Nutritional Epidemiology

Consumption of (n-3) Fatty Acids Is Related to Plasma Biomarkers of Inflammation and Endothelial Activation in Women[1]

Esther Lopez-Garcia,[*2] Matthias B. Schulze,[*] JoAnn E. Manson,[† ††‡‡] James B. Meigs,[**] Christine M. Albert,[‡ ‡‡] Nader Rifai,[§] Walter C. Willett,[*†] and Frank B. Hu[*† ††]

*Departments of *Nutrition and †Epidemiology, Harvard School of Public Health; Department of Medicine, Harvard Medical School, **General Medicine Division and ‡Cardiology Division, Massachusetts General Hospital and Harvard Medical School; ††The Channing Laboratory and ‡‡Division of Preventive Medicine, Harvard Medical School; and §Department of Laboratory Medicine, Children's Hospital and Department of Pathology, Harvard Medical School*

ABSTRACT   We evaluated the hypothesis that intake of (n-3) fatty acids is inversely associated with biomarkers of inflammation and endothelial activation. We conducted a cross-sectional study of 727 women from the Nurses' Health Study I cohort, aged 43–69 y, apparently healthy at time of a blood draw in 1990. Dietary intake was assessed by a validated FFQ in 1986 and 1990. C-reactive protein (CRP) levels were 29% lower among those in the highest quintile of total (n-3) fatty acids, compared with the lowest quintile; interleukin-6 (IL-6) levels were 23% lower, E-selectin levels 10% lower, soluble intracellular adhesion molecule (sICAM-1) levels 7% lower, and soluble vascular adhesion molecule (sVCAM-1) levels 8% lower. The intake of α-linolenic acid was inversely related to plasma concentrations of CRP ($\beta = -0.55$, $P = 0.02$), Il-6 ($\beta = -0.36$, $P = 0.01$), and E-selectin ($\beta = -0.24$, $P = 0.008$) after controlling for age, BMI, physical activity, smoking status, alcohol consumption, and intake of linoleic acid (n-6) and saturated fat. Long-chain (n-3) fatty acids (eicosapentaenoic and docosahexaenoic) were inversely related to sICAM-1 ($\beta = -0.11$, $P = 0.03$) and sVCAM-1 ($\beta = -0.17$, $P = 0.003$). Total (n-3) fatty acids had an inverse relation with CRP ($\beta = -0.44$, $P = 0.007$), IL-6 ($\beta = -0.26$, $P = 0.009$), E-selectin ($\beta = -0.17$, $P = 0.004$), sICAM-1 ($\beta = -0.07$, $P = 0.02$), and sVCAM-1 ($\beta = -0.10$, $P = 0.004$). These associations were not modified by intake of vitamin E, dietary fiber, *trans* fatty acids, or by the use of postmenopausal hormone therapy. In conclusion, this study suggests that dietary (n-3) fatty acids are associated with levels of these biomarkers reflecting lower levels of inflammation and endothelial activation, which might explain in part the effect of these fatty acids in preventing cardiovascular disease.   J. Nutr. 134: 1806–1811, 2004.

KEY WORDS: • (n-3) fatty acids • inflammation • endothelial activation • C-reactive protein • fish

Prospective cohort studies and secondary prevention trials indicate that higher intakes of (n-3) fatty acids from fish or plant sources lower the risk of cardiovascular disease (1). There are well-known mechanisms linking (n-3) fatty acids and cardiovascular disease, including reduction of serum triglycerides, decreased platelet aggregability, and antiarrhythmic effects (2). However, other mechanisms could also be involved. In vitro studies (3–6) showed that (n-3) fatty acids improve endothelial dysfunction, which is an early event in the development of atherosclerosis (7). However, clinical studies of (n-3) fatty acids and endothelial function are inconsistent (8–12). In addition, little information is available on the relation between dietary (n-3) fatty acid intake and plasma concentration of biomarkers of inflammation and endothelial activation in healthy individuals. In a previous study, Pischon et al. (13) found that the intake of (n-3) fatty acids was associated with plasma concentrations of biomarkers of inflammation, but they did not evaluate their effect on endothelial adhesion molecules, which reflect a more specific mechanism in endothelial activation.

