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Changes in plasma total saturated fatty acids and palmitic acid are related to pro-inflammatory molecule IL-6 concentrations after nutritional intervention for one year

Inés Domínguez-López [a,b,c,1], Camila Arancibia-Riveros [a,b,1], Rosa Casas [c,d], Anna Tresserra-Rimbau [a,b,c], Cristina Razquin [c,e], Miguel Á. Martínez-González [c,e], Frank B. Hu [f], Emilio Ros [c,g], Montserrat Fitó [c,h], Ramon Estruch [c,d], M. Carmen López-Sabater [a,b,c], Rosa M. Lamuela-Raventós [a,b,c,*]

[a] Department of Nutrition, Food Sciences and Gastronomy, XIA School of Pharmacy and Food Sciences, University of Barcelona, 08028 Barcelona, Spain
[b] INSA-UB, Nutrition and Food Safety Research Institute, University of Barcelona, 08921 Santa Coloma de Gramanet, Spain
[c] CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, 28029 Madrid, Spain
[d] Department of Internal Medicine, Hospital Clinic, Institut d'Investigació Biomèdica August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain
[e] Department of Preventive Medicine and Public Health, University of Navarra, IdiSNA, 31008 Pamplona, Spain
[f] Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA 02115, USA
[g] Department of Endocrinology, Hospital Clinic, IDIBAPS, Barcelona, Spain
[h] Unit of Cardiovascular Risk and Nutrition, Institut Hospital del Mar de Investigaciones Médicas (IMIM), 08007 Barcelona, Spain

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ABSTRACT

Systemic inflammation is associated with an increased risk of non-communicable diseases, such as cardiovascular diseases and diabetes. Circulating fatty acids (FA) are known to be related to these conditions, possibly through their role in inflammation, although different types of FAs can have opposite effects on inflammatory mediators. The aim of the present study was to analyze the association of plasma FAs with inflammatory biomarkers in a PREDIMED trial subsample after one year of intervention. In a one-year longitudinal study of 91 participants of the PREDIMED trial (Barcelona-Clinic center), plasma FAs and inflammatory biomarkers were analyzed using gas chromatography and ELISA, respectively. In baseline plasma, a multivariable-adjusted ordinary least squares regression model showed that n-3 polyunsaturated FAs concentrations were inversely associated with concentrations of soluble intercellular adhesion molecule-1 (sICAM-1) and E-selectin, whereas the level of the most abundant saturated FA, palmitic acid, was directly associated with concentrations of interleukin-6 (IL-6) (β = 0.48 pg/mL, 95% CI: 0.03, 0.93 per 1-SD increase, p-value = 0.037). After one year of nutritional intervention, changes of plasma diet-derived total saturated FAs and palmitic acid were directly associated with changes in IL-6 (β = 0.59 pg/mL [95% CI: 0.28, 0.89] per 1-SD, p-value = 0.001; β = 0.64 pg/mL, 95% CI: 0.31, 0.98, p-value = 0.001), respectively, after correction for multiple testing. Our findings suggest that saturated FAs of dietary origin, especially palmitic acid, are directly involved in the increase of IL-6 in plasma.

Abbreviations: ACE, angiotensin-converting enzyme; CDV, cardiovascular disease; CRP, C-reactive protein; DNL, de novo lipogenesis; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; E-Sel, E-selectin; FA, fatty acid; FAME, fatty acid methyl ester; ICAM-1, intercellular adhesion molecule-1; IL-6, interleukin-6; LPS, lipopolysaccharide; MedDiet, Mediterranean diet; MUFA, monounsaturated fatty acid; NSAID, non-steroidal anti-inflammatory drug; PPAR-Y, peroxisome proliferator-activated receptor y; PUFA, polyunsaturated fatty acid; sP-Sel, soluble P-selectin; SFA, saturated fatty acids; TLR-4, toll-like receptor 4; T2D, type 2 diabetes; VCAM-1, vascular cell adhesion molecule-1.

* Correspondence to: Av. Joan XXIII, 27-31, 08028 Barcelona, Spain.
E-mail addresses: idominguez@ub.edu (I. Domínguez-López), camilaarancibia@ub.edu (C. Arancibia-Riveros), rcasas1@clinic.cat (R. Casas), annatresserra@ub.edu (A. Tresserra-Rimbau), crazquin@unav.es (C. Razquin), mamartinez@unav.es (M.Á. Martínez-González), frank.hu@channing.harvard.edu (F.B. Hu), eros@clinic.cat (E. Ros), mfito@imim.es (M. Fitó), restruch@clinic.cat (R. Estruch), mclopez@ub.edu (M.C. López-Sabater), lamuela@ub.edu (R.M. Lamuela-Raventós).
[1] Equally contributing authors.

