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Red blood cell fatty acids and biomarkers of inflammation: A cross-sectional study in a community-based cohort

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ABSTRACT

Introduction: Inflammation and inflammatory biomarkers have emerged as integral components and predictors of incident cardiovascular (CV) disease. Omega-3 fatty acids, particularly eicosapentaenoic and docosahexaenoic acids (EPA and DHA) have anti-inflammatory properties, and have been variably associated with lower blood pressure, favorable blood lipid changes, and reduced CV events.

Methods and results: We examined the cross-sectional association of red blood cell (RBC) fatty acids, representative of body membrane fatty acid composition, with 10 biomarkers active in multiple inflammatory pathways in 2724 participants (mean age 66 ± 9 years, 54% women, 8% minorities) from the Framingham Offspring and minority Omni Cohorts. After multivariable adjustment, the RBC EPA and DHA content was inversely correlated (all $P \leq 0.001$) with 8 biomarkers: urinary isoprostanes ($r = -0.16$); and soluble interleukin-6 ($r = -0.10$); C-reactive protein ($r = -0.08$); tumor necrosis factor receptor 2 ($r = -0.08$); intercellular adhesion molecule-1 ($r = -0.08$); P-selectin ($r = -0.06$); lipoprotein-associated phospholipase-A2 mass ($r = -0.11$) and activity ($r = -0.08$). The correlations for monocyte chemoattractant protein-1 was −0.05, $P = 0.006$ and osteoprotegerin ($r = -0.06$, $P = 0.002$) were only nominally significant.

Conclusion: In our large community-based study, we observed modest inverse associations between several types of inflammatory biomarkers with RBC omega-3 fatty acid levels. Our findings are consistent with the hypothesis that omega-3 fatty acids have anti-inflammatory properties.

1. Introduction

Higher fish and fish oil consumption have been variably reported to be associated with decreased cardiovascular (CV) events [1], strokes [2,3], and dementia [4]. The major reason for the benefits of fish consumption is considered to be their content of omega-3 (ω-3) long chain polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [5–7]. The biological mechanisms responsible for the potential protective effects of EPA and DHA are incompletely understood.

The role of inflammation in atherosclerosis is well-established [8,9]. More recently studies have demonstrated associations of inflammatory markers with many other conditions such as hypertension [10], dyslipidemia [11], diabetes mellitus [12], atrial fibrillation [13], and chronic renal disease [14]. ω-3 PUFAs may modify inflammatory cascades favorably, which may be an

Abbreviations: RBC, red blood cell; PUFA, polyunsaturated fatty acid(s); EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

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important factor in their protective role, but published results in intervention studies have been inconsistent [15].

Other cohorts that have examined the correlations between omega-3 biostatus and inflammatory markers have been from cultures differing substantially in diet and lifestyle from the US (e.g., Tuscany, Japan, Korea, Finland) (Table 3), and although the findings are relatively consistent, the relations observed there may not reflect the US situation. The largest US study (prior to the present one) to examine these associations was the Multiethnic Study of Atherosclerosis (MESA) [16,17]. However, these investigators used plasma phospholipid EPA and DHA levels as their marker of exposure and examined relations with only six inflammatory biomarkers. The within person variability for EPA + DHA levels in plasma phospholipids is four times higher than that of red blood cells (RBC) [18], and as such, some true associations may have been missed. To avoid this concern and to expand upon the number of biomarkers examined in MESA, we used RBC EPA + DHA levels for our analyses which reflect relatively long-term tissue omega-3 status, much like hemoglobin A1c is used to monitor glycemic status [19]. We have assessed the relations between ω-3 PUFA status and 10 biomarkers representing different phases and pathways of inflammation in a large, community-based sample. We hypothesized that the RBC EPA + DHA content is inversely correlated with serum concentrations of several inflammatory biomarkers.