In this study, we examined the intakes of both α-linolenic acid (ALA),[3] an 18-carbon chain (n-3) fatty acid [18:3(n-3)] from plant sources, and the long-chain (n-3) fatty acids eicosapentaenoic [(EPA) 20:5(n-3)] and docosahexaenoic [(DHA) 22:6(n-3)] from fish, in relation to biomarkers of inflammation and endothelial activation, including C-reactive protein (CRP), interleukin-6 (IL-6), soluble tumor necrosis factor receptor 2

[3] Abbreviations used: AA, arachidonic acid; ALA, α-linolenic acid; CRP, C-reactive protein; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; IL-6, interleukin-6; LA, linoleic acid; MET-h/wk, metabolic equivalent hours per week; sICAM-1, soluble intracellular adhesion molecule 1; sTNFR-2, soluble tumor necrosis factor receptor 2; sVCAM-1, soluble vascular adhesion molecule 1.

(sTNFR-2), E-selectin, and soluble cell adhesion molecules (sICAM-1 and sVCAM-1) in apparently healthy women.

SUBJECTS AND METHODS

Subjects. The Nurses' Health Study cohort was established in 1976 with 121,700 female registered nurses residing in the United States. Every 2 y, follow-up questionnaires were sent to update information on potential risk factors and to identify newly diagnosed cases of chronic diseases. The present study included 727 women who were selected as control subjects for an earlier nested case-control study of diabetes. These women had not been diagnosed with cardiovascular disease, cancer, or diabetes mellitus at the time of the blood draw. The mean age of women at that time was 56 y (range, 43–69 y). All participants gave written informed consent, and the Harvard School of Public Health Human Subjects Committee Review Board approved the study protocol.

Blood collection and assessment of the biomarkers. Blood was collected between 1989 and 1990. Women willing to provide blood specimens were sent instructions and a phlebotomy kit. Sodium heparin was used as anticoagulant. Blood specimens were returned by overnight mail on ice and 97% arrived within 26 h of phlebotomy. The samples were centrifuged ($1200 \times g$, 15 min) on arrival to separate plasma from buffy coat and red cells, and were frozen in liquid nitrogen until analysis. Quality control samples were routinely frozen along with study samples to monitor changes due to long-term storage and assay variability. All markers were measured in the Clinical Chemistry Laboratory at Children's Hospital in Boston. CRP levels were measured via a high-sensitivity latex-enhanced immunonephelometric assay on a BN II analyzer (Dade Behring). IL-6 was measured by a quantitative sandwich enzyme immunoassay technique (Quantikine HS Immunoassay kit) and sTNFR-2 levels by an ELISA kit utilizing immobilized monoclonal antibody to human TNFR-2 (Genzyme). Levels of E-selectin, sICAM-1, and sVCAM-1 were measured by Theyare ELISA (R&D Systems). The interassay CV for each marker was as follows: CRP, 3.4–3.8%; IL-6, 5.8–8.2%; sT-NFR-2, 3.6–5.1%; E-selectin, 6.4–6.6%; sICAM-1, 6.1–10.1%; and sVCAM-1, 8.5–10.2%. Processing times did not substantially affect the concentration of the markers (14).

Assessment of dietary intake. In 1986 and 1990, a semiquantitative FFQ was mailed to participants. The FFQ included 116 food items with specified serving sizes that were described by using natural portions or standard weight and volume measures of the servings commonly consumed in this study population. For each food item, participants indicated their mean frequency of consumption over the past year in terms of the specified serving size by checking 1 of the 9 frequency categories ranging from "almost never" to "≥6 times/d." In 1986 and 1990, the dietary questionnaire included the following 4 fish and seafood items: 1) dark-meat fish such as mackerel, salmon, sardines, bluefish, or swordfish (84–140 g or 3–5 oz); 2) canned tuna (84–112 g or 3–4 oz); 3) other fish (84–140 g or 3–5 oz); and 4) shrimp, lobster, or scallops as the main dish (98 g or 3.5 oz). The mean daily intake of nutrients was calculated by multiplying the frequency of consumption of each item by its nutrient content per serving and totaling the nutrient intake for all food items. Nutrient intakes were adjusted for total energy intake by the residual approach (15). We calculated the means of nutrient intakes in 1986 and 1990 to represent long-term dietary consumption and reduce measurement error.

