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Relationship of Plasma Polyunsaturated Fatty Acids to Circulating Inflammatory Markers

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Aims: Persons with high intake of polyunsaturated fatty acids (PUFAs) have lower cardiovascular morbidity and mortality. The protective effect of PUFAs is mediated by multiple mechanisms, including their antiinflammatory properties. The association of physiological PUFA levels with pro- and antiinflammatory markers has not been established.

Methods and Results: In 1123 persons (aged 20–98 yr), we examined the relationship between relative concentration of fatty acids in fasting plasma and level of inflammatory markers. Adjusting for age, sex, and major confounders, lower arachidonic and docosahexaenoic acids were associated with significantly higher IL-6 and IL-1ra and significantly lower TGFβ. Lower α-linolenic acid was associated with higher C-reactive protein and IL-1ra, and lower eicosapentaenoic acid was associated with higher IL-6 and lower TGFβ. Lower docosahexaenoic acid was strongly associated with lower IL-10. Total n-3 fatty acids were associated with lower IL-6 ($P = 0.005$), IL-1ra ($P = 0.004$), and TNFα ($P = 0.040$) and higher soluble IL-6r ($P < 0.001$), IL-10 ($P = 0.024$), and TGFβ ($P = 0.0012$). Lower n-6 fatty acid levels were significantly associated with higher IL-1ra ($P = 0.026$) and lower TGFβ ($P = 0.014$). The n-6 to n-3 ratio was a strong, negative correlate of IL-10. Findings were similar in participants free of cardiovascular diseases and after excluding lipids from covariates.

Conclusions: In this community-based sample, PUFAs, and especially total n-3 fatty acids, were independently associated with lower levels of proinflammatory markers (IL-6, IL-1ra, TNFα, C-reactive protein) and higher levels of antiinflammatory markers (soluble IL-6r, IL-10, TGFβ) independent of confounders. Our findings support the notion that n-3 fatty acids may be beneficial in patients affected by diseases characterized by active inflammation. (***J Clin Endocrinol Metab* 91: 439–446, 2006**)

THERE IS EVIDENCE that a diet rich in polyunsaturated fatty acids (PUFAs) and, in particular, the omega-3 family (n-3), is associated with lower cardiovascular morbidity and mortality and reduced risk of sudden death, independent of other known cardiovascular risk factors (1–5). Studies have suggested that the protective effects of n-3 PUFA are mediated by multiple mechanisms, including their antiinflammatory properties (6).

Preclinical studies have shown that fatty acids modulate the inflammatory response by multiple mechanisms, including transcriptional down-regulation of proinflammatory cytokines and the vascular surface expression of endothelial leukocyte adhesion molecules (7, 8). In particular, in experimental and animal models, n-3 fatty acids inhibit the production of IL-1 and TNFα (7). An antiinflammatory effect of n-3 fatty acids is supported by the beneficial effect of n-3 fatty-acid supplementation in patients affected by diseases characterized by active inflammation, such as rheumatoid arthritis and Crohn's disease (9). In small groups of healthy volunteers, dietary supplementation with n-3 fatty acids was associated with reduced levels of IL-1β, thromboxane β_2, and prostaglandin E2 (10, 11) but not C-reactive protein (CRP) (12).

A recent study showed that dietary intake of n-3 and omega-6 (n-6) fatty acids in American men and women was inversely associated with plasma levels of soluble TNFα receptors 1 and 2 but not with other cytokines (13). This observation is important because it suggests that physiological levels of fatty acids modulate inflammation. However, studies on dietary intake should be complemented by studies that investigate the relationship between fatty acid plasma levels and serum levels of multiple inflammatory markers. In fact, although some studies found a good correlation between dietary intake of fatty acids and blood levels (14), others did not confirm this finding, reporting only a modest association (15), likely due to the fact that circulating fatty acid levels reflect the interplay among dietary intake, absorption, and metabolism.

Using data from a representative sample of the general population, we tested the hypothesis that circulating levels

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Abbreviations: AA, Arachidonic acid; ALA, α-linolenic acid; BMI, body mass index; CRP, C-reactive protein; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FAME, fatty acid methyl esters; HDL, high-density lipoprotein; IL-1ra, IL-1 receptor antagonist; InCHIANTI, Invecchiare in Chianti, aging in the Chianti area; LA, linoleic acid; LDL, low-density lipoprotein; n-3, omega-3 family; n-6, omega-6 family; PPaR, peroxisome proliferator activated receptor; PUFA, polyunsaturated fatty acid; sIL-6r, soluble IL-6 receptor.

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of selected PUFAs are associated with lower concentrations of proinflammatory cytokines and, possibly, higher levels of antiinflammatory cytokines. This information is important in furthering our understanding of the mechanisms by which fatty acids modulate cardiovascular risk and other clinical conditions characterized by a proinflammatory state.

