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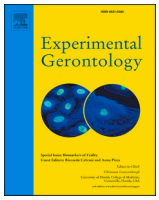

Polyunsaturated fatty acid status and markers of oxidative stress and inflammation across the lifespan: A cross-sectional study in a cohort with long-lived individuals

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ABSTRACT

Polyunsaturated fatty acids (PUFA) are known to have a regulatory effect on oxidative and inflammatory processes. This study aimed to identify the relationship between blood PUFA status and circulatory markers of oxidative stress and inflammation in a cohort of 172 subjects. The population was divided by sex and into three age groups: adults (18–64 years old, $n = 69$), older adults (65–89 years old, $n = 54$), and long-lived individuals (LLIs, 90–111 years old, $n = 49$). Whole blood PUFA content was quantified using gas chromatography. Additionally, serum levels of C-reactive protein (CRP), paraoxonase (PON), Trolox equivalent antioxidant capacity (TEAC), and malondialdehyde (MDA) were measured. Our results showed that a higher omega-3 (n-3) index in adult females was a predictor of lower MDA concentrations ($p = 0.038$). Conversely, total n-3 PUFA and total n-6 PUFA were positively related to MDA values among older adult females and LLI men ($p < 0.05$), while total n-6 PUFA was inversely correlated with MDA levels in LLI females ($p < 0.05$). Interestingly, increased concentrations of total n-3 PUFA and n-3 index were positively correlated with higher TEAC values in LLI men ($p = 0.007$), while the arachidonic acid (AA)/eicosapentaenoic acid (EPA) ratio was inversely correlated with TEAC values among LLI females ($p = 0.006$). These findings suggest that cellular antioxidant capacity is inversely correlated with changes in the AA/EPA ratio in long-lived females, whereas n-3 PUFA may enhance blood antioxidant capacity in long-lived men. Overall, our study highlights the complex, sex-specific interactions between PUFA profiles and oxidative stress and inflammatory markers across different age groups.

1. Introduction

Oxidative stress and dysregulated inflammation are increasingly recognized as being implicated in the pathophysiology of numerous diseases. Substantial evidence from large-scale studies shows that circulatory markers of oxidative stress and inflammation are risk indicators for several diseases, such as cardiovascular disease (CVD), metabolic disorders, cancer, and autoimmune and neurodegenerative disorders (Cardoso and Leal, 2020; Clearfield, 2005; Panda et al., 2022; Vona et al., 2019; Lakkur et al., 2015; Zahra et al., 2021; Furman et al., 2019). However, lifestyle modifications, such as a healthy diet, physical activity, stress management, and smoking cessation, have been reported to alleviate oxidative stress and inflammatory processes, and consequently can be an effective tool to prevent and manage non-communicable diseases (Husain et al., 2023; Beavers and Nicklas, 2011).

The effects of diet on oxidative stress and inflammatory processes have been extensively investigated in both observational and clinical studies. Evidence suggests that dietary patterns characterized by

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Table 1
Participant characteristics.

Variable	Total (mean ± SD)	Adults (mean ± SD)	Older adults (mean ± SD)	LLIs (mean ± SD)
Age (years)	68.5 ± 23.7	45.1 ± 13.7	71.4 ± 5.6	97.9 ± 5.0
Males/females	77/95	34/35	28/26	15/34
BMI (kg/m^2)	26.1 ± 4.9	25.17 ± 4.48	28.21 ± 4.60	25.10 ± 5.43
Total cholesterol (mg/dlL)	177.5 ± 33.3	179.28 ± 35.48	187.47 ± 28.54	163.29 ± 30.59
CRP (mg/dL)	3.5 ± 5.2	2.2 ± 3.5	3.2 ± 4.5	5.6 ± 7.2
TEAC (mM)	4178.8 ± 739.4	3845.2 ± 720.7	4120.9 ± 686.1	4714.7 ± 595.8
PON (U/L)	104.7 ± 61.6	123.4 ± 72.2	107.2 ± 63.4	79.1 ± 45.9
MDA (μmol/L)	2.5 ± 0.9	2.7 ± 0.9	2.58 ± 1.1	2.09 ± 0.8
Total n-3 PUFA (%)	5.3 ± 1.8	5.78 ± 1.77	5.03 ± 2.00	4.94 ± 1.51
DHA (%)	3.4 ± 1.2	3.71 ± 1.12	3.01 ± 1.19	3.22 ± 1.08
EPA (%)	0.8 ± 0.6	0.79 ± 0.66	0.84 ± 0.76	0.64 ± 0.41
n-3 index (%)	5.1 ± 1.7	5.54 ± 1.71	4.76 ± 1.93	4.70 ± 1.45
Total n-6 PUFA (%)	35.0 ± 4.0	36.99 ± 3.35	34.50 ± 4.33	32.75 ± 3.25
AA (%)	9.5 ± 2.1	10.07 ± 2.27	9.16 ± 2.10	9.08 ± 1.85
AA/EPA	17.8 ± 9.2	18.38 ± 10.02	16.49 ± 8.84	18.36 ± 8.41
n-6/n-3	7.3 ± 2.5	6.99 ± 2.17	7.87 ± 3.07	7.19 ± 2.14