The reproducibility and validity of the FFQs were described in detail elsewhere (16). The correlation coefficients between the calculated dietary fatty acids from the FFQ and the proportion of the fatty acids in adipose tissue were 0.34 ($P < 0.001$) for linolenic acid (LA), 0.37 ($P < 0.001$) for LA, 0.40 ($P < 0.001$) for *trans* fatty acids, and 0.16 ($P > 0.05$) for SFA (17). The correlation coefficient for linolenic acid intake between the 1986 and 1990 questionnaires was 0.48 ($P < 0.05$).

The calculation of EPA and DHA intake was described in detail elsewhere (18). Briefly, to calculate the intake of fish oil we assigned grams per serving as follows: 1.51 g for dark-meat fish, 0.42 g for canned tuna fish, 0.48 g for other fish, and 0.32 g for shrimp, lobster, or scallops. Within each category, these (n-3) fatty acid values were derived by weighting the mean values of (n-3) fatty acids for the most common types of fish according to the Harvard University Food Composition Database (compiled on 22 November 1993) derived from USDA sources and supplemented with manufacturer information. Intake of long-chain (n-3) fatty acids was primarily from fish (87% of the total intake) and secondarily from chicken (7%) and liver (2%), which is similar to the U.S. food supply data (19). Spearman rank correlation coefficients for the fish items between 2 questionnaires administered 1 y apart were 0.63 ($P < 0.05$) for dark-meat fish, 0.54 ($P < 0.05$) for canned tuna, 0.48 ($P < 0.05$) for other fish, and 0.67 ($P < 0.05$) for shrimp, lobster, or scallops as a main dish (20). The mean total fish intake was 3.7 servings weekly according to the questionnaire, and 3.6 servings weekly according to two 1-wk dietary records (Spearman rank correlation coefficient, 0.61; $P < 0.001$). The energy-adjusted intake of EPA from fish was correlated with the percentage of EPA in adipose tissue (Spearman rank correlation, 0.49; $P < 0.001$) (21). Information on fish oil supplements was not requested until 1990 in the Nurses' Health Study (22); at that point, the prevalence of consumption of this supplement was only 1.6%.

Assessment of other variables. Body weight and smoking status were assessed in 1990. BMI was calculated as weight (kg)/height^2 (m). Physical activity was assessed in hours per week spent on common leisure-time physical activities expressed as metabolic equivalent hours per week (MET-h/wk). Alcohol consumption was measured as mean intake (g/d) between 1986 and 1990. Standard portion sizes for alcoholic drinks were specified as a can/bottle or glass for beer (12.8 g of alcohol), 0.12 L or 4-oz glass for wine (11 g of alcohol), and 1 drink or shot for liquor (14 g of alcohol). In a validation study, the correlation between the reported alcohol intakes from an FFQ and from the mean of two 1-wk diet records was 0.86 ($P < 0.05$) (23). Hormone therapy use was ascertained among postmenopausal women, who were classified as never, past, or current users in 1990.

Statistical analysis. We used PROC GLM in SAS (24) to calculate the age-adjusted geometric means and their 95% CI for the biomarkers in each quintile of (n-3) fatty acid intake. We used the log-transformed biomarkers as the dependent variable and the consumption of (n-3) categorized in quintiles as the independent variable. Then, we calculated the exponential values of the means and the intervals obtained to back transform them.

Multiple linear regression analyses (PROC REG) were used to assess the relation between (n-3) fatty acid intake and plasma levels of endothelial biomarkers. We used log-transformed plasma concentrations of the biomarkers to better approximate normal distributions. In multivariate models, we adjusted for age (≤45, 46–50, 51–55, 56–60, 61–65, ≥66 y), BMI (<23.0, 23.0–24.9, 25.0–29.9, 30.0–34.9, ≥35.0 kg/m^2), physical activity (<1.5, 1.5–5.9, 6.0–11.9, 12.0–20.9, ≥21.0 MET-h/wk), smoking status (never, past, current 1–14 cigarettes/d, current ≥15 cigarettes/d), alcohol consumption (non-drinker, <5.0, 5.0–10.0, >10.0 g/d), and intakes of LA [18:2(n-6)] and SFA (in quintiles). We also adjusted the models for intakes of vitamin E, dietary fiber, and *trans* fatty acids (in quintiles), and for use of hormone therapy (premenopausal, never, past, current user). In addition, we examined whether the relation between (n-3) fatty acids and biomarkers was modified by LA intake through stratified analyses. Finally, we examined the relation between the consumption of the main food sources of (n-3) fatty acids and the markers.