Subjects and Methods

Study population and collection of blood samples.

Invecchiare in Chianti, aging in the Chianti area (InCHIANTI) is an epidemiological study conducted in two small towns of Tuscany, Italy. The rationale, design, and data collection methods of InCHIANTI are described elsewhere (16). In brief, in August 1998, 1270 persons aged 65 yr or older were randomly selected from the population registry of the two sites. Additionally, men and women randomly sampled from the age strata 20–29, 30–39, 40–49, 50–59, and 60–64 yr were sequentially invited to participate in the study, until at least 30 men and 30 women in each decade, ages 20–69 yr, had been enrolled.

Of the 1714 eligible persons, 640 men and 813 women (84.8%) agreed to participate and were interviewed. Of those, 595 men and 748 women (92.4%) provided blood samples. Data on plasma fatty acid and serum cytokine composition were obtained for 1180 participants (87.9%). Because poor cognitive status is associated with inflammatory conditions and strongly affects dietary intake (17), we also excluded 57 participants in whom we established a dementia diagnosis, based on the Diagnostic and Statistical Manual of Mental Disorders, version III-R criteria (18). Thus, the final study population included 1123 participants, none of whom had dietary supplementation of fatty acids.

The study protocol complies with the Declaration of Helsinki and was approved by the Italian National Institute of Research and Care on Aging Ethical Committee. Participants received an extensive description of the study and signed an informed participation consent that included permission to conduct analyses on the biological specimens collected and stored.

Laboratory analysis

Blood samples were collected in the morning after a 12-h fast. Aliquots of serum and plasma were immediately obtained and stored at −80 C. The samples used to measure circulating levels of cytokines and fatty acids had not been previously thawed.

Fatty acids were measured using a fasting plasma sample. A known amount of heptadecanoic acid (17:0) (Sigma Chemical Co., St Louis, MO) was added to each sample as an internal standard, and total lipids were extracted from 0.15 ml of plasma (19). In a pilot study, we had found that no traces of heptadecanoic acid were detectable in 25 plasma samples from InCHIANTI participants. Fatty acid methyl esters (FAME) were prepared through transesterification using Lepage and Roy's method (20), modified according to Rodriguez-Palmero *et al.* (21). Separation of FAME was carried out on an HP-6890 gas chromatograph (Hewlett-Packard, Palo Alto, CA) with a 30-m fused silica column (HP-225; Hewlett-Packard). FAMEs were identified by comparison with pure standards (NU Chek Prep, Inc., Elysian, MA). For quantitative analysis of fatty acids as methyl esters, calibration curves for FAME (ranging from C14:0 to C24:1) were prepared by adding six increasing amounts of individual FAME standards to the same amount of internal standard (C17:0; 50 μg). The correlation coefficients for the calibration curves of 20 fatty acids were in all cases higher than 0.998 in the range of concentrations studied. Fatty acid concentration was expressed as a percentage. Fatty acid percent area/area was also calculated. The coefficient of variation for all fatty acids was on average 1.6% for intraassay and 3.3% for interassay.

In the present analysis, we examined data on the concentration of PUFAs, which are characterized by two or more double bonds in the hydrocarbon chain. The n-3 and n-6 families of fatty acids account for more than 95% of total PUFAs and are named from the position of the first double bond, located on the third or sixth carbon, respectively, from the terminal methyl group (22). The total n-6 fatty acids included linoleic (LA) (C18:2-n6), eicosadienoic (C20:2n6), dihomo-g-linolenic (C20:3n6), and arachidonic (AA) (C20:4n6) acids, whereas the total n-3 fatty acids included α-linolenic (ALA) (C18:3n3), eicosapentaenoic (EPA) (C20:5n3), and docosahexaenoic (C22:6-n3) (DHA) acids.

Serum levels of IL-6, soluble IL-6 receptor (sIL-6r, 80 kDa), IL-1β, IL-1 receptor antagonist (IL-1ra), and TNFα were measured by ELISAs using commercial kits (BIOSOURCE International, Camarillo, CA). TGFβ and IL-10 levels were measured in duplicate using highly sensitive quantitative sandwich assays (Quantikine HS, R&D Systems, Minneapolis, MN). The lowest detectable concentrations were 0.1 pg/ml for IL-6, 8 pg/ml for sIL-6r, 0.01 pg/ml for IL-1β, 0.09 pg/ml for TNFα, 4 pg/ml for IL-1ra, 7 pg/ml for TGFβ, and 1.5 pg/ml for IL-10. The interassay coefficient of variation was 4.5% for IL-1ra and less than 8.0% for the other cytokines.

CRP was measured in duplicate using an ELISA colorimetric competitive immunoassay that used purified protein and polyclonal anti-CRP antibodies. The minimum detectable concentration was 0.03 mg/liter and the interassay coefficient of variation was 5.0%. Total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were assessed using commercial enzymatic tests (Roche Diagnostics, Mannheim, Germany).

