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Plasma and erythrocyte ω-3 and ω-6 fatty acids are associated with multiple inflammatory and oxidative stress biomarkers in breast cancer

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ABSTRACT

Objectives: The aim of this study was to assess the association of plasma and erythrocyte ω-3 and ω-6 fatty acids with multiple oxidative stress biomarkers in breast cancer patients.
Methods: Ninety-five women with recently diagnosed breast cancer and no previous treatment were selected for this study. The socioeconomic, clinical, and demographic profile of the patients was determined using a structured questionnaire and medical records. Body weight and height were measured to calculate body mass index. Blood samples were drawn after a 12-h fast for biochemical analyses. The oxidative stress biomarkers low-density lipoprotein (−) and its anti-low-density lipoprotein (−) antibodies, α-tocopherol, β-carotene, high-density lipoprotein cholesterol, thiobarbituric acid reactive substance, and adiponectin were measured. Plasma and erythrocyte fatty acids were assessed by gas chromatography. Factor and principal component analysis was used to identify three factors called factor 1 (anti-inflammatory), factor 2 (antioxidant), and factor 3 (oxidant). Linear regression, adjusted for confounding variables, was used to estimate the association of these factors with plasma and erythrocyte ω-3 and ω-6 fatty acids and their ratios.
Results: ω-3 series fatty acids showed a positive association with Factor 1. A positive association of plasma and erythrocyte ω-6 fatty acids with factors 1 and 2, respectively, was found. ω-6/ω-3 ratio (plasma) was inversely associated with the anti-inflammatory factor.
Conclusion: Polyunsaturated fatty acids of the plasma and erythrocyte ω-3 and ω-6 series were associated with multiple inflammatory and oxidative stress biomarkers in breast cancer.

Introduction

Dietary patterns, foods, nutrients, and bioactive components have been shown to exert multiple actions that can prevent and modify the carcinogenesis process. According the World Health Organization (WHO), ~35% of cancer cases can be explained by dietary factors [1–3]. High intake of saturated fats and refined sugars and low intake of fruit and vegetables are considered dietary determinants associated with breast oncogenesis, inflammation, and oxidation [2–6].

In breast cancer, continuous production of free radicals promotes imbalance in the antioxidant system, where biotransformation of DNA, RNA, lipids, and proteins leads to cell death, mutations, replication errors, and genomic instability [7–9]. This event is consistent with oxidative stress, in which the imbalance between oxidative products and antioxidant substances promotes multiple cell damage that can modify functions such as cell homeostasis and normal replication process [10]. In this context, antioxidant nutrients (vitamins A, C, and E and the minerals zinc and selenium) have modulating and protective effects against the development and growth of cancerous cells, and can significantly reduce the adverse effects produced by oxygen reactive species

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and inflammation [1,10–14]. The relationship between oxidative stress and breast cancer has been described previously [15] and it is directly associated with breast cancer aggressiveness. Previous research [16] detected a high content of thiobarbituric acid reactive substances (TBARS), classical biomarkers of lipid peroxidation, in patients with breast cancer compared with control group of women. Furthermore, reduced levels of tocopherols and carotenoids were found in female survivors of breast cancer [17]. More recently, modified lipids, such as oxidized low-density lipoprotein (oxLDL), were found to be associated with different breast and ovarian cancers [18,19]. The possible connection between lipoprotein metabolism and cancer has been the focus of studies carried out by Maranhao et al. [20–23]. Given that, and aiming to avoid bias associated with in vitro oxidation process, we developed a monoclonal antibody based on a natural modified LDL named electronegative LDL [LDL(−)], which has been extensively used in the cardiovascular field as an oxidative biomarker [24–26].

Contrary to the negative effects of oxidative stress caused by modified lipids, high-density lipoprotein cholesterol (HDL-C), adiponectin [27,28], and many cytokines are involved in chronic inflammatory response present in breast cancer [29,30].