RESULTS

Compared with women in the lowest quintile of ALA intake, those in the highest quintile had a slightly higher mean BMI ($P = 0.02$) and reported a higher consumption of alcohol ($P = 0.04$) (**Table 1**). In addition, women in the highest quintile of EPA and DHA intake were older ($P = 0.002$), reported higher alcohol consumption ($P = 0.05$), and were more likely to use postmenopausal hormone treatment ($P < 0.001$) than those in the lowest quintile. As the intake of ALA increased, so did the intakes of saturated, polyunsaturated, monounsaturated, and *trans* fats. However, higher intakes of EPA and DHA were related to lower intakes of

TABLE 1

Baseline characteristics according to quintiles of (n-3) fatty acid intake in the Nurses' Health Study[1]

	Quintile of ALA intake, g/d			P for trend	Quintile of EPA + DHA intake, g/d			P for trend
	1 (lowest)	3	5 (highest)		1 (lowest)	3	5 (highest)	
Median	0.73	0.95	1.25		0.07	0.19	0.45	
n	145	144	144		139	146	145	
Age, y	56 ± 7	56 ± 7	57 ± 7	0.34	55 ± 7	57 ± 6	57 ± 7	0.002
BMI, kg/m2	25 ± 5	26 ± 6	26 ± 6	0.02	27 ± 7	26 ± 6	26 ± 6	0.52
Physical activity,[2] MET-h/wk	13 ± 19	14 ± 16	12 ± 13	0.77	12 ± 16	11 ± 10	16 ± 18	0.91
Alcohol consumption, g/d	5 ± 9	6 ± 9	7 ± 11	0.04	5 ± 8	6 ± 10	7 ± 11	0.005
Current smoker, %	15	13	11	0.72	14	13	10	0.61
Current postmenopausal hormone therapy user, %	38	33	33	0.16	27	36	39	<0.001
Nutrient intake (energy-adjusted)								
Saturated fat, g/d	18 ± 3	20 ± 4	21 ± 4	<0.001	21 ± 4	20 ± 4	18 ± 4	<0.001
Polyunsaturated fat, g/d	9 ± 2	11 ± 2	13 ± 2	<0.001	11 ± 3	11 ± 2	11 ± 3	0.06
Monounsaturated fat, g/d	19 ± 3	22 ± 4	23 ± 4	<0.001	22 ± 4	21 ± 4	20 ± 4	<0.001
Trans fat, g/d	2 ± 1	3 ± 1	3 ± 1	<0.001	3 ± 1	3 ± 1	2 ± 1	<0.001
Protein, g/d	74 ± 14	75 ± 11	77 ± 12	0.02	67 ± 10	75 ± 9	85 ± 11	<0.001
Carbohydrate, g/d	212 ± 29	195 ± 27	180 ± 28	<0.001	199 ± 30	197 ± 27	191 ± 31	0.01
Fiber, g/d	17 ± 5	17 ± 4	19 ± 7	<0.001	17 ± 5	18 ± 5	20 ± 7	<0.001
Vitamin E, mg/d	6 ± 4	7 ± 3	8 ± 3	<0.001	6 ± 3	7 ± 3	8 ± 3	<0.001
LA, g/d	7 ± 2	9 ± 2	11 ± 2	<0.001	9 ± 2	9 ± 2	9 ± 2	0.15
Food consumption, serving/d								
Fish and other seafood	0.31 ± 0.23	0.31 ± 0.19	0.41 ± 0.39	<0.001	0.10 ± 0.06	0.30 ± 0.11	0.66 ± 0.36	<0.001
Salad dressing	0.08 ± 0.09	0.22 ± 0.20	0.53 ± 0.42	<0.001	0.18 ± 0.22	0.26 ± 0.28	0.36 ± 0.40	<0.001

[1] Values are means ± SD.
[2] MET, metabolic equivalent (energy need per kilogram of body weight per hour of activity divided by the energy need per kilogram of body weight per hour at rest).

saturated fat, monounsaturated, and *trans* fatty acids. The intakes of protein, fiber, and vitamin E were greatest in the top quintile of both ALA and EPA + DHA consumption. In contrast, carbohydrate intake was lowest in the highest quintiles of (n-3) intake. Finally, LA intake increased with increasing quintiles of ALA and was similar across quintiles of EPA + DHA.