Covariates

Participants were classified as nonsmokers or former smokers *vs.* current smokers based on self-report. Weight was measured using a high-precision mechanical scale. Standing height was measured to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight (kilograms)/height (square meters). Average daily intake of energy (kilocalories) and carbohydrates, proteins, total lipids and unsaturated, and monosaturated and polyunsaturated fatty acids (grams) were estimated by administering the European Prospective Investigation into Cancer and Nutrition food frequency questionnaire, which has been extensively validated in the Italian population (23).

Study participants responded to an extensive questionnaire on habitual physical activity and were classified as sedentary if they reported being completely inactive or performing low-intensity physical activity, such as short walking or light housekeeping activities totaling less than 2 h/wk.

A physician evaluated all participants. Diseases were ascertained according to standard, preestablished criteria that combined information from self-reported physician diagnoses, current pharmacological treatment, medical records, clinical examinations, and blood tests. Diseases included in the current analysis were coronary heart disease (including angina and myocardial infarction), congestive heart failure, cerebrovascular disease (including transient ischemic attack and stroke), diabetes, and hypertension. Diagnostic algorithms were modified versions of those created for the Women's Health and Aging Study (24). An ankle-arm index of 0.9 or less was considered indicative of peripheral arterial disease (25). Participants were asked to report all drugs taken at least once over the last 15 d. Using this information, we created a dichotomous variable indicating whether the participant received treatments that may affect circulating levels of fatty acids and/or inflammatory markers, including statins, other drugs aimed at reducing circulating lipids, steroids, nonsteroidal antiinflammatory drugs, and angiotensin-converting enzyme inhibitors. This condition is defined in the text as potentially confounding drug treatment.

Statistical analysis

Continuous variables are reported as mean ± SD and categorical variables as percentages. Log-transformed values of cytokines, except for TGFβ, were used in the analysis.

The relationships of specific fatty acids with potential covariates were explored by computing age- and sex-adjusted partial Pearson correlations. Further analyses were performed to test the mutually independent effects of total n-3 and n-6 fatty acids on inflammatory markers, after adjusting for age; sex; education; intake of energy, proteins, and carbohydrates; physical activity; BMI; smoking; low-density lipoprotein (LDL) cholesterol; HDL cholesterol; triglycerides; hypertension; diabetes; coronary heart disease; congestive heart failure; stroke; peripheral arterial disease; and potentially confounding drug treatment. All analyses were performed using the SAS statistical package (version 9.1; SAS Institute, Inc., Cary, NC) with a statistical significance level set at $P < 0.05$.

Results

The principal characteristics of the study population are reported in the first column of Table 1. Only data on total n-3 and n-6 PUFAs are shown. Both total n-3 and n-6 were negatively correlated with age (n-3: r = −0.13; n-6: r = −0.33) but not sex (n-3: r = 0.02; n-6: r = 0.03). Independent of age and sex, total n-3 PUFAs were positively correlated with education and HDL cholesterol and negatively correlated with triglycerides. Total n-6 PUFAs were positively correlated with total, LDL, and HDL cholesterol and negatively correlated with most of the other cardiovascular risk factors and cardiovascular diseases (Table 1). LA was positively and independently correlated with LDL cholesterol. LA (r = 0.31), AA (r = 0.28), EPA (r = 0.23), and DHA (r = 0.14) were positively correlated with HDL cholesterol. LA (r = 0.51) was positively correlated (r = 0.51), whereas AA (r = −0.34), EPA (r = −0.21), and DHA (r = −0.20) were negatively correlated with triglycerides. Other correlations of specific fatty acids with the variables reported in Table 1 were small (<0.09) and generally not statistically significant. In particular, neither n-3 nor n-6 fatty acids were independently correlated with parameters of dietary intake.

The mean plasma concentration of total fatty acids was 3200 ± 724 mg/liter (range 1295–6885), which is compatible with those reported in a group of middle-aged American women and blood donors (26, 27). The concentration tended to be higher at older ages (r = 0.08, $P < 0.0023$) with no substantial difference between men and women (men, 3155 ± 777 *vs.* women, 3236 ± 677 mg/liter, $P = 0.07$). As expected, the plasma concentration of total fatty acids was positively correlated with total cholesterol (r = 0.59, $P < 0.0001$) and triglycerides (r = 0.71, $P < 0.0001$) and negatively correlated with HDL cholesterol (r = −0.07, $P < 0.025$). Total fatty acids were not independently correlated with any of the inflammatory markers considered in this study. n-3 and n-6 fatty acids accounted for 3.4 and 33.1% of total fatty acids. The percentage of n-3 and n-6 fatty acids on total fatty acids was significantly lower in older participants, respectively, by 0.002% per year ($P < 0.0001$) for n-3 and 0.008% per year ($P < 0.0001$) for n-6, without substantial difference between men and women.