SD, standard deviation; LLIs, long-lived individuals; BMI, body mass index; CRP, C reactive protein; TEAC, Trolox equivalent antioxidant capacity; PON, paraoxonase; MDA, malondialdehyde; PUFA, polyunsaturated fatty acids; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

increased intake of anti-inflammatory and antioxidant-rich foods, such as fruits, vegetables, nuts, seeds, olive oil, and fatty fish, can reduce systemic inflammation and oxidative stress, thereby lowering the risk of chronic diseases (Aleksandrova et al., 2021). A systematic review of observational and intervention studies found that plant-based diets, including the Mediterranean diet, Dietary Approaches to Stop Hypertension (DASH) diet, the USDA Healthy Eating Index (HEI)-based diet, were associated with lower levels of oxidative stress and inflammation biomarkers (Aleksandrova et al., 2021). Adherence to the traditional Mediterranean diet is correlated to reduced levels of inflammation and coagulation markers, including C-reactive protein (CRP), interleukin-6 (IL-6), homocysteine, and fibrinogen (Chrysohoou et al., 2004; Smidowicz and Regula, 2015). Similarly, antioxidant-rich dietary interventions have been shown to confer protective effects by scavenging free radicals and enhancing antioxidant enzyme activities, leading to improved clinical outcomes. In a randomized controlled trial, individuals at high risk of CVD assigned to a traditional Mediterranean dietary pattern showed a decrease in malondialdehyde (MDA), a marker of lipid peroxidation, and oxidized low-density lipoprotein (LDL) levels (Fitó et al., 2007). In contrast, Western and fast-food diets high in saturated fats, processed foods, and refined sugars, can increase inflammatory mediators and consequently promote a state of chronic low-grade inflammation (Aleksandrova et al., 2021). Concurrently, these dietary patterns also enhance oxidative stress by increasing ROS production, and lipid peroxidation and interfering with antioxidant defense mechanisms (Fitó et al., 2007).

Among dietary components, the physiological role of polyunsaturated fatty acids (PUFA) has been extensively studied, indicating that their metabolism may impact clinical conditions associated with inflammatory and oxidative processes. Although there are conflicting results regarding the effects of PUFA on oxidative stress, it is proposed that the n-3 PUFA can improve total antioxidant capacity and mitigate oxidative damage (Xiao et al., 2022; Sakai et al., 2017). Experimental evidence indicated that PUFA can enhance the expression and activity of antioxidant enzymes, including superoxide dismutase (SOD), catalase, and glutathione peroxidase, through various signaling pathways (Davinelli et al., 2022). Likewise, n-3 PUFA intake is shown to enhance paraoxonase (PON) activity, which is an antioxidant enzyme associated with high-density lipoprotein (HDL) and plays a crucial role in protecting against the oxidation of LDL (Shekhanawar et al., 2013). These enzymes play a significant role in scavenging or eliminating free radicals, thereby establishing a crucial intracellular antioxidant system

Table 2
Characteristics of the population by sex.

Variable	Total (mean ± SD)		*P*-value
	Males	Females	
Age (years)	65.23 ± 21.81	71.15 ± 24.93	0.1
BMI (kg/m^2)	26.85 ± 3.64	25.51 ± 5.79	0.09
Total cholesterol (mg/dlL)	176.89 ± 33.19	177.93 ± 33.54	0.8
CRP (mg/dL)	4.52 ± 12.59	4.64 ± 7.65	0.9
TEAC (mM)	4048.16 ± 783.62	4263.08 ± 729.65	0.07
PON (U/L)	108.11 ± 58.47	105.02 ± 71.10	0.8
MDA (μmol/L)	2.77 ± 0.97	2.26 ± 0.94	**0.001**
Total n-3 PUFA (%)	5.46 ± 1.86	5.18 ± 1.76	0.3
DHA (%)	3.42 ± 1.19	3.30 ± 1.15	0.5
EPA (%)	0.79 ± 0.65	0.74 ± 0.63	0.6
n-3 index (%)	5.22 ± 1.79	4.92 ± 1.71	0.3
Total n-6 PUFA (%)	35.20 ± 3.91	34.84 ± 4.17	0.6
AA (%)	9.61 ± 2.18	9.42 ± 2.12	0.6
AA/EPA	17.41 ± 9.20	18.08 ± 9.25	0.6
n-6/n-3	7.10 ± 2.23	7.50 ± 2.68	0.3