2. Methods

2.1. Study sample

The Framingham Heart Study is a longitudinal community-based cohort study that was initiated in 1948. The selection criteria for the Framingham Offspring Cohort and the Framingham Omni Cohort have previously been described [20,21] (http://nhlbi.nih.gov/about/framingham). Briefly, adult children of the Original cohort were recruited in 1971 into the Framingham Offspring Cohort. To reflect the increased diversity of the community as the population has changed in Framingham, the ethnic/racial minority Omni cohort was recruited in 1994 [22]. We evaluated Framingham Offspring participants (n = 3021) who attended their eighth examination cycle (2005–2008) and Framingham Omni participants (n = 298) who attended their third examination (2007–2008). Participants were excluded in hierarchical order if they were missing RBC fatty acid measurements (n = 123), biomarker measurements (n = 323), or clinical covariates (n = 149). The study protocol was approved by the Institutional Review Board of the Boston University Medical Center. Informed consent was provided by all participants.

2.2. Omega-3 index

Blood was drawn after a 10–12 h fast into an EDTA tube, and RBCs were separated from plasma by centrifugation. The RBC fraction was frozen at −80 °C immediately after collection. RBC fatty acid composition was determined as described previously [23]. Briefly, RBCs were incubated at 100 °C with boron trifluoride-methanol and hexane to generate fatty acid methyl esters that were then analyzed by gas chromatography with flame ionization detection. The omega-3 index was defined as EPA + DHA as a percent of the total fatty acids in RBC membranes [24]. The coefficients of variation were 6.2% for EPA, 4.4% for DHA, and 3.2% for the omega-3 index.

2.3. Inflammatory biomarkers

We selected one urinary and nine serum biomarkers representing multiple inflammatory pathways: urinary 8-epi-PGF2α isoprostanes (normalized to creatinine), C-reactive protein (CRP), interleukin-6, intercellular adhesion molecule-1 (ICAM-1), lipoprotein-associated phospholipase-A2 (LpPLA2) activity and mass, monocyte chemoattractant protein-1 (MCP-1), osteoprotegerin, P-selectin, and tumor necrosis factor receptor 2 (TNFR2). The details of the rational for selection of these biomarkers, assays and measurements have been described previously [25]. The inter-assay coefficients of variation were less than 10% for all measurements [13].

2.4. Statistical analyses

Descriptive statistics are presented as percentage for categorical variables and as mean ± standard deviation for continuous variables. To normalize skewed distributions, analyses of inflammatory biomarkers were natural logarithmically transformed. Relationships of omega-3 index and logarithmic values of inflammatory biomarkers were evaluated using Pearson correlation coefficients. Multivariable models were adjusted for age, sex, cohort (Offspring vs. Omni populations), current smoking, systolic blood pressure, body mass index, blood levels of total cholesterol, high density lipoprotein cholesterol, triglycerides, glucose, and presence of diabetes, aspirin use (≥3 times per week), hormone replacement therapy, lipid drug treatment, blood pressure treatment, fish oil supplementation and/or self-reported dietary fish intake, and prevalent cardiovascular disease (myocardial infarction, stroke, congestive heart failure). Participants with prevalent CVD were excluded in a secondary analysis. We tested for effect modification by age and sex of the relations between omega-3 index and the inflammatory biomarkers. After Bonferroni correction for multiple analyses, the statistical significance was defined by two-tailed $p < 0.0013$. All statistical analyses were performed using SAS 9.2, (SAS Institute Inc., Cary, North Carolina).

3. Results

We evaluated 2724 eligible participants from the Framingham Offspring and Omni Cohorts. The mean age was 66 ± 9 years for both groups combined, and 53% of participants were women. Table 1 shows the baseline clinical characteristics of the

Table 1
Baseline clinical characteristics at examination (n = 2724).

