations: CI, confidence interval; CVD, cardiovascular disease.

[a]The above table includes factors associated with at least one biomarker. Factors not associated with any of the biomarkers of inflammation at the $\alpha = 0.05$ level are not presented, including race/ethnicity, regular aspirin use, non-aspirin NSAID use, use of cholesterol-lowering drugs, multivitamin use, history of diabetes, education, dietary fiber intake, dietary α-tocopherol intake, dietary β-carotene intake, supplemental fiber use, supplemental vitamin E use, supplemental vitamin C use, supplemental vitamin D use, supplemental β-carotene use, supplemental fish oil use, dietary + supplemental α-tocopherol consumption, dietary + supplemental vitamin C consumption, dietary + supplemental vitamin D consumption, and dietary + supplemental β-carotene consumption.

[b]eβ in a model in which the dependent variable is ln (biomarker). All models were adjusted for age (continuous), sex, and BMI (continuous), except the models for age as categories include age (categorical), sex, and BMI (continuous) and the models for BMI as categories include age (continuous), sex, and BMI (categorical). All dietary and dietary + supplement exposures were additionally adjusted for energy intake.

[c]Includes all individually significant factors ($P < 0.05$) for the biomarker in a single model.

consistent pattern was observed between increasing intakes of saturated fat and increasing concentrations of CRP, sTNFRII, TNFα, and IL1β, with concentrations more than two and a half times greater in the highest quartile of intake (vs. the lowest) for CRP and IL1β. In addition, IL6 concentrations were twice as high for those in the highest quartile of saturated fat, but this result was not statistically significant. Saturated fat has been hypothesized to contribute to inflammation through activation of toll-like receptor (TLR) pathways involved in innate immunity, particularly TLR-4 (71). TLRs, in turn, stimulate NF-κB, a transcription factor that regulates a battery of immune factors, including cytokines and chemokines (72).

A systematic review recently assessed the association of saturated fat and circulating concentrations of inflammation and adipokines. Data were available for several markers of inflammation, including intercellular and vascular adhesion molecules (ICAM-1 and VCAM-1, respectively), CRP, IL6, TNFα, and adiponectin (73). Higher intakes of saturated fat were positively associated with CRP, IL6, and ICAM1 (73). In another study, erythrocyte fatty acid composition in 55 healthy adults was compared with serum CRP and IL6 concentrations (74). In multivariate models, total saturated fatty acids were modestly associated with IL6 ($P = 0.05$) and CRP ($P = 0.06$), but palmitic and stearic acids, specifically, were significantly associated with IL6 ($P = 0.006$) and CRP ($P = 0.04$), respectively (74). Finally, a population-based dietary inflammatory index developed by Shivappa and colleagues (51), which scored the inflammatory potential for a variety of dietary components based on the extensive

primary literature evaluating dietary associations with a panel of inflammation markers (CRP, IL1β, IL4, IL6, IL10, and TNFα), found an overall positive association of saturated fat and inflammation. Taken together, these results align with our findings of higher inflammation concentrations with increased dietary intakes of saturated fat.

EPA and DHA omega-3 PUFAs, from diet or diet plus supplemental sources, were associated with lower concentrations of CRP, TNFα, and IL1β. Both omega-3 and omega-6 PUFAs are essential nutrients that are incorporated into tissue membranes and have a variety of physiologic roles, including mediation of inflammation (75). Although both classes of PUFAs are structural components of eicosanoid-related pathways, those derived from omega-6 are generally proinflammatory, whereas those produced from omega-3 tend to have opposing, less inflammatory effects (76, 77). The long-chain PUFAs EPA and DHA are found in fish oils (78). Moreover, omega-3 PUFAs are precursors of proresolving mediators, which serve to dampen the immune response (79). Most studies evaluating the association of omega-3 PUFAs and markers of inflammation do so in the context of disease risk, for example, cardiovascular disease, cancer, rheumatoid arthritis, etc. (80), rather than inflammation *per se*. According to the inflammatory index referenced earlier, there was substantial anti-inflammatory potential with omega-3 fatty acids among >2,000 studies evaluated (51).