There was a trend toward decreasing plasma concentrations of IL-6 and sVCAM-1 with increasing quintiles of ALA intake (IL-6: 2.1–1.8 ng/L, P for trend of medians = 0.02; sVCAM-1: 534–505 μg/L, P = 0.03) (**Table 2**). In addition, plasma concentration of sVCAM-1 decreased with increasing quintiles of EPA + DHA intake (sVCAM-1: 561–511 μg/L, P = 0.02). When both ALA and fish (n-3) fatty acids were added together in the same model, differences in plasma levels of biomarkers among quintiles of intake were larger, with significant trends for IL-6 (P = 0.008), sICAM-1 (P = 0.006), and sVCAM-1 (P = 0.002), and borderline trends for CRP (P = 0.05) and E-selectin (P = 0.07). For total (n-3) fatty acid intake, CRP levels were 29% lower among those in the highest quintile of (n-3) fatty acids, compared with the lowest quintile; Il-6 levels were 23% lower, E-selectin 10% lower, sICAM-1 7% lower, and sVCAM-1 levels 8% lower.

In multivariate models, ALA intake was inversely related to plasma concentration of CRP (β = −0.55, P = 0.02), IL-6 (β = −0.36, P = 0.01), and E-selectin (β = −0.24, P = 0.008) (**Table 3**). Fish (n-3) fatty acids had an inverse relation to sICAM-1 (β = −0.11, P = 0.03), and sVCAM-1 (β = −0.17, P = 0.003). Finally, when total (n-3) fatty acids were considered, an inverse relation existed with all biomarkers except sTNFR-2: CRP (β = −0.41, P = 0.006), IL-6 (β = −0.26, P = 0.009), E-selectin (β = −0.17, P = 0.004), sICAM-1 (β = −0.07, P = 0.02), and sVCAM-1 (β = −0.10, P = 0.004).

Due to potential competition between (n-6) and (n-3) fatty acids in the eicosanoid synthesis pathway (25), we analyzed whether (n-6) intake modified the associations between (n-3) fatty acids and the biomarkers. However, in stratified analyses, these associations were not significantly different between groups with low and high intakes of LA (median value as the cutoff point) (**Table 4**). In addition, we repeated the analyses in Table 3, adjusting the multivariate models for quintiles of arachidonic acid [20:4(n-6); AA]. The associations remained virtually the same, suggesting that the associations between (n-3) fatty acids and the biomarkers were also independent of AA intake.

Because the results of some studies support a role for vitamin E in preserving endothelium function due to its antioxidant properties (26), we also adjusted the models for total vitamin E intake (dietary and from supplements). But this additional adjustment had little effect on the results, suggesting that the effect of ALA, EPA, and DHA in the endothelium was independent of the antioxidant effect of this vitamin. Also, further adjustment for dietary fiber or *trans* fat intake did not substantially alter the results.

Several case-control studies and clinical trials found that hormone therapy increases plasma levels of CRP (27–30) and decreases levels of IL-6, E-selectin, sICAM-1, and sVCAM-1 (27,29). In addition, hormone therapy was related to an increase in flow-mediated vasodilation (31), which is indicative of an improvement in endothelial function. Thus, we performed analyses controlling for hormone therapy use. However, the associations between intake of (n-3) fatty acids and biomarkers were not altered.

Finally, we also performed analyses to test the relation between the consumption of fish and salad dressing [main sources of (n-3) fatty acids] and the concentrations of the biomarkers. The adjusted means of CRP and IL-6 decreased with an increase in the frequency of fish consumption and

TABLE 2

Age-adjusted geometric means (95% CI) of plasma concentrations of biomarkers of endothelial activation by quintiles of (n-3) fatty acid intakes in the Nurses' Health Study[1]