SD, standard deviation; BMI, body mass index; CRP, C reactive protein; TEAC, Trolox equivalent antioxidant capacity; PON, paraoxonase; MDA, malondialdehyde; PUFA, polyunsaturated fatty acids; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid. In bold the significant differences between males and females are reported.

Table 3
Correlation coefficients (B) and significance (*p*-value), by multiple linear regression analyses, between total blood PUFA (n-3 and n-6) and markers of oxidative stress and inflammation in adults, older adults, and LLIs, by sex.

	Adults	Older adults	LLIs
	B (*p*-value) males/females	B (*p*-value) males/females	B (*p*-value) males/females
CRP[a]			
Total n-3 PUFA (%)	0.07 (0.7)/−0.30 (0.6)	−0.37 (0.4)/0.83 (0.4)	1.48 (**0.038**)/−0.84 (0.4)
Total n-6 PUFA (%)	−0.06 (0.6)/0.04 (0.9)	0.23 (0.2)/0.15 (0.7)	0.27 (0.2)/−0.17 (0.8)
CRP[b]			
Total n-3 PUFA (%)	−0.06 (0.8)/−0.12 (0.8)	−0.35 (0.4)/1.06 (0.3)	2.54 (**0.012**)/−0.70 (0.5)
Total n-6 PUFA (%)	−0.09 (0.4)/0.001 (0.9)	0.19 (0.3)/0.06 (0.9)	0.23 (0.2)/0.36 (0.6)
TEAC[c]			
Total n-3 PUFA (%)	147.46 (0.052)/−0.30 (0.6)	−73.86 (0.5)/−137.48 (0.2)	852.56 (**0.007**)/36.92 (0.6)
Total n-6 PUFA (%)	42.47 (0.3)/0.04 (0.9)	−35.70 (0.4)/8.93 (0.8)	54.39 (0.4)/−102.81 (0.051)
PON[d]			
Total n-3 PUFA (%)	1.916 (0.7)/13.39 (0.2)	−0.276 (0.9)/−1.49 (0.9)	−28.37 (0.1)/12.61 (0.08)
Total n-6 PUFA (%)	0.39 (0.9)/6.04 (0.3)	5.573 (0.1)/−2.98 (0.4)	−3.994 (0.4)/3.89 (0.4)
MDA[e]			
Total n-3 PUFA (%)	0.03 (0.7)/−0.04 (0.8)	−0.006 (0.9)/0.27 (**0.019**)	1.06 (**0.024**)/−0.12 (0.1)
Total n-6 PUFA (%)	−0.05 (0.2)/−0.05 (0.5)	−0.02 (0.8)/0.096 (**0.01**)	0.09 (0.2)/−0.17 (**0.003**)
MDA[f]			
Total n-3 PUFA (%)	0.019 (0.8)/−0.090 (0.5)	0.015 (0.9)/0.272 (**0.011**)	0.640 (0.1)/−0.167 (0.06)
Total n-6 PUFA (%)	−0.047 (0.2)/−0.065 (0.4)	0.006 (0.9)/0.097 (**0.01**)	0.114 (0.3)/−0.152 (**0.008**)

B, beta coefficient; LLIs, long-lived individuals; PUFA, polyunsaturated fatty acid; CRP, C reactive protein; TEAC, Trolox equivalent antioxidant capacity; PON, paraoxonase; and MDA, malondialdehyde. In bold the significant p values are reported.
[a] Adjusted for BMI, cholesterol, PON, and MDA.
[b] Adjusted for BMI, cholesterol, TEAC, and MDA.
[c] Adjusted for BMI, cholesterol, CRP, and MDA.
[d] Adjusted for BMI, cholesterol, CRP, and MDA.
[e] Adjusted for BMI, cholesterol, CRP, and TEAC.
[f] Adjusted for BMI, cholesterol, CRP, and PON.

against oxidative stress (Wang et al., 2004a).