Of the plasma n-3 fatty acids, 13.6 ± 6.1% were ALA, 18.8 ± 5.0% were EPA and 67.7 ± 4.9% were DHA. Of the n-6 fatty acids, 75.3 ± 5.0% were LA and 24.4 ± 4.9% were AA. Levels of inflammatory markers according to quartiles of specific fatty acids are reported in Table 2. All mean values and statistical tests are adjusted for age and multiple confounders. Lower AA and DHA were associated with higher IL-6 and IL-1ra and lower TGFβ. Lower DHA was also associated with lower IL-10. Lower ALA was associated with higher CRP and IL-1ra, and lower EPA was associated with higher IL-6, lower TGFβ, and lower IL-10. Participants in the two lower quartiles of LA had significantly lower sIL-6r than those in the two upper quartiles. AA/EPA ratio was not associated with any of the different inflammatory markers. After removing lipids as covariates from these models, these results were substantially unchanged, except that the inverse association of IL-1ra with LA (adjusted values across quartiles: 149, 129,and 127 pg/ml; $P = 0.0003$) and EPA (143, 132, 128, and 130; $P = 0.0076$) became stronger and highly statistically significant (*cf.* Table 2). Restricting the analysis to the 432

TABLE 1. Characteristics of the study population (n = 1123) and their correlation with n-3 and n-6 fatty acids

	Mean ± SD or n (%)	Age and sex adjusted partial correlations r (*P* value)	
		With total n-3 FA[a]	With total n-6 FA
Age (yr)	68.2 ± 15.4		
<65	246 (21.9)		
65–74	504 (44.9)		
75–84	276 (24.6)		
≥85	97 (8.6)		
Sex (women)	620 (55.2)		
Years in school	6.6 ± 4.2	0.14 (<0.0001)	0.05 (0.13)
BMI (kg/m^2)	27.5 ± 4.4	−0.04 (0.13)	−0.14 (<0.0001)
Energy intake (kcal/d)	2027 ± 621	−0.07 (0.03)	−0.02 (0.47)
Carbohydrate intake (g/d)	261 ± 87	−0.06 (0.03)	−0.01 (0.69)
Protein intake (g/d)	79 ± 23	−0.05 (0.10)	−0.02 (0.46)
Total lipids intake (g/d)	69 ± 23	−0.05 (0.08)	−0.01 (0.86)
Saturated FA intake (g/d)	23 ± 9	−0.05 (0.09)	0.02 (0.45)
Monounsaturated FA intake (g/d)	35 ± 12	−0.02 (0.46)	−0.03 (0.29)
Polyunsaturated FA intake (g/d)	7 ± 2	−0.05 (0.11)	0.01 (0.82)
Total cholesterol (mg/dl)	215 ± 40	0.00 (0.95)	0.11 (<0.001)
LDL cholesterol (mg/dl)	134 ± 35	0.02 (0.58)	0.18 (<0.0001)
HDL cholesterol (mg/dl)	56 ± 15	0.14 (<0.0001)	0.38 (<0.0001)
Triglycerides (mg/dl)	123 ± 65	−0.21 (<0.0001)	−0.58 (<0.0001)
Current smoker	179 (16)	−0.03 (0.36)	−0.07 (0.030)
Sedentary	186 (17)	−0.06 (0.06)	−0.09 (0.004)
Coronary heart disease	58 (5.2)	−0.01 (0.69)	−0.06 (0.020)
Stroke	21 (1.9)	−0.02 (0.46)	−0.08 (0.003)
Congestive heart failure	62 (5.5)	−0.07 (0.02)	−0.09 (0.004)
Hypertension	500 (45.5)	−0.04 (0.21)	−0.08 (0.005)
Diabetes	78 (7.0)	0.01 (0.81)	0.03 (0.24)
Peripheral artery disease	139 (12.6)	−0.04 (0.15)	−0.08 (0.010)

FA, Fatty acids.
[a] Log-transformed values were used for the correlation analysis.