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1. Introduction

The Mediterranean diet (MedDiet) is characterized by a high consumption of extra virgin olive oil (with a high monounsaturated to saturated fat ratio), fruits, vegetables (excluding potatoes), legumes, whole grains, and tree nuts; a moderate-to-high consumption of fish, but low consumption of dairy products, red and processed meats and sweets. A huge accrual of epidemiological evidence and trials support that this traditional food pattern should be considered as an ideal healthy model worldwide, in part because it combines foods rich in antioxidants and nutrients with anti-inflammatory effects [1,2]. In this context, biological plausibility is strongly supported by the fact that different studies have reported that adherence to the MedDiet and/or consumption of its main components is inversely associated with systemic inflammation [3], and lower circulating levels of adhesion molecules, such as P-Selectin (P-Sel) or E-Selectin (E-Sel) [4,5], interleukin 6 (IL-6), TNF-α and C-reactive protein (CRP) [6,7], and white blood cell and platelet counts [8]. On the other hand, reduced adherence to the MedDiet has been directly associated with a worse profile of plasma inflammatory biomarkers [9] generated by immune cells, such as macrophages, lymphocytes and natural killer T cells, and dendritic and mast cells, which infiltrate the lesions caused by the inflammatory process and accelerate disease development [10,11].

Inflammation plays a key role in the pathophysiology of a wide range of diseases, including arthritis, asthma, atherosclerosis [12], autoimmune diseases [13], cancer [2], diabetes [14], and obesity [15], and anti-inflammatory foods and diets have a potential therapeutic role in these conditions [16]. Among the key components of the MedDiet are monounsaturated fats, particularly oleic acid provided by olive oil and olives, and fish and seafood products, which contain omega 3 polyunsaturated fatty acids (n-3 PUFAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), reported to have an anti-inflammatory effect through different metabolic pathways [17,18]. In contrast, saturated fatty acids (SFAs) derived mainly from meat and dairy sources, and, therefore, with lower intakes in the MedDiet, have been directly associated with inflammatory processes. The aim of the present study was to prospectively analyze the relationship between changes in plasma FAs and inflammatory biomarkers in a subsample of the PREDIMED study after one year of follow-up.

2. Methods

2.1. Study design

A prospective cohort analysis was carried out using baseline and one year data from the PREDIMED (PREvención con DIeta MEDiterránea) study, a large, parallel-group, multicenter, randomized, controlled, five-year clinical trial designed to assess the effect of MedDiets enriched in olive oil or nuts on cardiovascular disease (CVD) incidence (http://www.predimed.es) [19]. It was conducted in Spain from October 2003 to December 2010 and included 7447 participants at high cardiovascular risk. Eligible participants were men (55–80 years) and women (60–80 years) with type 2 diabetes (T2D) or at least three of the following major risk factors: current smoking, hypertension, dyslipidaemia, overweight/obesity or family history of premature CVD. A detailed description of methods and participants has been published elsewhere [4,19,20].

The present substudy of the PREDIMED trial consists of a random subsample of 91 participants from the PREDIMED-Hospital Clinic recruitment centre (Barcelona). To evaluate the effect of plasma fatty acids (FAs) on inflammatory status, we determined circulating inflammatory biomarkers and plasma FAs at baseline and after one year of follow-up. Participants with extreme total energy intakes (>3500 or <500 kcal/day in women or >4000 or <800 kcal/day in men) were excluded from the analysis. For this reason, one man that reported > 4000 kcal/day was not considered in this analysis.

2.2. Ethics statement

The Institutional Review Board (IRB) of the Hospital Clinic (Barcelona, Spain) accredited by the US Department of Health and Human Services (DHHS) update for Federal-wide Assurance for the Protection of Human Subjects for International (Non-US) Institutions #00000738 approved the study protocol on July 16, 2002. All participants provided informed consent and signed a written consent form.

2.3. Covariate assessment

Dietary intake was assessed using a validated, semi-quantitative 137-item food frequency questionnaire with the assistance of trained dietitians at baseline and yearly thereafter. Nutrient intakes were calculated from Spanish food composition tables [21].