Characteristic	Value
Age, years	66 ± 9
Female sex, %	53
Body mass index, kg/m^2	28.3 ± 5.4
Waist circumference, inches	40.0 ± 5.7
Smoking, %	8
Systolic blood pressure, mmHg	129 ± 17
Diastolic blood pressure, mmHg	74 ± 10
Total serum cholesterol, mg/dL	186 ± 37
High density lipoprotein-C, mg/dL	57 ± 18
Triglycerides, mg/dL	117 ± 68
Glucose, mg/dL	107 ± 24
Hypertension Treatment, %	49
Lipid drugs, %	43
Aspirin use ≥3 times week, %	44
Fish oil supplement use, %	11
Hormone replacement, % women	10
Diabetes mellitus, %	15
Prevalent cardiovascular disease, %	6

participants. Fish oil supplements were used by 11% of participants. The mean omega-3 index was 5.65 ± 1.73 for all participants.

The correlations between the omega-3 index and the inflammatory biomarkers are shown in Table 2. In age- and sex-adjusted models we observed statistically significant negative correlations between omega-3 index and all 10 inflammatory biomarkers. After multivariable adjustment, 8 correlations with the omega-3 index remained statistically significant. Correlations with monocyte chemoattractant protein-1 ($p = 0.006$) and osteoprotegerin ($p = 0.0018$) did not reach the Bonferroni critical value of $p < 0.0013$). The strongest correlations observed were for interleukin-6 ($r = -0.10$), isoprostane/creatinine ratio ($r = -0.16$), and LpPLA2 mass ($r = -0.11$). There were no significant effect modifications by sex or age (Supplemental Tables 1 and 2). In secondary analysis, the removal of 163 participants with CV disease (mean omega-3 index 5.43 ± 1.59) did not materially affect the correlations (Supplemental Table 3). Reported fish oil supplementation was associated with a higher omega-3 index (mean 7.9%) and a lower concentration of CRP, but there were no significant associations with the other nine biomarkers (Supplemental Table 4).

4. Discussion

In our large community-based study, we found significant inverse associations between the omega-3 index and inflammatory biomarkers adjusting for multiple confounders including fish oil intake. We did not observe effect modification by age or sex of the relations between the omega-3 index and inflammatory biomarkers, and this association persisted after exclusion of individuals with a history of CVD and/or fish oil supplementation with a pill (as shown in Supplemental Tables 3 and 4).

Several previous studies have examined the relation between the dietary intake of ω-3 PUFA and blood levels of ω-3 PUFA to inflammatory biomarkers (Table 3). Among the dietary studies, 6 of 8 found a significant inverse association between at least one inflammatory biomarker and ω-3 PUFA intakes, whereas in 10 of 12 biomarker-based studies, significant inverse relations for at least 1 inflammatory marker with EPA and/or DHA levels were observed. Similarly, a recent meta-analysis of 68 trials found overall significant reductions in CRP and IL-6 and marginally significant reductions in TNFα after ω-3 PUFA supplementation [15]. Treatment with omega-3-based pharmaceutical agents has also been shown to significantly lower $LpPLA_2$ levels [26–28]. Reported fish oil supplementation was associated with lower concentrations of CRP in our study (Supplemental Table 4), which is consistent with data from randomized clinical trials [31]. Our findings extend the available evidence using the omega-3 index to a larger number of inflammatory biomarkers representing major inflammatory mechanisms.

ω-3 PUFAs may affect multiple pathways resulting in the decreased production of inflammatory mediators. EPA and DHA have been shown to modulate the activity of major transcription factors controlling inflammatory responses such as nuclear factor κB [29], peroxisome proliferator activated receptors [30,31], and others [32]. The administration of ω-3 PUFAs also was associated with decreased production of potent pro-inflammatory autacoids derived from ω-6 PUFAs [33,34]. EPA and/or DHA have been reported to reduce certain interleukins and tumor necrosis factor-α [34,35], molecules produced by a wide variety of cells including those of the innate immune system, and are central to inflammatory responses [36–38]. Other markers assessed here, such as the isoprostanes [39], MCP-1 [40], ICAM-1 [41] and LpPLA2 [42] also play important roles in inflammatory processes and oxidative stress.