TABLE 2. Multivariate analysis of the relationship between specific n-3 and n-6 fatty acids and inflammatory markers

	LA (quartiles), %[a]					AA (quartiles), %[a]					ALA (quartiles), %[a]				
Quartiles limits	<22.33	22.33–24.95	24.96–27.51	>27.51[ref]	*P* for trend	<6.82	6.82–7.97	7.98–9.28	>9.28[ref]	*P* for trend	<0.304	0.304–0.381	0.382–0.493	>0.493[ref]	*P* for trend
Median	20.5	23.5	26.2	29.4		5.95	7.34	8.58	10.19		0.264	0.344	0.427	0.640	
IL-6 (pg/ml)	1.32	1.23	1.28	1.28	0.38	1.44[b]	1.31[b]	1.28	1.15	0.0024	1.35	1.28	1.31	1.20	0.12
sIL-6r (ng/ml)	83[c]	86[c]	90	97	0.06	84[b]	80	87	96	0.1452	87	87	90	92	0.50
IL-1β (pg/ml)	0.13	0.14	0.13	0.14	0.60	0.13	0.14	0.14	0.13	0.52	0.14	0.12	0.14	0.13	0.41
IL-1ra (pg/ml)	140	127	129	131	0.26	141[c]	140[c]	126	126	0.0008	143[c]	127	132	128	0.0248
TNF-α (pg/ml)	4.8	4.7	4.7	4.8	0.67	4.8	5.0	4.5	4.7	0.29	5.1	4.4	4.7	4.8	0.53
IL-10 (pg/ml)	3.3	3.6	4.8	3.5	0.91	3.4	3.7	4.4	3.5	0.98	3.3	3.7	3.8	3.9	0.17
TGF-β (ng/ml)	11.20[c]	11.90	12.00	12.60	0.38	10.60[b]	11.40	12.80	12.90	0.0003	11.8	11.7	11.5	12.7	0.35
CRP (mg/liter)	2.70	2.50	2.50	2.40	0.90	2.50	2.70[c]	2.70[c]	2.30	0.60	3.0[b]	2.6	2.3	2.4	0.0047

	EPA (quartiles), %[a]					DHA (quartiles), %[a]					AA/EPA ratio (quartiles)				
Quartiles limits	<0.491	0.491–0.585	0.586–0.707	>0.707[ref]	*P* for trend	<1.77	1.77–2.23	2.24–2.75	>2.75[ref]	*P* for trend	<11.40	11.40–13.20	13.21–15.95	>15.95[ref]	*P* for trend
Median	0.422	0.543	0.647	0.800		1.46	2.00	2.47	3.18		9.82	12.28	14.50	18.16	
IL-6 (pg/ml)	1.37[b]	1.41[b]	1.24	1.16	0.0033	1.43[b]	1.30	1.25	1.18	0.0075	1.22	1.34	1.31	1.25	0.51
sIL-6r (ng/ml)	86[c]	88	89	94	0.39	85[c]	87	91	94	0.19	89	88	84[c]	95	0.75
IL-1β (pg/ml)	0.13	0.14	0.14	0.13	0.85	0.13	0.14	0.13	0.13	0.61	0.13	0.14	0.14	0.14	0.44
IL-1ra (pg/ml)	138	132	129	131	0.20	143[b]	130	135	125	0.0021	131	136	133	133	0.59
TNF-α (pg/ml)	5.2	4.6	4.5	4.7	0.13	4.8	5.1	4.7	4.5	0.06	4.9	4.4	4.8	4.8	0.69
IL-10 (pg/ml)	3.0	3.7	4.0	4.1	0.07	2.9	3.8	3.6	4.7	0.003	4.4	3.5	3.9	3.3	0.11
TGF-β (ng/ml)	11.40[b]	11.00[b]	11.90[c]	13.10	0.0071	10.90[b]	11.50[c]	11.90[c]	13.20	0.0004	11.6	12.3	11.5	12.4	0.79
CRP (mg/liter)	2.60	2.60	2.40	2.50	0.97	2.80[c]	2.40	2.60	2.30	0.17	2.5	2.6	2.6	2.4	0.55

ref, Reference quartile.

[a] Mean values and statistics for the association of specific fatty acids with selected inflammatory markers are adjusted for age, sex, education, daily intake of energy, carbohydrates, proteins and lipids, physical activity, BMI, smoking, LDL cholesterol, HDL cholesterol, triglycerides, hypertension, diabetes, coronary heart disease, congestive heart failure, stroke, peripheral artery disease, and potentially confounding drug treatment.

[b] $P < 0.01$, compared with the reference quartile.

[c] $P < 0.05$, compared with the reference quartile.