Trained personnel took anthropometric measures by standard methods at baseline and after one year, including weight and height, from which body mass index (kg/m^2) was calculated. Physical activity (metabolic equivalent tasks per minutes per day, METs min/day) was assessed with a validated Spanish version of the Minnesota physical activity questionnaire [22].

2.4. Derivatization and analysis of plasma fatty acids

2.4.1. Sample preparation

Plasma EDTA samples were collected after an overnight fast, centrifuged, coded, and stored at − 80ºC until analysis. The FA profile was determined by fast gas chromatography after derivatization to their corresponding fatty acid methyl esters (FAMEs) [23]. Briefly, 20 μL of the internal standard tridecanoic acid (C13:0) methyl ester, purchased from Sigma-Aldrich (St. Louis, MO, USA), was added to 100 μL of plasma sample. One milliliter of sodium methylate (0.5% w/v), acquired from Sigma-Aldrich, was directly added and heated to 100 ºC for 15 min. After cooling, the samples were esterified with one milliliter of boron trifluoride-methanol reagent, purchased from Sigma-Aldrich, at 100 ºC for 15 min. Once the tubes were cooled, FAMEs were isolated by adding 500 μL of n-hexane (Sigma-Aldrich). After shaking, one milliliter of saturated sodium chloride solution purchased from Panreac Quimica SLU (Barcelona, Spain) was added. Finally, the tubes were centrifuged for 10 min at 3000 g. After drying with anhydrous sodium sulfate (Scharlab, Barcelona, Spain), the clear n-hexane top layer was transferred into an automatic injector vial equipped with a volume adapter of 300 μL.

2.4.2. Gas chromatographic conditions

Fast analyses were performed on a Shimadzu GC-2010 Gas Chromatograph (Shimadzu, Kyoto, Japan) equipped with a fame ionization detector and a Shimadzu AOC-20i Autoinjector. Separation of FAMEs was carried out on a capillary column (10 m x 0.10 mm i.d.) coated with an SGE-BPX70 cross-linked stationary phase (70% cyanopropyl polysilphenylene-siloxane, 0.20 μm film thickness) from SGE (SGE Europe Ltd., United Kingdom).

Operation conditions were as follows: the split-splitness injector was used in split mode with a split ratio of 1:100. The injector volume of the sample was 1 μL. The injector and detector temperatures were kept at 250 ºC and 270 ºC, respectively. The temperature program was as follows: initial temperature 150 ºC, increased at 25 ºC/min until 250 ºC (total run time: 4 min). Helium was used as the carrier gas, with linear velocity of 59.4 cm/s (average at 150 ºC). Data acquisition and processing were performed using Shimadzu-Chemstation software for GC systems.

Methyl ester peaks were identified by comparison of their relative retention times with those of the standards Supelco 37 Component FAME mix and PUFA No. 2 (Animal Source), purchased form Merck (Darmstadt, Germany). Results were expressed as relative percentages of total FA, and their means and standard deviation (SD) at baseline and

after one year of follow-up are shown in Supplementary Table S1.

2.5. Inflammatory biomarkers

Circulating inflammatory biomarkers were analyzed as described elsewhere [24]. In summary, baseline and one-year plasma samples were determined by using commercial ELISA kits for soluble (s) intercellular cell adhesion molecule-1 (sICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), sE-Sel, sP-Sel, and IL-6 (BLK and PelkinElmer Elast Amplification System). The samples were processed by a technician blinded to group allocation. High-sensitive CRP was determined in serum by particle-enhanced immunonephelometry, as reported [4]. Means and SD of circulating inflammatory biomarkers at baseline and after one year of follow-up are shown in Supplementary Table S1.

2.6. Statistical analyses

Analyses were performed with Stata 16.0 (Stata-Corp LP, Tx. USA). Baseline characteristics of the participants are presented as means + SD for continuous variables and percentages for categorical variables.

Statistical analyses were performed for FA subtypes according to their degree of saturation and series: SFAs (C14:0, C16:0 (palmitic acid), C17:0, and C18:0), monounsaturated fatty acids (MUFAs) (C16:1 n-7, C18:1 n-9, and C18:1 n-7), omega 6 polyunsaturated fatty acids (n-6 PUFAs) (C18:2 n-6, C18:3 n-6, C20:3 n-6, and C20:4 n-6) and n-3 PUFAs (C18:3 n-3, C20:5 n-3 (EPA), and C22:6 n-3 (DHA)).