In addition to oxidative stress, dietary fats appear to influence, via multiple pathways, the inflammatory and oxidative cascade in breast cancer. The balance between polyunsaturated fatty acids (PUFAs) of the ω-3 family (α-linolenic acid [ALA], eicosapentaenoic acid [EPA], docosahexaenoic acid [DHA], and docosapentaenoic acid [DPA]) and ω-6 (linoleic acid [LA], arachidonic acid [AA], and dihomo-γ-linoleic acid [DGLA]) controls the inflammatory processes by modulating desaturase, elongase, and cyclooxygenase and lipoxygenase enzymes, which lead to the formation of eicosanoids, resolvins, protectins, and maresins—essential in controlling inflammatory processes [14].

Therefore, the ratio of these two fatty acids (FAs) is essential for an adequate inflammatory response, given that ω-6 and its derivatives have been associated with increased leukotrienes and thromboxanes of the even series (more proinflammatory) and inflammatory cytokines (interleukin [IL]-1β, IL-6, tumor necrosis factor [TNF]-α), whereas ω-3 has an inhibitory role in nuclear factor (NF)-κB signaling, stimulates eicosanoid synthesis of the odd series (less proinflammatory), and induces apoptosis of tumor cells derived from breast cancer [14–21].

Although the epidemiologic evidence supports the protective role of antioxidant nutrients and lipids in the development of breast cancer, the interaction between these nutrients has not been extensively investigated because of limitations of intake assessment tools and the presence of potential epidemiologic (age, sex, smoking) and clinical (tumor staging, menopausal status, presence of morbidities, and cancer treatments) confounders [31,32].

In this scenario, the objective of the present study was to assess, using multivariate analysis, the association of plasma and erythrocyte ω-3 and ω-6 FAs with interaction among antioxidants, oxidation, and inflammation in women recently diagnosed with breast cancer who had no previous surgical intervention or neoadjuvant treatment.

Materials and methods

Study population

Women with breast cancer treated at the Mastology Clinic of the General Hospital of Fortaleza – HGF (Fortaleza, Ceará) between May 2011 and August 2012 were selected. Women >19 y of age, recently diagnosed with malignant cancer of the breast, clinical staging (CS) I to IV, without metastatic spread, other associated neoplasia, or previous antineoplastic treatment, and Karnofsky index ≥70 were eligible. The exclusion criteria were patients with uncontrolled chronic non-communicable or transmissible diseases, those receiving nutritional guidance or in use of drug therapy for weight control, and those presenting with neurologic or psychiatric problems.

Consecutive sampling was employed, in which each patient was admitted according the inclusion and exclusion criteria. This gave a final study sample of 95 women. The sample size was calculated based on correlation coefficients, with $\beta = 0.20$, $\alpha = 0.05$ (bilateral), yielding a minimum sample needed of 85 patients [33,34].

The study was approved by the Research Ethics Committee of the General Hospital of Fortaleza and University of São Paulo School of Public Health.

Socioeconomic and clinical profiles

The socioeconomic and demographic profile of the patients (age, marital status, education, and family income per capita in minimum wages) was collected using a structured questionnaire. The clinical history of the patients was taken from medical records and a direct interview collecting data on menopausal status (pre- or postmenopausal), smoking (active smoker, past smoker, and never smoked), family history of cancer and clinical/pathologic staging (TNM).

Anthropometric parameters

The anthropometric assessment included measuring current weight (CW) and height (m). CW was taken using digital scales (Control model; Plenna, São Paulo, Brazil) with a capacity of 150 kg and accuracy to the nearest 100 g. Height measurements were taken using a stadiometer (AlturaExata model; TBW, São Paulo, Brazil) with a limit of 2.10 m and accuracy to the nearest 1 mm. After calculation of body mass index (BMI; CW/height^2, kg/m^2), participants were classified as obese or non-obese, according to the nutritional status categories recommended by the World Health Organization (WHO) [35].