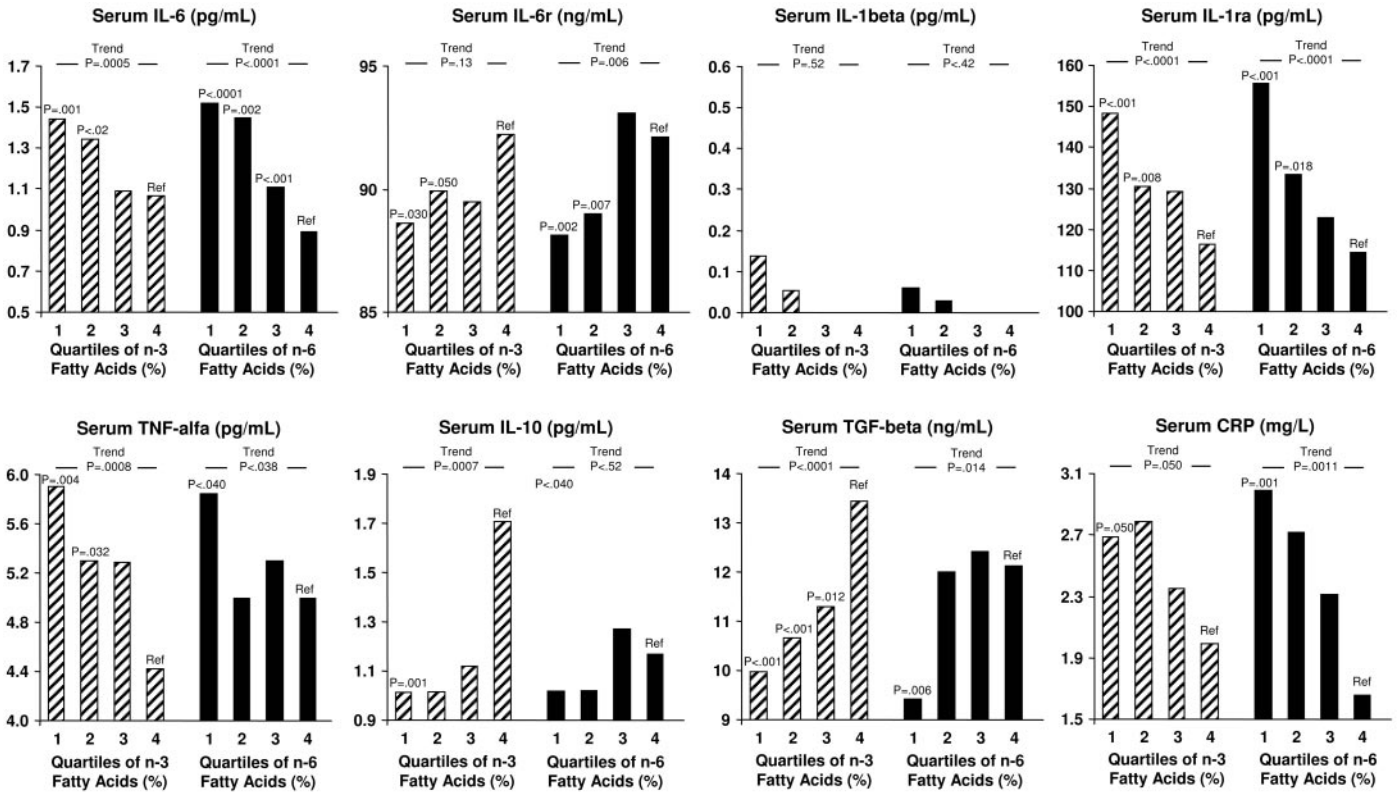

FIG. 1. Median serum levels of inflammatory markers according to total n-3 and n-6 PUFAs quartiles. Comparisons between groups and tests for trend are based on age- and sex-adjusted nonparametric ANOVA. The thresholds for quartile definition were 2.72, 3.27, and 3.93 for total n-3 fatty acids and 30.4, 33.13, and 36.26 for total n-6 fatty acids.

men and 569 women free of prevalent cardiovascular disease, all the associations that were statistically significant in Table 2 remained statistically significant, and in addition, the inverse relationship between LA and sIL-6r and between DHA and TNFα that were borderline statistically significant became statistically significant (respectively, $P = 0.020$ and $P = 0.040$).

Figure 1 shows median serum levels of inflammatory markers according to n-3 and n-6 quartiles. Adjusting for age and sex, lower total n-3 and n-6 PUFAs were associated with higher IL-6, IL1-ra, TNFα, and CRP and lower IL-6r, IL-10, and TGFβ levels. In most cases, the associations were highly statistically significant, with an evident dose-response relationship.

In subsequent models predicting inflammatory markers, we simultaneously entered both total n-3 and total n-6 PUFAs as well as multiple potential confounders and tested n-6 to n-3 ratio as predictor. From these models, we estimated the mean values of inflammatory markers, according to n-3 and n-6 quartiles and n-6 to n-3 ratio quartiles, which are reported and statistically compared in Table 3. Lower total n-3 PUFAs were still strongly and significantly associated with higher IL-6, IL-1ra, and TNFα and lower total sIL-6r, IL-10, and TGFβ but no longer with higher CRP. Lower n-6 PUFAs were independently associated with higher IL-1ra and lower TGFβ but no longer with the other inflammatory markers. The n-6 to n-3 ratio was positively associated with IL-6 and IL-1ra and, based on P value, was the strongest negative correlate of IL-10 and TGFβ. These findings were confirmed in analyses performed separately in men and women, in participants 65 yr and older and after restricting the study population to the 1001 participants free of coronary artery disease, congestive heart failure, stroke, and peripheral arterial disease.