Individual baseline values of plasma FAs and inflammatory biomarkers were normalized and scaled in multiples of 1 SD with Blom inverse normal transformation [25]. Changes in plasma FAs and inflammatory biomarkers (one-year value minus the baseline value) were calculated, and the resulting difference was also normalized and scaled.

Multivariable adjusted linear regression models were used to assess associations between plasma FAs and levels of inflammatory biomarkers (baseline) and their 1-year changes per 1-SD increase. Plasma FAs were introduced as independent variables in units of SD. Therefore, the beta coefficients in ordinary least squares regression models should be interpreted as the change in the biomarker per each SD of the respective FA. Two models with adjustment of increasing complexity were applied. Model 1 was minimally adjusted for age and sex. Model 2 was additionally adjusted for physical activity, smoking habit, educational level, total energy intake and medication (insulin, other glucose lowering drugs, statins, other lipid lowering drugs, angiotensin-converting enzyme (ACE) inhibitors, other antihypertensive drugs, antiplatelet therapy, and non-steroidal anti-inflammatory drugs (NSAIDs)). Model 3 was only developed for the analysis of SFAs and palmitic acid and included an index of de novo lipogenesis (DNL) calculated as the ratio of C16:0 / C18:2 n-6 to account for endogenous synthesis of SFAs as different from nutritional sources [26]. One-year changes in inflammatory biomarkers and plasma FAs were analyzed using the same models as for the baseline values but they were further adjusted for the intervention group in the trial (MedDiet+virgin olive oil; MediDiet+-nuts; Low-fat control) for the longitudinal study. The *p* values obtained were penalized for multiple comparisons using the procedure described by Simes [27].

3. Results

3.1. General characteristics

The main characteristics of the 91 participants are described in Table 1. Among them, 52 were women and 39 were men with a mean age of 68 + 5.8 and 66 + 5.8 years, respectively. By study design, the participants had a high burden of CVD risk factors: 82.4% had been diagnosed with T2D, 57.1% with hypertension and 61.5% with hypercholesterolemia. Consequently, medications targeting inflammatory pathways, such as ACE inhibitors, statins, or insulin, were used at high rates.

Table 1
Baseline characteristics of participants.

	Total	Men	Women
n	*91*	*39*	*52*
Age, years	67 ± 5.9	66 ± 5.8	68 ± 5.8
Weight, Kg	75 ± 11.8	82 ± 9.4	70 ± 10.9
BMI, kg/m^2	29 ± 3.7	28.6 ± 3.3	29.4 ± 4.0
Type 2 diabetes, n (%)	75 (82.4)	34 (87.2)	41 (78.8)
Hypertension, n (%)	52 (57.1)	17 (43.6)	35 (67.3)
Hypercholesterolemia, n (%)	56 (61.5)	25 (64.1)	31 (59.6)
Medication use, n (%)			
ACE inhibitors	23 (25.3)	8 (20.5)	15 (28.8)
Statins	30 (33.1)	9 (23.1)	21 (40.4)
Insulin	11 (12.1)	6 (15.4)	5 (9.6)
Antiplatelet therapy	15 (16.5)	7 (17.9)	8 (15.4)
NSAIDs	9 (9.9)	1 (2.5)	8 (15.4)
Current smoker, n (%)	15 (16.5)	12 (30.8)	3 (5.8)
Leisure-time physical activity, MET·min/week	261 ± 252.8	313 ± 289.7	222 ± 215.9
Educational level, n (%)			
Low	60 (65.9)	20 (51.3)	40 (76.9)
Medium / High	31 (34.1)	19 (48.7)	12 (23.1)

BMI, body mass index; ACE, angiotensin-converting enzyme; NSAIDs, non-steroidal anti-inflammatory drugs; MET, metabolic equivalent task.
Values are percentages for categorical variables and means ± SD for continuous variables.

Up to 16.5% of the participants were current smokers, 30.8% of which were men. The mean values of physical activity revealed that men were more physically active (313 ± 289.7 MET·min/week) than women (222 ± 215.9 MET·min/week). Overall, men had a higher level of education, which was classified as high or medium in 48.7%, whereas 76.9% of women had the lowest level.

3.2. Association between fatty acids and circulatory inflammatory biomarkers at baseline

As shown in Table 2, plasma n-3 PUFAs were inversely related to circulating levels of sICAM-1 and sE-Sel per 1-SD change when the multivariable adjustment model 2 was used (−0.30 ng/mL [95% CI: −0.59; -< 0.01] per 1-SD, *p*-value = 0.047; −0.31 ng/cL [−0.57; −0.05], *p*-value = 0.019). No significant associations were found between the plasma concentration of n-6 PUFAs and any inflammatory biomarker. A direct association was observed between MUFA concentrations and sVCAM-1 (0.31 ng/mL [0.03; 0.58] per 1-SD, *p*-value = 0.028).