Biochemical analyses

Blood samples were collected (20 mL) after a 12-h fast in Vacutainer tubes containing EDTA (1 mg/mL) as the anticoagulant and antioxidant. Blood samples were stored on ice and shielded from light until extraction of the plasma (1500g, 10 min, 4°C). Protease inhibitors were added to the plasma: aprotinin (2 μg/mL), benzamidine (2 mM) and phenylmethanesulfonyl fluoride (1 mM), plus butylated hydroxytoluene antioxidant (20 mM). The mixture was aliquoted and stored at −80 °C until analyses.

HDL-C and adiponectin

Cholesterol levels were determined for HDL-C (Labtest, Minas Gerais, Brazil) using the manual application of enzymatic reagents. Plasma adiponectin was analyzed by sandwich enzyme immunoassay using the human adiponectin enzyme-linked immunosorbent assay (ELISA) commercial kit (EMD Millipore, St Charles, MO, USA).

α-tocopherol and β-carotene

The fat-soluble antioxidants (α-tocopherol and β-carotene) were extracted from the plasma (200 μL) and analysis performed by high-performance liquid chromatography (HPLC; Shimadzu Inc., Tokyo, Japan) using a separation column – Synergy Fusion 5 μ-C8 (2) 150 × 4.6 mm coupled to a precolumn (Security Guard-HPLC Guard Cartridge System-KJO 4282), both from Phenomenex (Torrance, CA, USA). The effluent was monitored by SPD-10 AVVP UV-vis (340 nm) and RF-10 AXL (Shimadzu Inc.) The fluorescence detector was set at 295 nm (excitation) and 340 nm (emission). The curve was plotted using external standard (99%) containing concentrations of α-tocopherol of between 2 and 49 μmol/L and β-carotene of between 0.1 and 0.76 μmol/L.

TBARS

Plasma (50 uL) was added to a 1 mL solution of thiobarbituric acid solution (0.046 M), trichloroacetic acid (0.92 M), and hydrochloric acid (0.25 M), which was subsequently incubated in a boiling bain-marie (100°C) for 30 min. The solution was then centrifuged at 8000g for 15 min at 4°C, and supernatant (200 uL) readings taken at 535 nm. Quantification was performed using the standard curve built with 1,1,3,3 tetraethoxypropane (0.2–4 μmol of TBARS/L) [36]. Values were expressed in μmol of TBARS/mg of total protein. All analyses were carried out in duplicate.

Detection of LDL(−) and its antibodies

LDL(−) was detected by sandwich ELISA [37]. Results were expressed as the mean of sample absorbances minus the background. The standard curve was then applied and multiplied by the respective dilution. A calibration curve was built with human LDL(−) obtained by liquid chromatography (0.6–20 μg/mL). Results were expressed as U/L, with units representing 1 g/L of oxidized apolipoprotein B.

AntiLDL(−) and immunoglobulin G isotope antibodies were detected by capture ELISA [38]. The results were determined by applying the mean of absorbances of the samples, minus the background, to the standard curve plotted with the anti-LDL monoclonal antibody$^{(-)}$ (0.004–0.125 mU/L).

Plasma and erythrocyte fatty acids

Using the plasma (100 uL) and concentrate of red blood cells (500 uL) lysed with cold phosphate-buffered saline (1:10; v/v, 15 min, 3000g), lipid extraction was carried out with methanol and chloroform (2:1 vol/vol), where FAs were converted into methyl esters by addition of sodium methoxide [39]. Subsequently, the profile of the FAs was determined using gas chromatography/flame ionization detection (Shimadzu, CG-2010, Kyoto, Japan) based on a DB-FFAP capillary column (15 m × 0.100 mm × 0.1 mm; J&W Scientific from Agilent Technologies, Folsom, CA, USA). FAs were identified based on the profile of the external standard (FAME 37, code 47885, Sigma Chemical Co, St. Louis, MO, USA). The injection volume was 2 uL on an AOC 20 i automatic injector. The FAs were identified by comparing the retention times of the external standard against those of the samples. The internal standard methyl tricosanoate (C23:0, T9900, Sigma Chemical Co) was also used. Results were expressed as total percentage of FAs present in the samples [40].