Quintile	n	CRP	IL-6	sTNFR-2	E-selectin	sICAM-1	sVCAM-1
		mg/L	*ng/L*	*μg/L*	*ng/L*	*μg/L*	
ALA (range: *g/d*)							
Q1 (0.43–0.80)	145	1.7 (1.4,2.1)	2.1 (1.9,2.4)	2375 (2210,2554)	44.9 (41.8,48.3)	251 (241,261)	534 (513,557)
Q2 (0.81–0.90)	148	1.4 (1.1,1.7)	1.9 (1.7,2.2)	2469 (2298,2653)	43.6 (40.6,46.8)	255 (245,265)	548 (526,570)
Q3 (0.91–1.00)	144	1.4 (1.1,1.7)	1.8 (1.6,2.0)	2249 (2092,2419)	44.6 (41.5,48.0)	249 (240,260)	521 (500,543)
Q4 (1.01–1.15)	146	1.6 (1.4,2.0)	1.7 (1.5,1.9)	2172 (2021,2335)	44.4 (41.3,47.7)	240 (231,250)	531 (509,553)
Q5 (1.16–2.36)	144	1.3 (1.1,1.6)	1.8 (1.6,2.0)	2271 (2112,2442)	42.9 (39.9,46.1)	244 (234,254)	505 (484,526)
P for trend[2]		0.29	0.02	0.10	0.47	0.10	0.03
EPA + DHA (range: *g/d*)							
Q1 (0.01–0.09)	139	1.4 (1.2,1.7)	1.9 (1.7,2.2)	2385 (2214,2569)	47.6 (44.2,51.2)	259 (249,270)	561 (538,585)
Q2 (0.10–0.15)	151	1.9 (1.5,2.2)	2.0 (1.8,2.3)	2212 (2060,2375)	44.4 (41.4,47.6)	244 (234,253)	513 (493,535)
Q3 (0.16–0.22)	146	1.5 (1.3,1.9)	1.8 (1.6,2.0)	2294 (2133,2466)	43.1 (40.1,46.3)	250 (241,261)	539 (517,562)
Q4 (0.23–0.32)	146	1.3 (1.1,1.6)	1.9 (1.7,2.1)	2327 (2164,2501)	43.2 (40.3,46.4)	246 (236,256)	517 (496,539)
Q5 (0.33–1.67)	145	1.3 (1.1,1.6)	1.7 (1.5,1.9)	2323 (2160,2498)	42.4 (39.5,45.6)	241 (232,251)	511 (490,532)
P for trend[2]		0.07	0.11	0.87	0.06	0.06	0.02
Σ (n-3) (range: *g/d*)							
Q1 (0.54–0.97)	145	1.7 (1.4,2.1)	2.2 (1.9,2.4)	2453 (2281,2637)	46.7 (43.4,50.2)	254 (244,264)	548 (526,571)
Q2 (0.98–1.11)	145	1.3 (1.1,1.6)	1.8 (1.6,2.0)	2371 (2205,2549)	43.8 (40.8,47.1)	253 (243,263)	536 (515,559)
Q3 (1.12–1.23)	148	1.6 (1.3,2.0)	1.9 (1.7,2.2)	2217 (2064,2382)	44.1 (41.0,47.3)	250 (240,260)	534 (513,557)
Q4 (1.24–1.42)	144	1.6 (1.4,2.0)	1.8 (1.6,2.1)	2209 (2054,2376)	43.9 (40.8,47.1)	245 (236,255)	515 (494,537)
Q5 (1.43–3.33)	145	1.2 (1.0,1.4)	1.7 (1.5,1.9)	2289 (2129,2461)	42.0 (39.1,45.2)	237 (228,246)	505 (485,527)
P for trend[2]		0.05	0.008	0.12	0.07	0.006	0.002

[1] Mean nutrient intake between 1986 and 1990.
[2] *P* for trend of medians in each quintile.

salad dressing (*P* < 0.001 and *P* = 0.005, respectively). In addition, the adjusted means of E-selectin and sICAM-1 decreased with an increase in the frequency of consumption of total fish (*P* for trend = 0.04 and 0.06, respectively), but not to the same degree as observed with EPA and DHA.

DISCUSSION

In this study, we examined the relation between the intake of (n-3) fatty acids and plasma concentrations of biomarkers of inflammation and endothelial activation in apparently healthy women. Over the range of dietary intake in this population, we found an inverse relation between individual and total (n-3) fatty acids (ALA, EPA, and DHA) and plasma concentrations

of CRP, IL-6, E-selectin, sICAM-1, and sVCAM-1. CRP levels were 29% lower among those in the highest quintile of total (n-3) fatty acid intake, compared with the lowest quintile; Il-6 levels were 23% lower, E-selectin 10% lower, sICAM-1 7% lower, and sVCAM-1 levels 8% lower. These associations were independent of lifestyle and dietary covariates and were not modified by intake of LA.