Furthermore, PUFA modulate inflammation by serving as precursors of bioactive signaling lipids known as eicosanoids. Specifically, eicosanoids derived from n-3 PUFA have been shown to possess anti-inflammatory properties and reduce the production of inflammatory cytokines associated with several chronic diseases (Beavers and Nicklas, 2011). In the n-3 pathway, alpha-linolenic acid (ALA) transforms into eicosapentaenoic acid (EPA) by cyclooxygenases-2 and 5-lipoxygenase to form 3-series prostaglandins (B3, D3, E3, I3, and thromboxane A3), and 5-series leukotrienes (B5, C5, and D6), respectively. Subsequently, the EPA is elongated into docosahexaenoic acid (DHA), which is metabolized to autacoids such as D-series resolvins and protectins (neuroprotectin D1). The EPA and DHA-derived mediators have potent anti-inflammatory activities and play a significant role in the resolution of inflammation. On the other hand, in the n-6 pathway, linoleic acid (LA) is converted to dihomo-gamma-linolenic acid (DGLA) and arachidonic acid (AA) through a series of elongation and desaturation processes. AA synthetizes 2-series prostaglandins (A2, E2, I2, and thromboxane A2) and leukotrienes of the series 4 (B4, C4, and E4) by the action of cyclooxygenases-2 and 5-lipoxygenase, respectively. The AA-derived eicosanoids are involved in proinflammation and pro-platelet aggregation (Davinelli et al., 2021).

In this study, we aimed to evaluate the association between blood PUFA status with levels of oxidative stress and inflammation markers including CRP, PON, Trolox equivalent antioxidant capacity (TEAC), and MDA in a cohort of healthy adults, older adults, and LLIs from Sicily, Italy.

2. Methods

2.1. Participants

Detailed study design and participant recruitment have been previously reported (Aiello et al., 2021). Briefly, a total of 172 subjects from Western Sicily (Italy) were enrolled. The subjects were recruited from June 2017 to February 2019 within the project "Discovery of molecular and genetic/epigenetic signatures underlying resistance to age-related diseases and comorbidities (DESIGN)", funded by the Italian Ministry of Education, University and Research. The Ethics Committee of Palermo University Hospital approved the study protocol (Nutrition and Longevity, No. 032017). The study protocol was conducted following the Declaration of Helsinki and its amendments. Written informed consent was obtained from all study participants before enrolment. The 172 subjects (95 females and 77 men) who participated in this study were recruited at the University of Palermo (Italy) and the neighboring municipalities in Sicily. The cohort was divided into three age groups, adults (18–64 years old, $n = 69$), older adults (65–89 years old, $n = 54$), and long-lived individuals (LLIs, 90–111 years old, $n = 49$). The participants were relatively healthy and cognitively performant, as demonstrated by a normal score on the Mini-Mental State Examination scale (Aiello et al., 2021). Subjects diagnosed with chronic and acute diseases were excluded from the study.

Anthropometric measurements were obtained, including body weight and height which were used to determine the body mass index (BMI). Additionally, blood samples were collected from all the

Table 4
Correlation coefficients (B) and significance (*p*-value), by multiple linear regression analyses, between PUFA indices (n-3 index, AA/EPA and n-6/n-3) and markers of oxidative stress and inflammation in adults, older adults, and LLIs, by sex.

	Adults	Older adults	LLIs
	B (*p*-value) males/females	B (*p*-value) males/females	B (*p*-value) males/females
CRP[a]			
n-3 index (%)	0.06 (0.8)/−0.27 (0.6)	−0.4 (0.4)/0.32 (0.7)	1.77 (**0.038**)/−1.04 (0.3)
AA/EPA	−0.07 (0.1)/−0.05 (0.6)	−0.015 (0.8)/−0.09 (0.7)	−0.12 (0.4)/−0.3 (0.1)
n-6/n-3	−0.37 (0.4)/−0.12 (0.9)	0.33 (0.5)/−0.21 (0.8)	0.09 (0.9)/−2.05 (0.1)
TEAC[b]			
n-3 index (%)	14.13 (0.9)/−40.89 (0.9)	307.69 (0.4)/−222.26 (0.5)	1455.56 (0.6)/347.25 (0.3)
AA/EPA	−4.97 (0.8)/9.52 (0.4)	−10.5 (0.5)/−19.96 (0.5)	28.75 (0.3)/−45.63 (**0.006**)
n-6/n-3	135.0 (0.4)/−86.58 (0.4)	−71.28 (0.5)/−22.29 (0.8)	68.9 (0.7)/−129.36 (0.3)
PON[c]			
n-3 index (%)	2.02 (0.7)/14.2 (0.2)	−27.86 (0.5)/9.13 (0.7)	128.84 (0.6)/8.52 (0.8)
AA/EPA	0.29 (0.8)/−1.82 (0.3)	1.3 (0.4)/−3.63 (0.1)	0.89 (0.7)/−0.60 (0.7)
n-6/n-3	−12.91 (0.2)/11.96 (0.5)	10.07 (0.3)/1.57 (0.8)	−4.82 (0.8)/1.3 (0.9)
MDA[d]			
n-3 index (%)	0.38 (0.1)/−0.83 (**0.038**)	0.12 (0.9)/−0.39 (0.2)	3.54 (**0.003**)/−0.15 (0.8)
AA/EPA	−0.02 (0.2)/0.03 (0.1)	0.02 (0.6)/0.03 (0.4)	−0.06 (0.4)/0.01 (0.6)
n-6/n-3	−0.04 (0.8)/0.07 (0.7)	−0.02 (0.9)/0.06 (0.5)	−0.16 (0.7)/−0.11 (0.5)