Two groups were formed based on the identification of the FAs in the plasma: ω-3 FAs (sum of EPA, DHA, DPA) and ω-6 (sum of LA, AA, DGLA), as well as the ω-6/ω-3 ratio.

Statistical analysis

Qualitative variables were described as simple frequency and absolute numbers. Multiple oxidative stress biomarkers and quantitative variables were standardized using the same unit for performing the factor analysis. Factor analysis is a multivariate statistical analysis used for the identification of factors in a set of measurements. In this method, all the variables (biomarkers) are considered simultaneously, each one in relation to the others [41].

For this factor analysis, sample adequacy was checked using the Kaiser-Meyer-Olkin (KMO) index and Bartlett's test of sphericity. KMO values >0.50 and $P < 0.05$ were considered acceptable. The number of factors was determined using the following criteria: eigenvalues >1 and analysis of the Scree plot. There were three factors in the present study. Negative loadings indicated that the biomarker was inversely correlated with the factor; whereas positive loadings indicated a direct correlation. The larger the factor loading of a biomarker, the more the biomarker contributed to that factor [41].

Pearson's correlation was then applied between the factors and plasma and erythrocyte concentrations of the FAs. Linear regression adjusted for age, ethnicity/color, menopausal status, BMI, and smoking habit was used to estimate coefficients of regression (β), SE, and *P*-value, where factors were dependent variables and PUFAs the independent variable of interest. A level of significance of $P < 0.05$ was adopted for all statistical tests.

Results

Patients were predominantly 20 to 59 y of age (82.1%), of non-white ethnicity/color (73.7%), and had a family income of less than one minimum wage. Regarding clinical and lifestyle characteristics, most patients were premenopausal (51.6%), had a family history of breast cancer (69.5%), lobular carcinoma subtype (72.6%), and early stage (66.3%). Additionally, 56.8% of participants were non-smokers and 34.7% were obese (Table 1). A descriptive analysis of oxidative stress biomarkers and the plasma and erythrocyte FA profile of the participants assessed is presented in Table 2.

Three factors were identified through factor analysis. Factor 1, which was labeled *anti-inflammatory*, was positively correlated with HDL-C and adiponectin; factor 2, labeled as *antioxidant*, was correlated with β-carotene and inversely correlated with TBARS; factor 3, labeled as *oxidant*, was directly proportional-LDL(−) and anti-LDL(−) and inversely proportional to α-tocopherol (Table 3).

Plasma FAs of the ω-3 family showed a positive correlation with factor 1 (anti-inflammatory), including EPA ($r = 0.296$; $P = 0.004$), DHA ($r = 0.294$; $P = 0.004$), DPA ($r = 0.462$; $P < 0.001$), and the sum of EPA and DHA ($r = 0.318$; $P = 0.002$). No correlations were found between plasma ω-3 FA and factor 2 (antioxidant) or factor 3 (oxidant). A positive correlation was detected between plasma ω-6 FAs and factor 1 ($r = 0.398$; $P < 0.001$), including AA ($r = 0.545$; $P < 0.001$). A similar relationship was found between factor 3 and ω-6 ($r = 0.285$; $P = 0.005$) and LA ($r = 0.279$; $P = 0.006$). The ω-6/ω-3 ratio was negatively correlated with factor 1 ($r = -0.205$; $P = 0.046$; Table 4).

Table 1
Demographic, socioeconomic, clinical, and anthropometric description of breast cancer patients

Variables	n	%
Age group (y)		
20–59	78	82.1
≥60	17	17.9
Ethnicity/Color		
White	25	26.3
Brown, black and others*	70	73.7
Family income		
≤1 MW	57	60
≥2 MW	38	40
Menopause		
No	49	51.6
Yes	46	48.3
Smoking habit		
No	54	56.8
Active or former smokers	41	43.2
Family history of breast cancer		
No	29	30.5
Yes	66	69.5
Subtype		
Ductal	15	15.8
Lobular	69	72.6
Clinical stage		
I and II	63	66.3
III and IV	19	20.0
Body mass index (kg/m^2)		
<30	62	65.3
≥30	33	34.7

MW, minimum wage.
Results expressed as absolute and relative values.
*Others: Yellow/indigenous.

