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Posted Date: 29 May 2025

doi: 10.20944/preprints202505.2301.v1

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 doi:10.20944/preprints202505.2301.v1

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Red Blood Cell Omega-6 Fatty Acids and Biomarkers of Inflammation in the Framingham Offspring Study

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Abstract: Chronic inflammation is recognized as an important risk factor for a variety of health disorders. Omega-6 polyunsaturated fatty acids (n-6 PUFAs), particularly linoleic (LA) and arachidonic acid (AA), have been shown to be either pro- or anti-inflammatory, and researchers have advocated both for and against reducing their dietary intake. This study sought to correlate the levels of ten inflammation-related biomarkers across multiple pathways with red blood cell (RBC) membrane levels of the major dietary and circulating n-6 PUFAs. This study included 2,777 participants (mean age 66±9 years, 54% women, 9.8% minorities) from the Framingham Offspring and minority-enriched Omni Cohorts. After multivariable adjustment, RBC LA was inversely correlated (all $p \leq 0.05$) with five markers of inflammation, receptors, or pathways: C-reactive protein (r=-0.06); soluble interleukin-6 (r=-0.15); intercellular adhesion molecule-1 (r=-0.09); monocyte chemoattractant protein-1, and (r=-0.07); P-selectin (r=-0.07). RBC AA was inversely correlated (all $p \leq 0.05$) with soluble interleukin-6 (r=-0.10); intercellular adhesion molecule-1 (r=-0.14); monocyte chemoattractant protein-1, and (r=-0.06); and osteoprotegerin (r=-0.07). Lipoprotein associated phospholipase A2 mass and activity, urinary isoprostanes, and tumor necrosis factor receptor 2 were not significantly correlated with LA or AA. In our large community-based study, we observed weak but statistically significant inverse associations between several types of inflammatory biomarkers with RBC n-6 PUFAs. Our findings do not support the hypothesis that omega-6 fatty acids are proinflammatory.

Keywords: linoleic acid; arachidonic acid; inflammatory markers; C-reactive protein; interleukin-6; intercellular adhesion molecule-1; monocyte chemoattractant protein-1; P-selectin; osteoprotegerin

1. Introduction

The roles that dietary fats play in the etiology of multiple chronic diseases, particularly cardiovascular disease (CVD), are topics of ongoing controversy. The only class of dietary fatty acids (FAs) that is universally believed to be unhealthy is the industrially-produced trans FAs [1]. The convention of grouping FAs into classes by carbon chain length and saturation does not imply similar biochemical effects and physiological functions. For example, some dietary saturated FAs (e.g., palmitic acid) have adverse effects on serum cholesterol levels while others in the same family are neutral (e.g., stearic acid) [2–4]. Circulating levels of oleic acid, a major component of olive oil (and thus the Mediterranean diet) and also produced through *de novo* lipogenesis [5], have been positively associated with risk for depression [6], CVD [7], and total mortality [8] bringing into question their cardioprotective reputation [5]. Because some clinical trials have not demonstrated a reduction in CVD risk with marine-derived omega-3 polyunsaturated fatty acids (n-3 PUFAs), eicosapentaenoic

 doi:10.20944/preprints202505.2301.v1

acid (EPA) and docosahexaenoic acid (DHA) once believed to be cardioprotective [9] are now viewed by some [10] but not all [11,12] as being ineffective.

Relative to health benefits, the most controversial dietary FAs are the omega-6 (n-6) PUFAs. Some scientists point to the potential harms of arachidonic acid (AA, C20:4n6) as it is the precursor to multiple pro-inflammatory mediators [13]. AA can be synthesized from the major dietary and tissue n-6 PUFA, linoleic acid (LA, C18:2n6). Major food sources of LA in the western diet are seed oils (e.g., soybean, corn, cottonseed oils) and to a certain extent, chicken (via their feed) [14], and these have, in recent years become the focus of much adverse publicity owing to both their being "processed" and providing LA. Some researchers have called for a marked reduction in LA intake, based strongly on the view that LA is pro-inflammatory [13,15,16]. The concern about LA and seed oils is illustrated by the recent Cleveland Clinic Health Newsletter story entitled, "Seed Oils: Are they actually toxic [17]?"