Although our findings do not establish causality, they do support the hypothesis that ω-3 PUFAs may have anti-inflammatory actions that might contribute to the clinical protective effects seen with increased intake of ω-3 rich food, especially fish. The effect of ω-3 PUFAs on inflammatory pathways may lead to their use therapeutically in diseases associated with chronic inflammation such as inflammatory bowel disease [43], asthma [44], rheumatoid arthritis [45] and cardiovascular disease [46]. However, studies have failed to consistently demonstrate improvement in various inflammatory conditions with ω-3 PUFAs supplementation (see review by Calder [47]). Thus, further studies, perhaps exploring higher doses and/or longer treatment periods, are required to more fully evaluate the role that ω-3 PUFAs may play in the management of chronic inflammatory conditions. Such studies might track the effects of change in erythrocyte ω-3 PUFAs on inflammatory biomarkers over time. Further research is also needed to better discern the biological mechanisms by which ω-3 PUFAs may modify the inflammatory cascade.

4.1. Strengths and limitations

We examined a large community-based cohort with rigorous ascertainment of clinical risk factors, inflammatory biomarkers, and ω-3 PUFA status. The inclusion of the minority Framingham Omni cohort in addition to the Offspring cohort increases the diversity of the study population and therefore improves the generalizability of the findings. To our knowledge, our study is the largest to examine the relations between circulating ω-3 PUFA levels and inflammatory markers. The present study is also unique in including up to 10 different inflammatory biomarkers associated with widely varying pathways and phases of inflammation.

There are several limitations to our study. In an observational cross-sectional study, the associations between RBC ω-3 PUFAs with inflammatory markers cannot establish temporality or causality, and cannot exclude the possibility of residual confounding or that higher blood omega-3 levels may simply be markers of an overall healthier lifestyle. Secondly, the correlations coefficients were low to moderate in magnitude (even though highly statistically significant), we cannot establish that they are clinically relevant. Thirdly, participants were mainly middle-aged to older adults from Framingham, Massachusetts. Thus, our findings may not necessarily be representative of individuals that are younger or from other geographic areas.

Table 2
Age- and sex-adjusted and multivariable-adjusted correlations between the inflammatory biomarkers and erythrocyte omega-3 index.

Adjustment	Age and sex		Multivariable[a]	
	r	*p*-value	r	*p*-value
Biomarkers				
C-reactive protein	−0.12	<0.001	−0.08	<0.001
Interleukin-6	−0.11	<0.001	−0.10	<0.001
Intercellular adhesion molecule-1	−0.14	<0.001	−0.08	<0.001
Urinary isoprostanes/creatinine ratio	−0.18	<0.001	−0.16	<0.001
LpPLA2 Activity	−0.10	<0.001	−0.08	<0.001
LpPLA2 Mass	−0.13	<0.001	−0.11	<0.001
Monocyte chemoattractant protein-1	−0.08	<0.001	−0.05	0.006
Osteoprotegerin	−0.07	<0.001	−0.06	0.0018
P-selectin	−0.11	<0.001	−0.06	<0.001
Tumor necrosis factor receptor 2	−0.08	<0.001	−0.08	<0.001

[a] Multivariable model: Age; sex; cohort (Offspring vs. Omni) current smoking; systolic blood pressure; body mass index; total cholesterol, high density lipoprotein cholesterol, triglycerides, glucose, diabetes, aspirin use (≥3 times per week); hormone replacement therapy; lipid lowering treatment; blood pressure, fish oil supplement or self-reported diet intake, prevalent cardiovascular disease (myocardial infarct, stroke, congestive heart failure).

Table 3
Previous observational studies on the relationship of omega-3 polyunsaturated fatty acids and inflammatory markers.