B, beta coefficient; LLIs, long-lived individuals; PUFA, polyunsaturated fatty acid; CRP, C reactive protein; TEAC, Trolox equivalent antioxidant capacity; PON, paraoxonase; and MDA, malondialdehyde; AA, arachidonic acid; EPA, eicosapentaenoic acid. In bold the significant p values are reported.
[a] Adjusted for BMI, cholesterol, PON, and MDA.
[b] Adjusted for BMI, cholesterol, CRP, and MDA.
[c] Adjusted for BMI, cholesterol, CRP, and MDA.
[d] Adjusted for BMI, cholesterol, CRP, and PON.

participants after a 12-hour fasting period. Following standard procedures, hematological analyses were performed immediately, including serum total cholesterol. The samples were kept at −80 °C until further analyses.

employed. FAMEs were identified by comparison with a standard mixture (Nu-Chek-Prep, Elysian, MN, U.S.A.). The results were analyzed using Shimadzu system GC Solutions software. The concentration of PUFA was expressed as a percentage of total fatty acids:

$$\text{PUFA\%} = (\text{surface peak corresponding to the PUFA/sum of all the peak surfaces corresponding to the total mixture of the fatty acids}) \times 100$$

2.2. Determination of inflammatory and oxidative stress markers

The CRP levels were determined using immunoturbidimetry method, as previously described (Aiello et al., 2021). This is a rapid and reliable method to detect and measure inflammation in the body. A reagent containing an antibody that recognizes CRP was added to the blood sample. The turbidity of the protein-antibody complex was measured by spectrophotometry, where the turbidity amount was proportional to the CRP level in the mixture. The quantitative value of CRP was reported in milligrams per deciliter (mg/dL). Similarly, PON, TEAC, and MDA levels were evaluated, as previously reported (Succu et al., 2014; Gambino et al., 2020).

2.3. Determination of blood PUFA

PUFA were extracted from whole blood samples and analyzed using gas chromatography, as previously described (Ali et al., 2023). Initially, fatty acid methyl esters (FAMEs) were prepared through a direct transesterification reaction, using boron trifluoride-methanol (BF3-MeOH) (12 % *w/v*, 1.5 M from Acros Organics, Geel, Belgium) and heating the mixture at 100 °C for 60 min. Subsequently, the FAMEs were extracted using n-Hexane (Carlo Erba Reagents, S.r.l., France).

For the separation of individual FAMEs, a GC-2010 gas chromatograph (Shimadzu, Kyoto, Japan) with a flame ionization detector, was

The n-3 index was estimated as the proportion of DHA and EPA content in erythrocytes relative to the total amount of fatty acids. Additionally, the AA/EPA and n-6/n-3 ratios were calculated.

2.4. Statistical analysis

Correlations between PUFA and oxidative and inflammatory markers among the age groups were estimated by multiple linear regression analyses adjusted for sex, total cholesterol, and BMI, or, when stratified by sex, adjusted for total cholesterol, and BMI. Data were analyzed using STATA v.16 (StataCorp LLC, College Station, TX, USA). A *p*-value of 0.05 or less is considered statistically significant.