Erythrocyte ω-3 FA levels were not correlated with any of the factors. Erythrocyte ω-6 FA were positively correlated with factor 2 ($r = 0.309$; $P = 0.003$) and factor 3 ($r = 0.211$; $P = 0.044$), including AA with factor 1 ($r = 0.242$; $P = 0.020$) and factor 2 ($r = 0.330$; $P = 0.001$; Table 4).

After adjusting for confounding variables, plasma ω-3 FAs showed a positive association with factor 1 (anti-inflammatory), including EPA (β = 1.23; $P = 0.002$), DHA (β = 1.23; $P = 0.002$), DPA (β = 1.23; $P = 0.002$), and the sum of EPA and DHA (β = 1.23; $P = 0.002$). Plasma ω-6 FAs showed a direct association with factor 1 (β = 0.11; $P < 0.001$), whereas the opposite pattern was seen for the ratio of ω-6 to ω-3 (β = −0.14; $P = 0.032$). Plasma ω-6 and ω-3 FAs were not associated with factors 2 or 3 (Table 5).

On the erythrocyte analyses, DGLA showed a negative association with factor 1 (anti-inflammatory; β = −0.39; $P = 0.043$), whereas a positive association was found between ω-6 acids (β = 0.11; $P = 0.005$), including AA (β = 0.10; $P = 0.005$), and factor 2 (antioxidant). FAs of the ω-6 and ω-3 family showed no association with factor 3 (Table 5).

Discussion

The objective of the present study was to assess the association of ω-3 and ω-6 FAs with multiple oxidative stress biomarkers in breast cancer. Factor analysis was used to estimate three factors with different oxidant and inflammatory characteristics. Results revealed that FAs of the ω-3 family showed an association with antioxidant and anti-inflammatory biomarkers, whereas those of the ω-6 family showed a controversial effect on the oxidative and inflammatory process.

The positive correlation of plasma ω-3 FAs with anti-inflammatory factors corroborate the results of the study by Iyengar et al. [42], in which a similar association was observed. The authors found

Table 2
Description of oxidative biomarkers and plasma and erythrocyte fatty acids profile

Biochemical analyses	Mean	SD	Median	Minimum value	Maximum value
LDL(−), U/L	4.08	5.17	2.38	0.51	36.86
Anti-LDL(−), um/L	4.69	3.04	4.39	0.67	20.70
α-tocopherol, μmol/L	11.18	2.79	10.87	4.39	19.65
β-carotene, μmol/L	0.47	0.36	0.38	0.06	1.72
HDL-C, mg/dL	34.04	9.18	35.00	6.00	52.50
TBARS, μmol/mg of protein	0.79	0.21	0.77	0.40	1.72
Adiponectin, μg/mL	8.97	5.33	7.15	1.41	27.25
Plasma fatty acids, %					
ω-3	4.69	1.17	4.49	2.64	8.33
EPA	0.62	0.25	0.57	0.25	1.51
DHA	2.56	0.89	2.46	1.12	5.28
DPA	0.88	0.23	0.86	0.47	1.63
EPA+DHA	3.18	1.06	3.03	1.39	6.79
ω-6	34.19	4.43	33.95	21.72	43.37
Linoleic	22.35	3.70	22.41	13.25	31.28
Arachidonic	9.23	2.62	9.02	4.23	18.44
DGLA	2.61	0.67	2.51	1.16	4.68
ω-6/ω-3	7.63	1.60	7.74	3.45	11.35
Erythrocyte fatty acids, %					
ω-3	6.59	1.77	6.33	3.23	12.78
EPA	0.77	0.89	0.53	0.25	5.95
DHA	3.59	1.29	3.36	1.39	8.85
DPA	2.23	0.54	2.18	1.24	3.85
EPA+DHA	4.36	1.50	4.06	1.99	9.43
ω-6	33.62	2.79	33.85	26.49	39.42
Linoleic	10.89	1.55	10.89	7.04	14.91
Arachidonic	20.56	3.01	20.66	11.93	27.53
DGLA	2.17	0.56	2.16	0.95	4.67
ω-6/ω-3	5.44	1.41	5.42	2.72	9.36