For the analysis of SFAs and palmitic acid, a third adjustment model was generated including a DNL index. As the concentrations of total plasma SFAs may be influenced by dietary intake and DNL, this correction was applied to subtract the possible effect of endogenous metabolism on this FA subtype. However, DNL was not taken into consideration for the analysis of unsaturated FAs, as it does not directly affect their synthesis. Total plasma SFAs exhibited a direct association with circulating levels of sE-Sel and IL-6 per 1-SD increase (0.26 ng/cL [0.02; 0.49], *p*-value = 0.033; 0.33 pg/mL [0.05; 0.61], *p*-value = 0.021), but the changes were not significant after adjustment for DNL.

Individual FAs that may interfere with the inflammatory process were also analyzed: the anti-inflammatory EPA and DHA, and palmitic acid, which activates inflammatory pathways. In the fully adjusted model, significant inverse associations were found between DHA and sICAM-1 and sE-Sel (−0.33 ng/mL [−0.60; −0.06], *p*-value = 0.019; −0.31 ng/cL [−0.56; −0.07], *p*-value = 0.014), and between EPA and sICAM-1 (−0.33 ng/mL [−0.62; −0.03], *p*-value = 0.031). Plasma palmitic acid was directly associated with circulating IL-6, whether considering DNL (0.48 pg/mL [0.03; 0.93], *p*-value = 0.037) or not (0.35 pg/mL [0.09; 0.61], *p*-value = 0.009) per 1-SD increase.

At baseline, no plasma FA was significantly associated with any

Table 2
Multivariable-adjusted associations between plasma fatty acids and circulating inflammatory biomarkers at baseline (per 1-SD increase in FA).