In contrast, the American Heart Association (AHA) in 2009 issued a Science Advisory that reviewed the role of n-6 PUFAs in CVD and recommended that n-6 PUFA intake should be at least 5% to 10% of energy. The Advisory concluded, "To reduce n-6 PUFA intakes from their current levels would be more likely to increase than to decrease risk for coronary heart disease (CHD)" [18]. In 2012, Johnson and Fritsche conducted a systematic review of the effects of LA on inflammatory biomarkers and stated: "We conclude that virtually no evidence is available from randomized, controlled intervention studies among healthy, non-infant human beings to show that addition of LA to the diet increases the concentrations of inflammatory biomarkers [19]". Two major studies that pooled *de novo* analyses of individual data from multiple cohorts found circulating LA levels to be inversely associated with risk for CVD [20] and for type 2 diabetes mellitus (T2DM) [21], and a recent report from the UK Biobank found strong inverse associations between plasma LA levels and total and cause-specific mortality [22].

In regard to the other major n-6 PUFA, when AA was fed to mice with experimental colitis, there was no increase in bowel inflammation compared with the oleic acid-rich control diet, and the AA-treated mice had less diarrhea [23]. Supplementation trials with AA in humans have not observed any increase in proinflammatory metabolites [24,25]. Finally, although higher AA levels in adipose tissue were directly associated with risk for CHD events in the Diet, Cancer and Health study [20], when pooled together with 29 other cohorts with data on *in vivo* AA levels, this FA was unrelated to risk for CVD [20]. Similarly in a paper from the Cardiovascular Health Study, plasma phospholipid AA was directly association with risk for incident T2DM, but when combined with similar data from 19 other cohorts, the overall relationship was null [21].

The purpose of the present study is to further explore the hypothesis that LA and/or AA are pro-inflammatory by examining the cross-sectional association between red blood cell (RBC) membrane levels of LA and AA and ten biomarkers representing different phases and pathways of inflammation in a large, community-based sample. Based on our reading of the prior literature, we hypothesized that overall, LA would be inversely correlated with several inflammatory biomarkers, and that no relationship would be found with AA. This study took the same approach as Fontes et al. [26] who reported the associations between RBC n-3 PUFA levels and these ten inflammatory biomarkers in this cohort.

2. Materials and 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 been described previously [27,28] (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 [29]. We evaluated Framingham

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Offspring participants (n = 2,985) who attended their eighth examination cycle (2005-2008) and Framingham Omni 1 participants (n = 285) who attended their third examination (2007-2008). Participants were excluded in hierarchical order if they were missing RBC fatty acid measurements (n = 143), biomarker measurements (n = 342), or clinical covariates (n = 12). 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. Red Blood Cell Linoleic Acid and Arachidonic Acid

Blood was drawn after a 10-12 hour fast into an EDTA tube, and RBCs were separated from plasma by centrifugation. The RBC fraction was frozen at -80ºC immediately after collection. RBC fatty acid composition was determined as described previously (OmegaQuant Analytics, Sioux Falls, SD, USA) [30]. Briefly, RBCs were incubated at 100ºC with boron trifluoride-methanol and hexane to generate fatty acid methyl esters that were then analyzed by gas chromatography with flame ionization detection. The coefficients of variation were 5.3% for LA and 2.3% for AA.

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 (Lp-PLA2) activity and mass, monocyte chemoattractant protein-1 (MCP-1), osteoprotegerin, P-selectin, and tumor necrosis factor receptor 2 (TNFR2) (Table 1). The details of the rational for selection of these biomarkers, assays and measurements have been described previously [31]. Briefly, test kits used for quantification were ACE Competitive EIA (Cayman Chemical) for 8-epi-PGF2α isoprostanes; Dade Behring BN100 nephelometer for CRP; Quantitative ELISA (R&D systems) for interleukin-6, ICAM-1, MCP-1, P-selectin, and TNFR2; Quantitative ELISA (diaDexus) for LpPLA2 activity and mass; and Quantitative ELISA (Biomedica Gesellschaft mbH) for osteoprotegerin [31]. The inter-assay coefficients of variation were less than 10% for all measurements [19,31].