DGLA, dihomo-γ-linoleic; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; HDL-C, high-density lipoprotein cholesterol; LDL(−), electronegative LDL; TBARS, thiobarbituric acid reactive substances.

that ω-3 PUFAs were associated with reduced inflammation in cancerous cells in vitro, promoted by changes in cell membrane permeability and inhibition of cyclooxygenase-2 and lipoxygenase synthesis.

Incorporation of ω-3 (EPA and DHA) into the cellular structure is believed to change cellular signaling by modifying the lipid profile of the cell membranes. An experimental study [43] on breast cancer cells (MCF-7 and MDA-MB-231) showed that PUFAs are associated with a reduction in tumor proliferation, whereas EPA contributed to cell apoptosis and DHA to reduced total cholesterol in the cells, favoring inhibition of carcinogenesis. The results of the cited study are supported by the findings of the present investigation, showing a positive relationship of PUFAs, including EPA ($P = 0.004$), DHA ($P = 0.004$), DPA ($P < 0.001$), and sum of EPA and DHA ($P = 0.002$), with factor 1 (anti-inflammatory). This profile demonstrates the role of ω-3 FAs in modulating the anti-inflammatory properties of HDL-C and adiponectin.

The relationship between PUFA ω-3 content in the lipid membrane and risk for malignant neoplasia of the breast was explored in an in vitro study, in which higher EPA concentrations observed in cell membrane of women with breast cancer was associated with lower risk for this neoplasia compared with women with lower EPA levels (odds ratio, 0.51; $P = 0.003$) [44].

With regard to plasma and erythrocyte ω-6 FAs, the present study showed a controversial effect of these FAs, which exhibited a positive association with factors 1 and 2 (anti-inflammatory and antioxidant) and with factor 3 (oxidant). This profile can be explained by a study [43] in which AA had either an anti-inflammatory or pro-inflammatory effect in breast cancer cells. However, a population-based cross-sectional study [40,45] involving 300 individuals found that plasma levels of ω-6 and AA were lower than in individuals from the less inflamed cluster, which may indicate the proinflammatory role of these FAs. Conversely, some findings have shown ω-6 FAs to have an antitumoral effect by arresting cell proliferation in the initial G1 phase and cell replication phase through the expression of the p21 protein, a tumor-suppressing protein [46]. The anticarcinogenic role of ω-6 FAs was reinforced by increased caspase 3 and caspase 9 activity, genes responsible for cell apoptosis.

Table 3
Factor loadings of latent variables derived by factor analysis

Standardized variables*	Factor 1 Anti-inflammatory	Factor 2 Antioxidant	Factor 3 Oxidant
LDL(−)	0.190	−0.002	0.668
Anti-LDL(−)	−0.138	0.231	0.509
α-tocopherol	−0.143	0.241	−0.740
β-carotene	0.218	0.763	−0.189
HDL-C	0.805	−0.095	0.072
TBARS	0.133	−0.797	−0.159
Adiponectin	0.761	0.135	0.089
% Explained variance	19.5	19.4	19

HDL-C, high-density lipoprotein cholesterol; LDL(−),electronegative LDL; TBARS, thiobarbituric acid reactive substances.
Factor loadings ≥0.5 or ≤ −0.5 were considered significant.
*Principal component and factor analysis. KMO = 0.504; Bartlett's Test of Sphericity = 0.007.