Study	Study population	Result
Omega-3 intake		
Pischon et al., 2003 [48]	859 healthy subjects	n-3 PUFA intake inversely associated with soluble-TNF receptors 1 and 2 but not IL-6 or CRP
He at al. 2009 [49]	5677 healthy subjects	2 of 9 markers (IL-6 and MMP-3) inversely related to n-3 PUFA intake
Lund et al., 2013 [50]	1212 healthy subjects	n-3 PUFA intake not associated with inflammatory markers
Niu 2006 [51]	971 Japanese age >70	Intake of n-3 PUFA inversely associated with CRP
Zampelas 2005 [52]	3042 Greek adults	Fish intake inversely associated with CRP, TNF-α, IL-6, SAA, and leukocyte count
Lopez-Garcia 2004 [53]	727 healthy female nurses	Compared with lowest quintile of n-3 FA intake, those in highest had lower CRP, E-selectin, sICAM-1, and s-VCAM-1
Murakami 2008 [54]	443 female Japanese dietetic students	Intake of n-3 PUFA inversely associated with CRP
Poudel-Tandukar 2009 [55]	512 healthy Japanese	Intake of EPA and DHA not associated with CRP
Omega-3 Biomarkers		
Farzaneh-Far et al., 2009 [56]	992 subjects with CAD	Erythrocyte n-3 PUFA inversely associated with CRP and IL-6
Grenon et al., 2013 [57]	64 subjects with peripheral arterial disease	Erythrocyte n-3 PUFA inversely associated with CRP but not IL-6, ICAM-1, TNF-α
Kalogeropoulos et al., 2010 [58]	374 healthy subjects	Plasma n-3 PUFA inversely associated with CRP, IL-6, TNF-α, fibrinogen
Baek et al., 2013 [59]	80 depressed Koreans vs 80 controls	Erythrocyte EPA + DHA inversely associated with iNOS, TNF-α
Ferrucci et al., 2006 [60]	1123 older Italians	Plasma DHA inversely associated with IL-6, IL-10, TGF-β, IL-1ra
Steffen et al., 2012 [16,17]	2448 MESA participants	Plasma phospholipid n-3 PUFA inversely associated with IL-6, TNF-r1 and LpPLA2
Reinders et al., 2011 [61]	1395 healthy Finns	Serum n-3 PUFA inversely associated with CRP
Sekikawa et al., 2010 [62]	297 men (Japanese in Japan; Japanese in US, and Whites in US)	Levels of 8 cytokines were not related to serum n-3 PUFA levels across cohorts
Micallef et al., 2009 [63]	124 healthy Australians	Plasma n-3 PUFA inversely associated with CRP
Labonte et al., 2014 [64]	744 Quebec Cree	RBC DPA n-3, but not EPA/DHA, was associated with CRP, IL-6 and TNF-α
Steffen et al., 2013 [16]	2246 MESA participants	Plasma phospholipid EPA + DHA was inversely associated with $LpPLA_2$ mass and activity
Schmidt et al., 2008 [65]	301 patients with CAD	Adipose tissue n-3 PUFA was inversely correlated with $LpPLA_2$ mass

PUFA = polyunsaturated fatty acid; IL-6 = interleukin-6; MMP-3 = matrix metalloproteinase-3; CRP = C-reactive protein; TNF = tumor necrosis factor; ICAM = intercellular adhesion molecule; VCAM = vascular cell adhesion molecule; CAD = coronary artery disease; MESA = Multi-ethnic study of atherosclerosis.

5. Conclusion

Our community-based study identified a small-to-modest inverse association between erythrocyte ω-3 EPA + DHA levels and eight major biomarkers of inflammation, representing a wide variety of inflammation pathways. Our data are consistent with the hypothesis that long chain ω-3 PUFA may promote anti-inflammatory processes, which may result in a reduction of CV events.

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Competing interests

Dr. Harris is currently an employee of Health Diagnostic Laboratory, Inc., and the President of OmegaQuant Analytics, LLC. Both of these laboratories offer RBC omega-3 fatty acid testing for clinical and research purposes, respectively.

Conflicts of interest

W.S.H. is a member of the scientific advisory board of Aker Biomarine. He is the President of OmegaQuant Analytics, LLC, and is a Senior Research Scientist at Health Diagnostic Laboratory, Inc., two companies that offer blood omega-3 fatty acid testing. Other authors have no conflicts of interest to disclose.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2015.03.043.

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