Table 1. Summary of the inflammatory markers examined (all serum or plasma unless otherwise noted)

Inflammatory marker	Units	Role in inflammation
Urinary 8-EPI-Isoprostanes/Creatinine	0.18 – 0.40 µg/g creatinine [32] [1]	A stable biomarker of oxidative stress and inflammation formed from non-enzymatic free radical-catalyzed peroxidation of AA [33]
C-Reactive Protein	≤ 3 mg/L [34]	An acute-phase protein produced in the liver; levels rise rapidly and acutely during inflammation [35]
Interleukin-6	1 – 5 pg/mL [36]	A pleotropic cytokine that exhibits both anti-inflammatory and pro-inflammatory effects [37,38]; an essential component for CRP production via hepatocytes [39].
Intercellular Adhesion Molecule 1	100 – 300 ng/mL [40,41]	A cell surface glycoprotein that mediates and facilitates leukocytes to sites of inflammation [42]; levels are upregulated during inflammation [43]
Lp-PLA2 Activity	225 nmol/min/mL [44,45]	A proinflammatory enzyme from the phospholipase A2 family; a potential marker of vascular inflammation [46,47]
Lp-PLA2 Concentration	≤ 200 ng/mL [48]	
Monocyte Chemoattractant Protein 1	127 – 274 pg/mL [49] [2]	A protein that is upregulated by pro-inflammatory stimuli; attracts monocytes, neutrophils, and lymphocytes to sites of inflammation [50]
Osteoprotegerin	13 – 84 pg/mL [51] [2]	A pro-inflammatory soluble decoy receptor and a member of the TNF receptor that potentially acts

 doi:10.20944/preprints202505.2301.v1

		through NF-κB activation [52]; inhibits bone resorption by preventing RANKL from engaging RANK receptors [53]
P-Selectin	19 – 521 ng/mL [54]	A member of the selectin adhesion molecule expressed on activated endothelial cells and platelets; facilitate and mediate leukocytes at inflammation sites [55]
Tumor Necrosis Factor Receptor 2	1951 – 3430 pg/mL [56–58] [2]	A cytokine that exhibits both anti-inflammatory (e.g. neuroprotection) [59] and pro-inflammatory effects (e.g. promotes tumor cell proliferation) [60]

Lp-PLA2 = Lipoprotein-associated phospholipase-A2. [1] Reported as pg/mmol in the Framingham Heart Study [31]. [2] Not absolute, levels may vary due to age, genetics, and overall health

2.4. Statistical Analysis

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 RBC LA and AA and logarithmic values of inflammatory biomarkers were evaluated using partial correlation coefficients. In addition, the associations between these biomarkers and RBC n-3 PUFA EPA + DHA, i.e. the Omega-3 Index (O3I), originally reported in Fontes et al. [26], were re-evaluated controlling for AA and LA levels, to confirm that the relationships originally observed remained after adjustment for these additional PUFAs. We conducted a series of multivariable models adjusting for different subsets of subject characteristic variables and FA exposures: Model 1 adjusted for age and sex, Model 2 adjusted for all Table 2 demographic covariates, Model 3 consisted of Model 2 covariates + AA, and Model 4 included Model 3 covariates + O3I. Statistical significance was defined by two-tailed $p<0.05$ for each FA vs biomarker comparison. We also looked for evidence of non-linearity or statistical interaction (age, sex, race/ethnicity) in Model 3. Given the exploratory nature of the analyses, we used a Bonferroni corrected statistical significance threshold of 0.005 (0.05/10 inflammatory markers) for tests of non-linearity and statistical interaction. Tests of non-linearity were conducted by fitting a cubic spline to the FA exposure and comparing that model using a nested F-test to the same model with a linear term for the FA exposure. Tests of interaction were conducted by evaluating the statistical significance of an interaction term between the FA exposure and (separately) age, sex or race/ethnicity (non-Hispanic White vs. other). All statistical analyses were performed using R Statistical Software (v4.2.0; R Core Team 2022).

3. Results

3.1. Descriptive Statistics

We evaluated 2,777 eligible participants from the Framingham Offspring and Omni Cohorts. The mean age was 66 years and 54% were female (Table 2). The intercorrelations between RBC LA, AA, and the O3I are in a footnote to Table 1, while additional descriptive statistics for RBC LA, AA, and the O3I are reported in Table S1.