Table 4
Correlation between multiple oxidative stress biomarkers and plasma and erythrocyte polyunsaturated fatty acids

	FactorAnti-inflammatory		Factor 2 Antioxidant		Factor 3 Oxidant	
	r	*P*-value	*r*	*P*-value	*r*	*P*-value
Plasma fatty acids*						
ω-3	0.344	0.001	0.015	0.887	0.122	0.240
EPA	0.296	0.004	0.030	0.771	0.002	0.987
DHA	0.294	0.004	0.000	0.998	0.111	0.285
DPA	0.462	<0.001	0.102	0.326	0.115	0.269
EPA+DHA	0.318	0.002	0.007	0.943	0.093	0.368
ω-6	0.398	<0.001	0.052	0.618	0.285	0.005
Linoleic	0.078	0.454	0.055	0.599	0.279	0.006
Arachidonic	0.545	<0.001	0.049	0.639	0.106	0.306
DGLA	0.064	0.535	−0.150	0.145	−0.074	0.476
ω-6/ω-3	−0.205	0.046	−0.034	0.740	0.044	0.672
Erythrocyte fatty acids*						
ω-3	−0.029	0.782	0.029	0.783	0.008	0.943
EPA	−0.134	0.204	−0.141	0.179	−0.040	0.707
DHA	0.012	0.907	0.067	0.525	0.028	0.789
DPA	0.095	0.367	0.168	0.110	0.023	0.831
EPA+DHA	−0.069	0.514	−0.026	0.802	0.001	0.994
ω-6	0.154	0.143	0.309	0.003	0.211	0.044
Linoleic	−0.124	0.239	−0.075	0.475	0.075	0.476
Arachidonic	0.242	0.020	0.330	0.001	0.174	0.096
DGLA	−0.190	0.070	−0.027	0.798	−0.096	0.361
ω-6/ω-3	0.028	0.795	0.069	0.510	0.030	0.778

DGLA, dihomo-γ-linoleic; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid.
*Pearson's correlation.

Further supporting the bimodal role of the ω-6 family, studies have shown a positive association of ω-6 with the promotion and progression of cancer [47,48]. This association was seen in an in vitro study [49], where a positive association was identified between increased proliferation of breast cancer cells and the amount of ω-6 (LA and AA) in phospholipid membrane ($P < 0.04$). A similar profile was found in another study [50], where ω-6 intake was positively associated with breast cancer and the development of estrogen receptor + progesterone receptor-type tumors ($P = 0.02$).

The disparities across studies suggests that the biological effects of ω-6 FAs and their derivatives in carcinogenesis is potentially influenced by the balance with other nutrients, including FAs from the ω-3 series. In the present study, the plasma ω-6/ω-3 ratio was negatively associated with an anti-inflammatory/antioxidant profile. This result is supported by a meta-analysis [51] based on six case–control and five cohort studies in which a high proportion of ω-3 was directly associated with lower risk for breast cancer (relative risk, 0.90; 95% confidence interval, 0.82–0.99). Although the results indicated that the ideal proportion can vary according to the disease and its stage of development, there is no ideal ratio for the prevention and management of breast cancer as exists in other chronic diseases [52].

The results of the present study are strengthened by the use of a combination of multiple biomarkers involved in different metabolic pathways associated with oxidative stress and inflammation. The

Table 5
Association between multiple oxidative stress biomarkers and plasma and erythrocyte polyunsaturated fatty acids