Four dietary factors were only associated with CRP: dietary vitamins C and D and fiber from diet plus supplements were associated with decreased CRP, and dietary γ-tocopherol intake was associated with increased CRP. Through donation of an electron, vitamin C functions as a cofactor in several enzymatic reactions and neutralizes free radicals (81). Of note, while dietary vitamin C was significantly associated with lower CRP concentrations, dietary plus supplemental vitamin C was not, suggesting that the association may be due to other constituents in foods high in vitamin C, for example, polyphenols, rather than vitamin C specifically. Vitamin D plays a role in immune function, and deficiency has been associated with a number of inflammatory diseases, including inflammatory bowel disease (82), rheumatoid arthritis (83), and multiple sclerosis (83). With respect to inflammatory cytokine production, binding of vitamin D_3 to its receptor inhibits NF-κB activation and signaling (83). Dietary fiber may lower inflammation through several mechanisms, including slower absorption of glucose and regulation of insulin sensitivity (84), and inducing favorable shifts in gut microbiota, which play a role in immunity through interaction with toll-like receptors in the gastrointestinal tract (85). Our results for vitamins C and D and fiber are in agreement with the inflammatory index (51). γ-tocopherol, the most common form of vitamin E, was not included due to limited research in humans. Other dietary factors associated with inflammation in our analysis were observed, but there were no consistent patterns and thus may have been chance findings.

Several limitations of this analysis should be noted. Most exposures were based on self-report and are subject to measurement error. However, we attempted to reduce this error in the dietary exposures by using the average of two FFQ and to reduce the error in relation to supplement use by recording information taken directly from product bottles in the homes of the participants. The biomarkers also are subject to error. The ILs and TNFα were multiplexed on a 4-plex assay, which may have had less precision than measuring each marker independently. As evidenced in the correlation matrix (Table 2), biomarkers included in the 4-plex assay (TNFα, IL1β, IL6, and IL8) were correlated, while no specific patterns emerged for the remaining biomarkers. Furthermore, the biomarkers which were multiplexed had higher CVs, although all CVs were <15%. As samples were stored for several years, some degradation may have taken place. Whereas CRP has been shown to be a stable marker over time and is not sensitive to diurnal fluctuations, less information is known about the other markers. These effects would likely have attenuated the associations between the exposures and biomarkers.

We also note that some of the significant trends for the dietary exposures were not linear. For example, the second quintile of vitamin C intake was associated with higher CRP while the third and fourth were associated with lower CRP concentrations. This may suggest that protective effects are only observed with higher intakes. For saturated fat, we observed the converse; the second quintile was associated with lower concentrations of some inflammation biomarkers, but intakes above that level were associated with increased inflammation biomarker concentrations. This suggests that any confounding effect of these factors may not be best modeled as linear.

Regarding the data analyses, our sample size was small, leading in many instances to wide confidence intervals. Furthermore, adjustment for multiple comparisons was not made, as our goal was to look for similarities in associations across the biomarkers and to inform researchers on what factors should be considered as potential confounders to be adjusted for in future studies. However, some of our findings, particularly those in the *P* value range of 0.01 to 0.05 could be due to chance. Although we did adjust for *a priori* selected confounders in our analyses, for example, age, sex, BMI, and energy intake, residual confounding by other factors may bias our results. For example, saturated fat or n-3 PUFA may be markers of unhealthy or healthy lifestyle or dietary habits, respectively, and these observed associations should be interpreted with caution. Although a higher percentage of variance was explained with all of the significant factors for CRP ($R^2 = 0.44$) and sTNFRII ($R^2 = 0.30$), the variance explained for the other markers was modest ($R^2 < 0.10$).

Finally, as our study participants were primarily Caucasian, our results may not be generalizable to other populations. Although other authors have noted that little variation in CRP is observed across different races (14, 19, 28), it is not clear whether the same might be expected for the other biomarkers.

In summary, age, BMI, dietary fat, and EPA+DHA intakes were the variables most consistently associated with circulating inflammation biomarkers, although the biomarkers did not vary in unison to dietary factors and other exposures. Some markers, for example, IL6, seem to be less perturbed by outside influences, whereas others were more responsive to lifestyle factors, for example, CRP and sTNFRII. This may reflect differential regulation, whereby various exposures are affecting different points in the pathways. These results suggest that numerous factors may contribute to variation in inflammation biomarker concentrations, but likely differ depending on the biomarker being evaluated.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Writing, review, and/or revision of the manuscript: S.L. Navarro, E.D. Kantor, X. Song, G.L. Milne, J.W. Lampe, M. Kratz, E. White
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): X. Song, E. White
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