Table 2. Participant characteristics from the Framingham Study

Characteristics	% or mean ± SD
Sex (% female)	54.1
Age (years)	65.9 ± 9.0
Race/ethnicity	
Non-Hispanic White (%)	89.7
NH Black (%)	3.5
NH Asian (%)	2.3

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NH Other (%)	0.7
Hispanic (%)	3.1
Current smoker (%)	7.3
Systolic Blood Pressure (mmHg)	129 ± 17
Body mass index (kg/m^2)	28.4 ± 5.5
Total Cholesterol (mg/dL)	186 ± 37
HDL Cholesterol (mg/dL)	57 ± 18
Triglycerides (mg/dL)	117 ± 68
Glucose (mg/dL)	107 ± 24
Aspirin Usage (% reporting ≥3 times a week)	43.6
Prevalent dyslipidemia medication (%)	45.1
Prevalent hypertension medication (%)	49.7
Prevalent diabetes (%)	13.6
Prevalent cardiovascular disease (CVD) (%)	16.0
Hormone Replacement Therapy (%)	13.4
Exposures[1]	
RBC Linoleic Acid (LA, %)	11.04 ± 1.71
RBC Arachidonic Acid (AA, %)	16.57 ± 1.60
Omega-3 Index (O3I, RBC EPA+DHA, %)	5.57 ± 1.71
Outcomes	
Isoprostanes/Creatinine (mg/mg)	11.2 ± 6.2
C-Reactive Protein (mg/L)	3.2 ± 7.3
Interleukin-6 (pg/mL)	2.6 ± 3.0
Intercellular Adhesion Molecule 1 (ng/mL)	294.4 ± 104.4
Lp-PLA2 Activity (nmol/min/mL)	137.4 ± 34.9
Lp-PLA2 Mass (ng/mL)	199.5 ± 49.7
Monocyte Chemotactic Protein 1 (pg/mL)	381.5 ± 131.3
Osteoprotegerin (pmol/L)	5.0 ± 1.6
P-Selectin (ng/mL)	41.2 ± 13.2
Tumor Necrosis Factor Receptor 2 (pg/mL)	2591.1 ± 1055.1

Lp-PLA2 = Lipoprotein-associated phospholipase-A2

[1] The correlation between plasma LA% and AA% was -0.47; between LA% and the O3I -0.23, and between AA% and the O3I -0.40.

3.2. The Correlations Between Linoleic Acid, Arachidonic Acid, and Inflammatory Biomarkers

The relationships between RBC LA levels (after accounting for variation in inflammatory markers accounted for by demographic and medical history variables, as well as AA and O3I; Model 3) were statistically significant and inverse for five markers: CRP, IL-6, ICAM-1, MCP-1 and P-selectin (Table 3). There were no inflammatory biomarkers that were positively and significantly associated with RBC LA after removing the association of AA and the O3I with these biomarkers.

Table 3. Partial correlations (95% CI) between RBC LA and inflammatory markers in the Framingham Study with differing levels of covariate adjustment

	Model 1	Model 2	Model 3	Model 4
Isoprostanes/Creatinine	-0.025 (-0.062, 0.013)	0.004 (-0.036, 0.044)	0.035 (-0.010, 0.080)	-0.048 (-0.102, 0.005)
CRP	**-0.063 (-0.101, -0.026)****	**-0.042 (-0.079, -0.005)***	-0.012 (-0.054, 0.029)	**-0.061 (-0.111, -0.011)***
Interleukin-6	**-0.105 (-0.141, -0.069)****	**-0.056 (-0.093, -0.019)****	**-0.042 (-0.084, -0.000)***	**-0.146 (-0.195, -0.096)****
ICAM-1	-0.035 (-0.073, 0.003)	0.014 (-0.024, 0.052)	-0.004 (-0.047, 0.039)	**-0.088 (-0.139, -0.037)****
LpPLA2 Activity	**0.099 (0.063, 0.135)****	**0.046 (0.014, 0.078)****	**0.059 (0.022, 0.095)****	0.034 (-0.010, 0.077)
LpPLA2 Mass	**0.117 (0.080, 0.155)****	**0.042 (0.004, 0.079)***	**0.068 (0.025, 0.111)****	0.027 (-0.025, 0.078)
MCP-1	-0.030 (-0.068, 0.007)	-0.020 (-0.060, 0.020)	-0.022 (-0.067, 0.023)	**-0.068 (-0.122, -0.015)***
Osteoprotegerin	**0.060 (0.026, 0.093)****	**0.071 (0.035, 0.106)****	**0.063 (0.023, 0.103)****	0.015 (-0.033, 0.063)
P-selectin	**-0.038 (-0.075, -0.000)***	-0.037 (-0.077, 0.003)	-0.014 (-0.059, 0.031)	**-0.067 (-0.121, -0.013)***

 doi:10.20944/preprints202505.2301.v1

TNFR2	-0.017 (-0.053, 0.019)	0.018 (-0.020, 0.056)	0.024 (-0.019, 0.067)	-0.028 (-0.080, 0.024)

*p<0.05; **p<0.01; Model 1 = Age, Sex only; Model 2 = All Table 2 variables (except fatty acids); Model 3 = All Table 2 variables + AA; Model 4 = All Table 2 variables + AA + O3I.