Discussion

Our findings are consistent with the hypothesis that n-3 fatty acids have antiinflammatory properties (6, 7, 11, 28). IL-6 and TNFα are generally considered proinflammatory cytokines and the potent antiinflammatory properties of IL-10 and TGFβ are well known (29, 30). The negative association of total n-3 fatty acids with IL-1ra (a competitive inhibitor of the proinflammatory cytokine IL-1) and the positive association with sIL-6r (that in certain conditions enhances the biological activity of IL-6) requires discussion. At a molecular level, IL-1ra is a natural antagonist of the proinflammatory cytokine IL-1 (31), and in animal models of chronic inflammation, the administration of IL-1ra prevents tissue damage (32). Despite this, as a circulating biomarker, IL-1ra is considered an acute-phase protein and a more reliable measure of proinflammatory state than IL-1 (33). Similar triggers induce the production of IL-1 and IL-1ra, but IL-1 is produced locally and only small quantities spill in the serum, whereas IL-1ra is produced by the liver in large quantities and fully released into the circulation. For example, in experimental endotoxemia in humans, IL-1 increases in the circulation only by a factor of 2–2.5, whereas IL-1ra increases by a factor of 10–20 (32, 34). This may also explain why we did not find any association between fatty acids and IL-1β in our study.

The role of sIL-6r in inflammation is still unclear. In gen-

TABLE 3. Multivariate analysis of the relationship between total n-3 and n-6 fatty acids and inflammatory markers

	Total n-3 fatty acids, % (quartiles)[a] (mean ± SD, 3.4 ± 1.0)			*P* for trend	Total n-6 fatty acids, % (quartiles)[a] (mean ± SD, 33.1 ± 4.6)			*P* for trend	n-6/n-3 fatty acids, % (quartiles) (mean ± SD, 10.5 ± 3.1)			*P* for trend
Quartiles limits	<2.7	2.7–3.3	3.4–3.9	>3.9[ref]	<30	30–33	34–36.3	>36[ref]	<8.4–10.2	10.2–12.1	>12.1[ref]	
Median	2.3	3.0	3.5	4.5	28.0	31.6	34.7	38.2	7.3	9.3	11.0	13.8	
IL-6 (pg/ml)	1.38[b]	1.29	1.24[c]	1.14	0.0048	1.26	1.29	1.29	1.20	0.56	1.13[b]	1.29	1.24	1.39	0.0057
sIL-6r (ng/ml)	79[b]	84[b]	91	97	0.0003	88	85	90	88	0.74	89	87	88	86	0.53
IL-1β (pg/ml)	0.13	0.14	0.13	0.13	0.53	0.13	0.13	0.14	0.13	0.56	0.13	0.13	0.13	0.14	0.93
IL-1ra (pg/ml)	140[b]	131	130	121	0.0044	143	128	128	123	0.0258	122[b]	126[c]	132	142	0.0007
TNF-α (pg/ml)	5.1[c]	4.6	4.9	4.3	0.0404	4.8	4.9	4.6	4.5	0.32	4.3	4.7	5.0	4.9	0.08
IL-10 (pg/ml)	2.6	4.1	4.2	4.7	0.0236	4.2	4.0	3.1	3.3	0.07	4.5[b]	4.0[a]	3.9	2.9	0.0002
TGF-β (ng/ml)	11.1[b]	11.4[b]	12.0	13.1	0.0012	10.6	12.0	12.3	12.7	0.014	13.1	12.1	11.2	11.0	0.0005
CRP (mg/liter)	2.51	2.40	2.54	2.24	0.35	2.53	2.28	2.55	2.32	0.77	2.30	2.45	2.45	2.48	0.46

ref, Reference quartile.

[a] Mean values and statistics for the association of n-3 and n-6 fatty acids with selected inflammatory markers are mutually independent. All mean values and statistical tests are adjusted for age, sex, education, daily intake of energy, proteins, lipids and carbohydrates, physical activity, BMI, smoking, LDL cholesterol, HDL cholesterol, triglycerides, hypertension, diabetes, coronary heart disease, congestive heart failure, stroke, peripheral artery disease, and potentially confounding drug treatment.

[b] $P < 0.01$, compared with the reference quartile.

[c] $P < 0.05$, compared with the reference quartile.

eral, a specific IL-6 receptor is expressed by the membranes of hepatocytes, monocytes/macrophages, and some leukocytes. However, the sIL-6r/IL-6 complex can stimulate a much wider range of cell types that have the gp130 protein, the inner portion of the IL-6 receptor, on their membrane. Recent literature suggests that this mechanism is active only when plasma levels of soluble gp130 are low, which is probably a rare condition. When high levels of gp130 and sIL-6r are present, a hexameric complex is created (2*IL-6 + 2*sIL-6r + 2*gp130), which tends to precipitate and has no biological activity (35). Thus, in this condition, sIL-6r levels are antiinflammatory (36). IL-6 and sIL-6r have shown opposite biological activity in several instances. Recently we reported that IL-6 is associated with insulin resistance, whereas sIL-6r has the opposite effect (37).