In a 3-y follow-up study, Ridker et al. (32) found that women who had subsequent cardiovascular events had baseline plasma levels of CRP 50% higher than those women who were free of the disease. In addition, these women also had higher baseline levels of IL-6 and sICAM (27 and 9%, respectively). In our study, the decrease in plasma levels of the

TABLE 3

Multiple linear regression models for the relation between (n-3) fatty acid intakes (g/d) and log-transformed biomarkers of endothelial activation in the Nurses' Health Study[1,2]

	ALA		EPA + DHA		Σ (n-3)	
	Age-adjusted	Multivariate-adjusted[3]	Age-adjusted	Multivariate-adjusted[3]	Age-adjusted	Multivariate-adjusted[3]
Log CRP, *mg/L*	−0.25 (0.20)	−0.55 (0.02)	−0.70 (0.005)	−0.42 (0.08)	−0.38 (0.009)	−0.41 (0.006)
Log IL-6, *ng/L*	−0.28 (0.02)	−0.36 (0.01)	−0.29 (0.05)	−0.24 (0.11)	−0.26 (0.003)	−0.26 (0.009)
Log sTNFR-2, *μg/L*	−0.13 (0.08)	−0.14 (0.14)	−0.03 (0.75)	−0.05 (0.61)	−0.08 (0.13)	−0.08 (0.19)
Log E-selectin, *ng/L*	−0.13 (0.07)	−0.24 (0.008)	−0.21 (0.02)	−0.17 (0.07)	−0.15 (0.007)	−0.17 (0.004)
Log sICAM-1, *μg/L*	−0.06 (0.15)	−0.07 (0.18)	−0.13 (0.01)	−0.11 (0.03)	−0.08 (0.01)	−0.07 (0.02)
Log sVCAM-1, *μg/L*	−0.06 (0.17)	−0.08 (0.14)	−0.17 (0.002)	−0.17 (0.003)	−0.09 (0.004)	−0.10 (0.004)

[1] Values are β coefficients (*P*-values).
[2] Mean intake between 1986 and 1990.
[3] Adjusted for age (≤45, 46–50, 51–55, 56–60, 61–65, ≥66 y), BMI in 1990 (<23.0, 23.0–24.9, 25.0–29.9, 30.0–34.9, ≥35.0 kg/m^{2}), physical activity in 1990 (<1.5, 1.5–5.9, 6.0–11.9, 12.0–20.9, ≥21 MET-h/wk), smoking status (never smoker, past smoker, current 1–14 cigarettes/d, current ≥15 cigarettes/d), mean alcohol consumption between 1986 and 1990 (nondrinker, 0–4.9, 5.0–10.0, >10.0 g/d), and quintiles of (n-6) LA and SFA.

TABLE 4

Multiple linear regression models for the relation between total n-3 fatty acids (g/d) and log-transformed biomarkers, stratified by (n-6) linoleic fatty acid intake in the Nurses' Health Study[1-3]

	Lower (n-6) linoleic acid intake ($<5.1\%$ energy)	Higher (n-6) linoleic acid intake ($\geq5.1\%$ energy)	P for interaction
Log CRP, mg/L	−0.47 (0.04)	−0.39 (0.04)	0.78
Log IL-6, ng/L	−0.21 (0.18)	−0.23 (0.06)	0.94
Log sTNFR-2, μg/L	−0.04 (0.64)	−0.10 (0.28)	0.72
Log E-selectin, ng/L	−0.14 (0.15)	−0.20 (0.007)	0.60
Log sICAM-1, μg/L	−0.05 (0.33)	−0.08 (0.08)	0.40
Log sVCAM-1, μg/L	−0.12 (0.04)	−0.10 (0.02)	0.94

[1] Values are β coefficients (P-values).
[2] Mean intake between 1986 and 1990.
[3] Adjusted for age (≤45, 46–50, 51–55, 56–60, 61–65, ≥66 y), BMI in 1990 (<23.0, 23.0–24.9, 25.0–29.9, 30.0–34.9, ≥35.0 kg/m²), physical activity in 1990 (<1.5, 1.5–5.9, 6.0–11.9, 12.0–20.9, ≥21 MET-h/wk), smoking status (never smoker, past smoker, current 1–14 cigarettes/d, current ≥15 cigarettes/d), mean alcohol consumption between 1986 and 1990 (nondrinker, 0–4.9, 5.0–10.0, >10.0 g/d), and quintiles of SFA.

biomarkers from the lowest to highest quintiles of (n-3) intake ranged from 8 to 29%, a difference comparable to that found in women with and without a major cardiovascular event.