The relationships of RBC AA levels (after accounting for variation in inflammatory markers for all other variables, including LA and O3I) were statistically significant and inverse for four markers: IL-6, ICAM-1, MCP-1 and osteoprotegerin (**Table 4**). There were no inflammatory biomarkers that were positively and significantly associated with RBC AA after removing the influence of LA and the O3I on these biomarkers.

Similar analyses as the above were conducted using the O3I as the exposure in order to confirm that the previously published results by Fontes et al. [26] were not altered by additional adjustment for LA and AA (which was not done in the original paper). The original findings were confirmed with all marker associations being significant (p<0.01) except that the inverse relationship with MCP-1 was, although still inverse, no longer statistically significant. (r = -0.038, p = 0.06; Table S2).

Table 4. Partial correlations (95% CI) between RBC AA and inflammatory markers in the Framingham Study with differing levels of covariate adjustment

	Model 1	Model 2	Model 3	Model 4
Isoprostanes/Creatinine	**0.069 (0.032, 0.106)****	**0.048 (0.010, 0.086)***	**0.064 (0.020, 0.107)****	-0.037 (-0.093, 0.019)
CRP	**0.093 (0.056, 0.130)****	**0.066 (0.031, 0.101)****	**0.061 (0.021, 0.101)****	0.003 (-0.049, 0.055)
Interleukin-6	**0.094 (0.058, 0.129)****	**0.047 (0.012, 0.083)****	0.029 (-0.011, 0.068)	**-0.096 (-0.147, -0.044)****
ICAM-1	-0.010 (-0.047, 0.027)	-0.034 (-0.071, 0.002)	-0.036 (-0.077, 0.005)	**-0.137 (-0.190, -0.084)****
LpPLA2 Activity	**-0.059 (-0.094, -0.023)****	-0.000 (-0.031, 0.031)	0.026 (-0.009, 0.061)	-0.004 (-0.049, 0.041)
LpPLA2 Mass	**-0.044 (-0.081, -0.006)***	0.023 (-0.013, 0.060)	**0.054 (0.013, 0.095)****	0.004 (-0.049, 0.058)
MCP-1	0.002 (-0.035, 0.038)	0.006 (-0.032, 0.044)	-0.004 (-0.047, 0.039)	**-0.060 (-0.116, -0.004)***
Osteoprotegerin	-0.029 (-0.062, 0.004)	**-0.044 (-0.078, -0.010)***	-0.016 (-0.054, 0.022)	**-0.074 (-0.124, -0.024)****
P-selectin	0.023 (-0.014, 0.060)	**0.053 (0.015, 0.091)****	**0.047 (0.004, 0.090)***	-0.017 (-0.073, 0.039)
TNFR2	-0.017 (-0.053, 0.019)	0.018 (-0.020, 0.056)	0.024 (-0.019, 0.067)	-0.028 (-0.080, 0.024)

*p<0.05; **p<0.01; Model 1 = Age, Sex only; Model 2 = All Table 1 variables (except fatty acids); Model 3 = All Table 1 variables + AA; Model 4 = All Table 1 variables + AA + O3I.

3.3. Tests of Non-Linearity and Interaction

One FA-inflammatory marker showed some evidence of non-linearity, AA and osteoprotegerin (p = 0.0005), with Q4/Q5 of AA levels showing much stronger inverse associations with osteoprotegerin than Q1-Q3 (see Table S3). There was no evidence of statistically significant interaction with age (continuous) or sex (male vs. female), though for one FA there was a statistically significant interaction with race (p = 0.001). ICAM-1 showed stronger inverse association with AA levels in non-whites and Hispanics [95% CI (-0.85, -0.32); n = 266], as compared to non-Hispanic whites [95% CI,(-0.16, -0.05); n = 2490]; see Table S4).