Similarly to Pischon *et al.* (13), we found some evidence that n-6 fatty acids may be antiinflammatory with no evidence of the proinflammatory activity previously suggested by many authors. However, our data suggest that the immunomodulatory effect of PUFAs may be influenced by the n-6 to n-3 ratio, which in our study was the strongest negative correlate of IL-10 and TGFβ, two powerful antiinflammatory cytokines. Pischon *et al.* found no significant association between plasma PUFA concentrations and serum CRP levels, and in our study only ALA was an independent negative correlate of CRP. This particular finding is somewhat puzzling and difficult to interpret because the production of CRP is mainly regulated by IL-6 (38), and ALA was not a significant independent correlate of IL-6. Interestingly, in a recent trial, supplementation of ALA *vs.* LA for 2 yr significantly reduced serum CRP but had no effect on other inflammatory markers (39).

Our findings show that plasma levels of AA and omega-3 PUFAs, which probably reflect higher dietary intake, are associated with lower serum concentrations of certain proinflammatory cytokines and lower concentrations of certain antiinflammatory cytokines. Therefore, these findings support the view that AA and n-3 PUFAs may modulate the inflammatory response by acting both on the proinflammatory and antiinflammatory arms of the cytokine network. Such a modulatory effect on multiple signaling pathways suggests a direct regulatory effect on gene expression. Interestingly, short-term infusion of n-3 lipid emulsion markedly suppresses monocytic generation of TNFα, IL-1, IL-6, and IL-8 in response to endotoxin (40).

The mechanism by which AA and n-3 fatty acids may inhibit the production of proinflammatory cytokines has been intensively investigated. Fatty acids can bind to the peroxisome proliferator activated receptors (PPaR)α and PPaRγ, which regulate the transcription of target genes (7, 41). PPaRs can also repress gene transcription by interfering with signaling molecules, such as nuclear factor-κB, therefore inhibiting the production of proinflammatory cytokines (42, 43). De Caterina and colleagues (8, 44) found that polyunsaturated fatty acids have antiinflammatory properties and hypothesized that they exert this effect because they have an unsaturated double bond, which, regardless of the n-3 or n-6 position, inactivates reactive oxygen species and prevents their interaction with nuclear factor-κB. This hypothesis is consistent with our findings suggesting that n-3 and n-6 fatty acids have both antiinflammatory properties.

The most important limitation of this study is the cross-sectional nature of our analysis. Although the consistency of the effect across multiple inflammatory markers is suggestive of causality, the correlation reported in this study does not prove the link between PUFAs and inflammatory markers but suggests that the physiological concentration of PUFAs reflects the severity of inflammation independently of other risk factors. In addition, although our analysis was adjusted for a number of potential confounders, we cannot exclude the possibility that other factors affect both n-3 fatty acids and cytokine concentrations. The diet of the Tuscany population is particularly poor of polyunsaturated fatty acids (45). In the InCHIANTI study, the average estimated daily intake of PUFAs was 7.4 g, which is lower than the intake reported for other populations (46) and even compared with other Italian populations (47). On the contrary, the intake of monounsaturated fatty acids in our population tended to be high (>50% of total lipids), likely the result of the large consumption of olive oil in Italy and, in particular, in the Tuscany region. The generalizability of our findings to other populations with different dietary intake should be confirmed by other studies.

Our finding that n-3 and n-6 account for a significantly lower percentage of total fatty acids in older persons may explain the mild proinflammatory state that is often found in the elderly and is not completely accounted for by cardiovascular risk factors and morbidity (48). If this hypothesis is correct, nutritional intervention may contrast this age-related trend to a proinflammatory state.

This study also has several strengths. To our knowledge, this is the first investigation of the relationship between plasma concentrations of fatty acids and multiple proinflammatory and antiinflammatory cytokines based on a representative sample of the general population. Different fatty acids were directly measured in plasma and not estimated from dietary reports. Because no participants were using dietary supplements, our findings are based on physiological plasma concentrations; this information is more precise and provides a more objective measure of fatty acid exposure, which depends on both intake and metabolism. Finally, information on multiple potential confounders, including dietary intake, was available for all participants.

Because serum levels of specific fatty acids can be easily modified by a different selection of foods in the diet or dietary supplementation, physicians should consider dietary interventions to suppress production of proinflammatory compounds as part of the prevention and treatment of diseases in which inflammation exerts adverse effects on clinical progression.

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