The relevance of the inflammatory and endothelial activation biomarkers in the atherogenic process was suggested by several studies. CRP and IL-6 are markers of systemic inflammation and independent predictors of cardiovascular disease in healthy women (32). Recent data suggest that CRP plays an active role in atherogenesis (33). In addition, the soluble TNF receptor, which is induced by TNF and other cytokines, is an indicator of inflammatory processes (34) and has been associated with obesity, coronary heart disease, and angina (35,36). Moreover, E-selectin, sICAM-1, and sVCAM-1 are surface and soluble cell adhesion molecules overexpressed when the endothelium encounters inflammatory stimuli. Higher levels of E-selectin and sICAM-1 were also observed in patients with coronary heart disease (37), and baseline plasma levels of sICAM-1 are predictors of myocardial infarction among apparently healthy men (38). Finally, sVCAM-1 is expressed mainly in atherosclerotic plaques, and is considered a marker of advanced atherosclerosis (39).

A possible biochemical pathway by which (n-3) fatty acids may inhibit inflammation and endothelial activation is by decreasing the baseline production of hydrogen peroxide (40). This happens because the multiple double bonds in the carbonated structure of the (n-3) fatty acids allow them to react with active oxygen species. Hydrogen peroxide is a critical activator of the nuclear factor-κB system of transcription factors, which controls the coordinated expression of adhesion molecules and of leukocyte-specific chemoattractants upon cytokine stimulation (41).

In vitro studies showed that not all PUFA have the same effect in the endothelium. De Catarina et al. (6,40–42), using adhesion molecules (E-selectin, sICAM-1, and sVCAM-1) and soluble proinflammatory proteins (IL-6 and IL-8) as biomarkers of endothelial activation, concluded that the ability of PUFA to reduce endothelial activation increased with the number of unsaturations, and that this ability did not depend on chain length. Thus, DHA (with 6 double bonds) was the most potent fatty acid inhibitor of endothelial activation in vitro (6). However, this was not tested by in vivo studies. Our work suggests that ALA and fish (n-3) fatty acids had similar inverse associations with the biomarkers of inflammation and endothelial activation.

Dietary fish oil is 2.5–5 times more effective than ALA in modulating eicosanoid metabolism and altering tissue phospholipid fatty acid composition (43). Although the human body is able to convert a portion of ALA into EPA and DHA, this conversion is inefficient and depends on the quantity of (n-6) intake because (n-6) fatty acids compete with (n-3) fatty acids for δ-6-desaturase in the pathway for eicosanoid synthesis. Eicosanoids derived from (n-3) fatty acids (mainly prostaglandin I₃ and thromboxane A₃) are less thrombogenic than those derived from (n-6) fatty acids. Theoretically, a higher intake of (n-6) fatty acids may attenuate the beneficial effects of (n-3) fatty acids. However, our data did not suggest that a higher intake of LA (up to ~5.1%, 95% CI: 2.6–7.6, of total energy in this population) modified the relation between intake of total (n-3) fatty acids and plasma concentrations of inflammatory and endothelial biomarkers. Moreover, Pischon et al. (13) found that the associations between (n-3) fatty acids and inflammatory biomarkers, such as sTNF-R1 and R2, were more evident among men with a higher intake of (n-6) fatty acids.

Our study has several limitations. First, because it is cross-sectional, we cannot infer causality from our results. Second, there could be some degree of error in the measurement of food consumption, nutrient content of foods, and in biochemical measures. However, the dietary questionnaire was shown to reflect long-term intake, and the marker measures are stable over time. In addition, the use of the repeated measurement of food consumption enabled us to reduce within-person random error.

In conclusion, this study suggests that dietary (n-3) fatty acids are associated with levels of biomarkers reflecting healthy endothelial function, which might explain in part the effects of these fatty acids in preventing cardiovascular disease.

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