4. Discussion

In our large community-based study, RBC LA was inversely associated with CRP, IL-6, ICAM-1, MCP-1, and P-selectin, while RBC AA was inversely associated with IL-6, ICAM-1, MCP-1 and osteoprotegerin, all after adjusting for multiple confounders including mutual n-6 PUFA biomarker adjustments and the O3I. Importantly, neither of these n-6 PUFAs was significantly and positively associated with any of the ten inflammatory biomarkers.

Several previous studies have examined the relation between the dietary intake of n-6 PUFA and blood levels of n-6 PUFA to inflammatory biomarkers. Among the dietary studies [61–64], three of four found a significant inverse association between n-6 PUFA intakes and CRP [62–64], while all ten biomarker-based studies reported significant inverse relations for at least one inflammatory marker

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with total n-6, LA, or AA levels [65–75]. Higher LA is most commonly reported to be associated with lower levels of CRP [62–64,66,67,70–73] though two studies reported links with higher Lp-PLA2 [68,74]. Findings for AA tend to be mixed, with mostly no significant associations with inflammatory biomarkers [62,63,70,72,73], but inverse associations with IL-6 [65] and Lp-PLA2 mass and activity [68,74]. Discrepancies between studies could often be attributed to differences in populations, lipid compartment analyzed (e.g., plasma vs RBC), and assay methods and the time of blood draw for both FAs and the inflammatory markers. Our findings extend the available evidence by additionally exploring a larger number of inflammatory biomarkers representing major inflammatory mechanisms. Though, it is important to note that correlations were mostly weak, even when statistically significant.

n-6 PUFA metabolism is closely linked to n-3 PUFAs. In conjunction, their metabolites affect multiple pathways pleiotropically and may result in the production of both anti-inflammatory and pro-inflammatory mediators [76]. In the context of n-6 PUFAs, LA and AA metabolites, i.e., oxylipins and eicosanoids produced via cyclooxygenase (COX), 5-lipoxygenase (5-LOX), 12-LOX, and 15-LOX pathways [76], exhibit pro-aggregatory effects, e.g., thromboxanes, as well as anti-aggregatory, anti-inflammatory and vasodilatory effects, e.g., epoxy-eicosatrienoic acids, prostaglandin E2,which induces IL-6 production, lipoxin A4, prostacyclin. An 8-week supplementation of soy oil (~50% LA) elevated levels of Lp-PLA2 activity in healthy Korean adults [77]. However, meta-analyses of randomized controlled trials found no significant effect of higher LA intake on a wide range of inflammatory biomarkers, including CRP, IL-6, ICAM-1, P-selectin, and MCP-1 [19,78]. Similarly, levels of proinflammatory metabolites did not increase with AA supplementation in human trials [24,25,79,80], even though experimental studies suggest that AA elevates the expression of ICAM-1[81] and increases osteoclastogenesis [82]. In observational studies, LA is inversely associated with mortality, cancer, T2DM, and other types of death [20,21,83–85], while associations between AA and CVD [86], cancer [85], diabetes [21], atrial fibrillation [87], and death [88] remain generally neutral. Collectively, based on the evidence above, our findings do not support the hypothesis that n-6 PUFAs are pro-inflammatory.

Our findings, although observational, do not establish causality. Instead, they suggest that n-6 PUFAs are not pro-inflammatory (refuting at least one of the ‘concerns’ of seed oils), and based on several biomarkers, they actually have an anti-inflammatory signature. LA is an essential fatty acid. Recommendations to reduce it in the diet could lead to an inadequate intake resulting, in the extreme, in an essential fatty acid deficiency manifested by dermatitis, alopecia, poor wound healing [89] and growth stunting in children [90]. Modeling exercises studying the potential effects on population LA intakes of ‘high mono’ seed oils (e.g., safflower and sunflower hybrids) have raised concerns that the health benefits of LA (beyond preventing classic deficiency symptoms) may be threatened by the increased use of these low LA oils [91]. Thus, our findings support current dietary guidelines [92] to prioritize LA-rich oils, including soybean, corn, and sunflower oils, and to decrease saturated fat intake (i.e., palmitic acid, stearic acid, predominant FAs in beef tallow or butter).