	Factor 1 Anti-inflammatory			Factor 2 Antioxidant			Factor 3 Oxidant		
	β*	SE	*P*-value	β*	SE	*P*-value	β*	SE	*P*-value
Plasma fatty acids									
ω-3	0.33	0.08	<0.001	0.01	0.09	0.892	0.08	0.09	0.358
EPA	1.23	0.39	0.002	0.03	0.42	0.938	0.17	0.41	0.679
DHA	0.38	0.11	0.001	0.01	0.12	0.951	0.10	0.12	0.383
DPA	1.97	0.42	<0.001	0.30	0.48	0.527	0.42	0.47	0.374
EPA+DHA	0.33	0.09	<0.001	0.01	0.10	0.944	0.08	0.10	0.408
ω-6	0.11	0.02	<0.001	0.02	0.03	0.485	0.05	0.03	0.066
Linoleic	0.04	0.03	0.273	0.03	0.03	0.311	0.06	0.03	0.082
Arachidonic	0.21	0.03	<0.001	0.01	0.04	0.879	0.04	0.04	0.378
Dihomo-γ-linoleic	0.08	0.15	0.594	−0.21	0.16	0.196	−0.13	0.16	0.408
ω-6/ω-3	−0.14	0.07	0.032	−0.01	0.07	0.903	0.02	0.07	0.764
Erythrocyte fatty acids									
ω-3	−0.02	0.06	0.756	0.01	0.06	0.860	0.01	0.06	0.877
EPA	−0.17	0.12	0.155	−0.15	0.12	0.196	−0.04	0.12	0.715
DHA	0.03	0.08	0.749	0.06	0.08	0.490	0.03	0.08	0.748
DPA	0.13	0.20	0.535	0.23	0.20	0.252	0.08	0.20	0.709
EPA+DHA	−0.04	0.07	0.561	−0.01	0.07	0.847	0.00	0.07	0.960
ω-6	0.04	0.04	0.310	0.11	0.04	0.005	0.06	0.04	0.164
Linoleic	−0.07	0.07	0.347	−0.04	0.07	0.590	0.02	0.07	0.810
Arachidonic	0.06	0.04	0.076	0.10	0.04	0.005	0.05	0.04	0.182
DGLA	−0.39	0.19	0.043	−0.02	0.20	0.909	−0.21	0.20	0.284
ω-6/ω-3	0.01	0.08	0.921	0.06	0.08	0.476	−0.01	0.08	0.913

DGLA, dihomo-γ-linoleic; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid.
*Linear regression adjusted for age, ethnicity/color, menopause, body mass index, smoking habit.

use of factor analysis allowed assessment of the synergistic effect of all of the biomarkers and naming of the factors according to the significance of factor loading and metabolic route of each marker. Another aspect that strengthens the results of this study was the analysis of AFs in two body compartments (plasma and erythrocytes), corresponding to current and habitual intake, respectively.

To our knowledge, this is the first study to demonstrate that LDL (−) and its antibodies can be important markers of oxidative stress in breast cancer. The results showed that modified LDL was directly associated with factor 3 (oxidant), corroborating the findings of a previous study [53] that examined the effect of modified LDL on cell proliferation in breast cancer and found that LDL(−) induced proliferation in all breast cancer cell lines tested. The stimulation was more intense in MDA MB 231 and HTB lines ($P = 0.003$ and $P = 0.029$, respectively). TBARS [26], an indicator of lipid peroxidation, also showed this association with breast cancer, with levels inversely correlated with the antioxidant profile (factor 2) and directly correlated with the oxidant profile (factor 3).

Counteracting oxidation products, adiponectin and HDL-C (factor 1) have an anti-inflammatory effect mediated by the action of paraoxonase-1 (PON1). Studies suggest that changes in this enzyme are associated with increased risk for various types of cancer, with low PON1 activity found in breast cancer patients [54,55]. Adiponectin, an adipokine secreted by adipose tissue, exerts an anti-inflammatory action by lowering sera levels of proinflammatory adipokines, such as IL-6 and TNF-α [56].

Although promising, the results from the present study cannot be used to infer causality among the variables and further studies are warranted. Moreover, the present study did not include a control group, which can be considered a limitation. Therefore, further studies should consider the use of a control group.

Conclusions

The PUFAs of the plasma and erythrocyte ω-3 and ω-6 family were associated with multiple oxidative stress biomarkers in breast cancer. Therefore, the consumption of foods rich in ω-3 should be encouraged to reap all their potential benefits in reducing oxidative stress and to achieve a diet with a balanced ω-6/ω-3 ratio.

Based on the study results, it can be concluded that ω-3 and ω-6 PUFAs modulate the critical processes of breast carcinogenesis with oxidation and inflammation, where the balance between these factors is important for optimizing their antitumoral roles. The present results confirm the benefits of regular dietary intake of antioxidant nutrients for women with breast cancer. In addition, these findings are relevant for clinical practice as they highlight the importance of continuous assessment of the quality of fat in the diet of women with breast cancer from diagnosis- to treatment.

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