3. Results

3.1. Characteristics of the participants

Characteristics of the participants are presented in Table 1. Briefly, the 172 Sicilian subjects included in this study had an age range of 18–111 years. Subjects in the older adult age group were overweight with a BMI of 28.2 ± 4.6 kg/m^2. Subjects in the LLIs group showed lower total cholesterol levels than the other age groups. In addition, LLIs showed a relatively high concentration of CRP (5.6 ± 7.2) and a slightly

low level of PON (79.1 ± 45.9). Regarding the PUFA status, the mean values of n-3 and n-6 PUFA were respectively 5.3 ± 1.8 and 35.0 ± 4.0, with an n-6/n-3 ratio of 7.3 ± 2.5. The average n-3 index was 5.1 ± 1.7, whereas the mean value of the AA/EPA ratio was 17.8 ± 9.2.

The population included 77 (45 %) men and 95 (55 %) females. Table 2 shows the characteristics of the population categorized by sex and highlights their differences. While there were no significant differences between men and females for most of the variables examined, the MDA levels were significantly higher in men compared to females ($p = 0.001$). The detailed characteristics and statistical differences between the age groups are described previously (Aiello et al., 2021; Ali et al., 2023).

3.2. Association between PUFA status and markers of oxidative stress and inflammation by sex

The correlation between blood PUFA levels and their indices with markers of oxidative stress and inflammation in adults, older adults, and LLIs groups by sex is displayed in Tables 3 and 4.

Among males and females of the adult group, we found no evidence of a significant correlation between total n-3 PUFA, total n-6 PUFA, AA/EPA ratio, and n-6/n-3 ratio with the markers of oxidative stress and inflammation. However, a negative correlation was identified between blood n-3 index and MDA values ($p = 0.038$) in adult females but not adult males (Table 4). The statistical analysis revealed higher percentages of total n-3 PUFA and n-6 PUFA predictors of higher MDA concentrations ($p < 0.05$) in older adult females (Table 3). No significant relationship was observed between blood PUFA status and CRP, TEAC, and PON among males and females in the older adult group (Tables 3 and 4).

In LLI males higher levels of total n-3 PUFA and n-3 index were linked to higher levels of PCR ($p < 0.05$, Tables 3 and 4). However, this correlation was not observed in females of the LLI group. We observed that a higher percentage of total n-3 PUFA in LLI males is a predictor of higher TEAC values ($p = 0.007$, Table 3). Similarly, LLI females exhibited a negative correlation between AA/EPA ratio and TEAC levels ($p = 0.006$, Table 4). Additionally, our analysis revealed that higher levels of total n-3 PUFA and n-3 index were linked to higher MDA concentration in LLI males but not females ($p < 0.05$, Tables 3 and 4). In contrast, a significant negative correlation between total n-6 PUFA and MDA levels was observed in LLI females ($p = 0.008$, Table 3).

4. Discussion

The present study evaluated the association between blood PUFA status and circulatory markers of inflammation and oxidative stress in a Sicilian cohort of healthy adults, older adults, and LLIs. Our results showed that blood n-3 index is negatively linked with MDA values in adult females, while higher total n-3 and n-6 PUFA were associated with higher MDA concentrations in older adult females. Additionally, higher total n-3 PUFA and n-3 index are associated with increased CRP levels in LLI males. Blood total n-3 PUFA was positively associated with TEAC value in LLI males, while AA/EPA ratio was negatively related to TEAC values among LLI females. Lastly, our analysis revealed that higher levels of total n-3 PUFA and n-3 index are correlated with increased MDA concentration in LLI males. In contrast, total n-6 PUFA showed a significant inverse correlation MDA level in LLI females.