The independent effects of LA and AA and their metabolites on the less studied Lp-PLA2, urinary isoprostanes, osteoprotegerin, and TNFR2 leave much room for further investigation. Such studies could explore the role of LA or AA in the management of chronic inflammatory conditions in patients versus healthy subjects, using higher doses and/or longer treatment periods, and track the effects of change in RBC n-6 PUFAs on inflammatory biomarkers over time.

The strengths of this study include the use of a well-characterized and large community-based cohort with rigorous ascertainment of clinical risk factors, inflammatory biomarkers, and individual RBC LA and AA levels. The inclusion of the minority Framingham Omni cohort in addition to the Offspring cohort improves the generalizability of the findings. To our knowledge, our study is one of the largest to examine the relationships between circulating individual RBC n-6 PUFA levels and inflammatory markers. The present study is also unique in including up to ten different inflammatory biomarkers associated with widely varying pathways and phases of inflammation.

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There are also limitations. Observational study, especially cross-sectional ones, cannot ascribe causal connections between RBC n-6 PUFAs with inflammatory markers, nor can they exclude the possibility of residual confounding. As noted, the correlations coefficients, although statistically significant and inverse, were nevertheless low. Hence, the clinical relevance of these findings is unclear. 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.

5. Conclusions

Our community-based study identified small, significant, inverse associations between RBC LA and AA levels with six major biomarkers of inflammation, representing a wide variety of inflammation pathways. Our results suggest that LA is more likely to be anti- than pro-inflammatory, and present efforts to reduce its intake are ill-advised.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Table S1: Descriptive statistics for linoleic acid, arachidonic acid, and omega-3 index; Table S2: Partial correlations between the omega-3 index and 10 inflammatory biomarkers with and without adjustment for RBC linoleic acid and arachidonic acid (All biomarkers log-Transformed and standardized); Table S3: The association between arachidonic acid and osteoprotegerin by quintiles; Table S4: The association between arachidonic acid and intercellular adhesion molecule 1 stratified by ethnicity

Author Contributions: Conceptualization, W.S.H., N.L.T., K.H.J., P.M.K. and N.A.R.; Methodology, W.S.H., N.L.T., N.A.R., H.T.M.L.; Software, N.L.T., N.A.R.; Validation, N.L.T., N.A.R.; Formal Analysis, N.A.R.; Investigation, W.S.H., N.L.T., K.H.J., P.M.K., N.A.R. and H.T.M.L.; Resources, W.S.H., N.L.T., N.A.R.; Data Curation, N.L.T., N.A.R.; Writing – Original Draft Preparation, W.S.H., N.L.T., N.A.R., H.T.M.L.; Writing – Review & Editing, W.S.H., N.L.T., K.H.J., P.M.K. N.A.R., H.T.M.L.; Visualization, N.A.R., N.L.T., H.T.M.L.; Supervision, W.S.H.; Project Administration, W.S.H., N.L.T., and H.T.M.L.; Funding Acquisition, W.S.H.

Funding: This research was supported in part by R01 HL089590 (WSH, PI), and by a grant from the Soy Nutrition Institute.

Institutional Review Board Statement: The study protocol was approved by the Institutional Review Board of the Boston University Medical Center. (H-32132; March 18, 2016).

Informed Consent Statement: Informed consent was provided by all participants.

Data Availability Statement: Data used in this study were obtained from the Framingham Offspring study. Investigators should contact the study office to gain access to the data.

Conflicts of Interest: W.S.H. is the founder and President of OmegaQuant Analytics, LLC which offers blood fatty acid testing to researchers, healthcare providers and consumers. K.H.J. is an employee of OmegaQuant Analytics. N.L.T., N.A.R., P.M.K. and H.T.M.L. have no conflicts of interest to disclose.

Abbreviations

The following abbreviations are used in this manuscript:

RBC	Red blood cell
PUFA	Polyunsaturated fatty acid(s)
LA	Linoleic acid
AA	Arachidonic acid
O3I	Omega-3 index (RBC eicosapentaenoic acid + docosahexaenoic acid)
FA	Fatty acid
CRP	C-reactive protein
ICAM-1	Intercellular adhesion molecule-1
Lp-PLA2	Lipoprotein-associated phospholipase-A2
MCP-1	Monocyte chemotactic protein-1

 doi:10.20944/preprints202505.2301.v1

TNFR2	Tumor necrosis factor receptor-2

 doi:10.20944/preprints202505.2301.v1

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