Overall, we did not find a correlation between blood PUFA status and serum CRP in adult and older adult subjects. However, total n-3 PUFA and n-3 index were positively associated with CRP levels in LLI males but not females. CRP is an acute-phase protein and a sensitive marker of inflammation, synthesized mainly by hepatocytes in response to proinflammatory mediators (Smidowicz and Regula, 2015; Luan and Yao, 2018). While healthy people have small amounts of CRP in their serum, increased levels and activation of CRP is described as a predictor of CVD events and other age-related disorders (Bassuk et al., 2004; Schwingshackl and Hoffmann, 2014). The effects of PUFA on inflammation have been extensively investigated in numerous studies, suggesting that PUFA status might modulate inflammation and metabolic function in the body (Davinelli et al., 2021). In general, n-3 PUFA are described to promote the synthesis of anti-inflammatory eicosanoids, while n-6 PUFA may contribute to inflammation by the synthesis of proinflammatory eicosanoids. Specifically, EPA and DHA-derived mediators exhibit anti-inflammatory properties and are involved in the resolution of inflammation (Schweitzer et al., 2021). In contrast, AA-derived eicosanoids play a role in various physiological actions, including proinflammation, pro-platelet aggregation, and immune responses (Davinelli et al., 2021). The findings presented in this study may seem paradoxical when compared to previous observational research, which demonstrated a negative correlation between n-3 PUFA and CRP levels (Poudel-Tandukar et al., 2009; Yoneyama et al., 2007; Murakami et al., 2008; Muka et al., 2015). Nevertheless, it's crucial to note that our investigation does not include data on the dietary intake of n-3 PUFAs, and our observed associations are contingent upon the n-3 PUFA status of the subjects. In addition, we estimated CRP which serves as a valuable marker for detecting inflammation and is routinely measured in clinical practice. However, it lacks specificity for a comprehensive clinical evaluation, as CRP levels increase in response to inflammation and several other factors (Farhan-Alanie et al., 2010). Hence, while our results provide insight into the relationship between n-3 PUFA and CRP levels, further research is needed to understand the complex interplay of dietary intake, blood n-3 PUFA status, and inflammation, considering the diverse factors influencing CRP levels.

On the other hand, our results are consistent with previous data suggesting PUFA levels do not influence CRP values in healthy subjects with low serum CRP levels (Madsen et al., 2003). The participants in our study were relatively healthy considering the age-related physiological decline in organ and system function. None of them had chronic invalidating diseases, such as cancers, autoimmune diseases, or dementia, nor did they suffer from acute diseases like infectious diseases (Aiello et al., 2021). The LLIs cohort showed higher CRP and lower total n-3 PUFA compared to young and adult age groups, although the increase was not significant among centenarians of the LLIs group (Aiello et al., 2021; Ali et al., 2023). Several previous studies have consistently reported elevated CRP concentrations in LLIs when compared to their controls, which indicates a chronic inflammatory state observed in LLIs (Hausman et al., 2012).

Although the potential health benefits of n-3 PUFA are widely recognized, PUFA are highly susceptible to peroxidation due to their degree of unsaturation. Therefore, we evaluated the association between PUFA status and MDA level. MDA is an important marker of oxidative stress that can be produced as a result of PUFA peroxidation from the membrane. We found that blood n-3 index is negatively associated with MDA in adult females. However, higher total n-3 and n-6 PUFA were associated with higher MDA concentration in older adult females. Increased total n-3 PUFA and n-3 index were linked with higher MDA concentration in LLI males, while total n-6 PUFA showed a significant inverse association with MDA level in LLI females. Consistent with our findings, previous evidence suggests that the membrane susceptibility to lipid peroxidation increases progressively with advancing age (Rabini et al., 2002). Nevertheless, nonagenarians and centenarians exhibit resilience mechanisms against the age-associated increase in membrane peroxidation and oxidative damage. Centenarians show lower levels of lipid peroxides in their cell membrane compared to elderly individuals and similar to those of adults (Rabini et al., 2002). In addition, healthy centenarians exhibit lower plasma levels of thiobarbituric acid reactive substances and lipid hydroperoxides compared to elderly subjects, demonstrating a lower degree of oxidative stress and lipid peroxidation (Paolisso et al., 1998). Consistent with these findings, we previously reported that the MDA level was not significantly higher in LLIs compared to adults and older adults (Aiello et al., 2021). PUFA in membrane phospholipids are particularly prone to peroxidation due to

their degree of unsaturation, and longer-lived species are often found to have a lower membrane PUFA/monounsaturated fatty acids (MUFA) ratio than shorter-lived ones. Our LLIs group had a distinctively higher level of MUFA, and lower levels of total n-3 and n-6 PUFA than adults and older adults, as well (Ali et al., 2023).

While PUFA are particularly prone to peroxidation, it is shown that certain n-3 PUFA, specifically EPA and DHA, can decrease MDA levels and protect against oxidative stress by significantly enhancing antioxidant defense mechanisms (Thorlaksdottir et al., 2006; Buonocore et al., 2020). We evaluated the association between blood PUFA status with TEAC level. Blood TEAC assessment is an assay that measures the capacity of blood antioxidants to neutralize free radicals and reduce oxidative stress damage, evaluating the overall blood antioxidant status of an individual (Wang et al., 2004b; Fischer et al., 2005). Aging is characterized by increased pro-oxidant factors and decreased antioxidant mechanisms. However, certain groups of LLIs have been found to have enhanced antioxidant defense systems compared to elderly subjects. Indeed, we previously reported that TEAC values increased in LLIs compared to adults and older adults (Aiello et al., 2021). In addition, our analysis in this study showed that total n-3 PUFA is positively correlated with TEAC value in LLI males. In contrast, AA/EPA ratio was negatively associated with TEAC values among LLI females. Our findings align with previous findings showing that the percentages of total n-3 PUFA and EPA are positively associated with plasma TEAC, while n-6 PUFA and AA are inversely related to TEAC (Thorlaksdottir et al., 2006). It is described that EPA may enhance total cellular antioxidant capacity by 50–70 %, through enhancing mitochondrial function and biogenesis, and upregulation of major antioxidant enzymes (Xiao et al., 2022). On the other hand, AA/EPA ratio reflects the balance between two molecules that compete for the same enzymes to be converted to bioactive eicosanoids. This ratio is considered a marker of cellular inflammation because the balance between AA and EPA is crucial for regulating the synthesis of inflammatory mediators (Davinelli et al., 2021). The AA-derived inflammatory mediators can induce oxidative stress, reduce the total antioxidant capacity, and decrease the activity of antioxidant enzymes (Ma et al., 2022). Additionally, n-3 PUFA, particularly EPA and DHA, have been shown to increase the expression and activity of antioxidant enzymes, such as SOD, catalase, and glutathione peroxidase (Davinelli et al., 2022). The n-3 PUFA can exert their antioxidant effects through mechanisms involving modulation of intracellular signaling pathways and transcription factor activity, such as nuclear factor erythroid 2-related factor 2 (NRF2), peroxisome proliferator-activated receptors (PPARs) and nuclear factor κB (NF-κB) (Davinelli et al., 2022).

Our study has some limitations. Firstly, since our study is cross-sectional, we cannot infer causality of the results. Secondly, due to our small sample size, statistical power might not have been sufficient to detect all associations between PUFA status and the markers of oxidative stress and inflammation. Additionally, we did not explore additional inflammatory markers that could have provided a more comprehensive understanding of the association between PUFA status and inflammation. Although PUFA can affect the synthesis of both pro-inflammatory and anti-inflammatory cytokines, which play a central role in inflammaging, we did not evaluate their levels in our cohort. Conversely, we estimated CRP as an inflammatory marker due to its routine measurement in clinical practice. However, CRP lacks specificity, as it can increase in response to various conditions. Our study has several strengths as well. We studied a well-characterized healthy population of adults, older adults, and LLIs. Although dietary PUFA intake was not assessed, it is shown that the proportion of blood PUFA is influenced by their dietary intake (Thorlaksdottir et al., 2006). By directly assessing blood PUFA levels instead of relying on dietary questionnaires, we also minimized potential biases related to reporting and recall of dietary assessment.

5. Conclusions

In conclusion, after adjustment for potential confounding factors, our cross-sectional study on Sicilian adults, older adults, and LLIs suggests a correlation between blood PUFA status and markers of oxidative stress and inflammation. Specifically, we observed that higher levels of n-3 index in adult females are associated with lower MDA values. Conversely, higher total n-3 and n-6 PUFA was related to higher MDA values among older adult females. Similarly, increased total n-3 PUFA and n-3 index were associated with increased MDA concentration in LLI males, while total n-6 PUFA was inversely correlated with MDA level in LLI females. Furthermore, higher total n-3 PUFA and n-3 index were associated with increased CRP levels in LLI males. Lastly, our findings suggest that increased blood concentrations of total n-3 PUFA and n-3 index are positively associated with TEAC value in LLI males, while the AA/EPA ratio was inversely associated with TEAC values among LLI females. Together, our findings may provide additional evidence for the importance of adequate intake of n-3 PUFA as a strategy to enhance total antioxidant capacity and may mitigate the risk of associated chronic diseases.

Ethics approval and consent to participate

This study was approved by The Institutional Ethics Committee "Paolo Giaccone" University Hospital (Reference No. 032017) and was performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants before the study began.

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CRediT authorship contribution statement

Anna Aiello: Writing – original draft. **Giulia Accardi:** Conceptualization. **Anna Calabrò:** Investigation. **Ciriaco Carru:** Data curation. **Alessandro Cannavo:** Data curation. **Calogero Caruso:** Writing – review & editing. **Giuseppina Candore:** Funding acquisition. **Giovanni Scapagnini:** Writing – review & editing. **Graziamaria Corbi:** Writing – original draft, Methodology, Data curation, Conceptualization. **Sawan Ali:** Formal analysis. **Sergio Davinelli:** Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare no conflicts of interest.

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