		sICAM-1, ng/mL *n = 68*	*p*-value	Adjusted *p*-value[d]	sVCAM-1, ng/mL *n = 78*	*p*-value	Adjusted *p*-value[d]	sE-Sel, ng/cL *n = 72*	*p*-value	Adjusted *p*-value[d]
n-3 PUFA	*B (CI) - Model 1*	-0.23 (−0.44; −0.03)	0.026	0.218	-0.18 (−0.36; 0.01)	0.066	0.302	-0.29 (−0.53; −0.04)	0.022	0.218
	B (CI) - Model 2	-0.30 (−0.59; -< 0.01)	0.047	0.219	-0.22 (−0.50; 0.06)	0.116	0.348	-0.31 (−0.57; −0.05)	0.019	0.173
n-6 PUFA	*B (CI) - Model 1*	-0.02 (−0.24; 0.20)	0.840	0.979	-0.16 (−0.35; 0.04)	0.114	0.336	-0.18 (−0.43; 0.07)	0.163	0.380
	B (CI) - Model 2	0.02 (−0.25; 0.29)	0.877	0.952	-0.22 (−0.47; 0.02)	0.074	0.259	-0.22 (−0.45; 0.01)	0.061	0.233
MUFA	*B (CI) - Model 1*	-0.02 (−0.24; 0.21)	0.875	0.979	0.22 (0.01; 0.43)	0.037	0.222	0.13 (−0.11; 0.37)	0.291	0.535
	B (CI) - Model 2	-0.05 (−0.31; 0.22)	0.728	0.913	0.31 (0.03; 0.58)	0.028	0.173	0.17 (−0.07; 0.40)	0.163	0.360
SFA	*B (CI) - Model 1*	0.14 (−0.08; 0.37)	0.209	0.418	0.11 (−0.10; 0.32)	0.293	0.535	0.22 (−0.02; 0.47)	0.072	0.302
	B (CI) - Model 2	0.11 (−0.16; 0.38)	0.404	0.738	0.17 (−0.06; 0.41)	0.148	0.355	0.26 (0.02; 0.49)	0.033	0.173
	B (CI) - Model 3	0.31 (−0.09; 0.71)	0.126	0.407	0.02 (−0.43; 0.45)	0.970	0.992	0.21 (−0.15; 0.58)	0.247	0.623
EPA	*B (CI) - Model 1*	-0.25 (−0.46; -0.04)	0.017	0.218	-0.16 (−0.37; 0.04)	0.121	0.336	-0.20 (−0.43; 0.05)	0.105	0.336
	B (CI) - Model 2	-0.33 (−0.62; −0.03)	0.031	0.173	-0.21 (−0.49; 0.08)	0.152	0.355	-0.17 (−0.41; 0.08)	0.188	0.395
DHA	*B (CI) - Model 1*	-0.25 (−0.46; −0.04)	0.020	0.218	-0.19 (−0.38; −0.01)	0.036	0.222	-0.29 (−0.53; −0.05)	0.018	0.218
	B (CI) - Model 2	-0.33 (−0.60; −0.06)	0.019	0.173	-0.25 (−0.50; 0.01)	0.060	0.233	-0.31 (−0.56; −0.07)	0.014	0.173
Palmitic acid	*B (CI) - Model 1*	0.11 (−0.11; 0.32)	0.323	0.565	0.06 (−0.15; 0.28)	0.572	0.828	0.19 (−0.05; 0.43)	0.123	0.336
	B (CI) - Model 2	0.04 (−0.23; 0.32)	0.736	0.913	0.11 (−0.14; 0.36)	0.370	0.706	0.22 (−0.3; 0.46)	0.087	0.281
	B (CI) - Model 3	0.15 (−0.29; 0.60)	0.491	0.992	-0.18 (−0.63; 0.26)	0.418	0.924	0.12 (−0.29; 0.53)	0.552	0.992
		sP-Sel, ng/cL *n = 64*	*p*-value	Adjusted *p*-value[d]	CRP, μg/mL *n = 72*	*p*-value	Adjusted *p*-value[d]	IL-6, pg/mL *n = 54*	*p*-value	Adjusted *p*-value[d]
n-3 PUFA	*B (CI) - Model 1*	0.06 (−0.18; 0.29)	0.639	0.866	< 0.01 (−0.22; 0.24)	0.956	0.979	-0.09 (−0.40; 0.22)	0.564	0.828
	B (CI) - Model 2	< 0.01 (−0.34; 0.35)	0.977	0.977	-0.01 (−0.25; 0.22)	0.905	0.952	0.19 (−0.30; 0.34)	0.907	0.952
n-6 PUFA	*B (CI) - Model 1*	0.14 (−0.08; 0.36)	0.199	0.418	-< 0.01 (−0.22; 0.22)	0.986	0.986	-0.10 (−0.42; 0.22)	0.526	0.818
	B (CI) - Model 2	0.15 (−0.05; 0.36)	0.139	0.355	- 0.06 (−0.33; 0.21)	0.657	0.913	-0.23 (−0.54; 0.09)	0.151	0.355
MUFA	*B (CI) - Model 1*	-0.17 (−0.38; 0.49)	0.128	0.336	-0.03 (−0.29; 0.24)	0.850	0.979	- < 0.01 (−0.31; 0.29)	0.949	0.979
	B (CI) - Model 2	-0.10 (−0.37; 0.17)	0.442	0.774	0.06 (−0.24; 0.36)	0.694	0.913	0.05 (−0.29; 0.39)	0.756	0.913
SFA	*B (CI) - Model 1*	-0.01 (−0.32; 0.29)	0.946	0.979	0.07 (−0.14; 0.29)	0.492	0.795	0.22 (−0.05; 0.49)	0.108	0.336
			0.693	0.913		0.542	0.876	0.33 (0.05;0.61)	0.021	0.173

(*continued on next page*)

Table 2 (*continued*)

	B (CI) - Model 2	0.07 (−0.27; 0.41)			0.07 (−0.17; 0.32)					
	B (CI) - Model 3	0.13 (−0.39; 0.64)	0.621	0.992	0.08 (−0.35; 0.51)	0.704	0.992	0.39 (−0.07; 0.86)	0.093	0.357
EPA	*B (CI) - Model 1*	0.08 (−0.14; 0.30)	0.489	0.795	-0.03 (−0.25; 0.18)	0.754	0.979	-0.27 (−0.63; 0.10)	0.153	0.378
	B (CI) - Model 2	0.06 (−0.31; 0.43)	0.761	0.913	-0.07 (−0.30; 0.16)	0.522	0.876	-0.09 (−0.53; 0.35)	0.689	0.913
DHA	*B (CI) - Model 1*	-0.1 (−0.22; 0.20)	0.923	0.979	0.01 (−0.20; 0.22)	0.956	0.979	-0.05 (−0.39; 0.30)	0.773	0.979
	B (CI) - Model 2	-0.06 (−0.36; 0.23)	0.674	0.913	-0.03 (−0.24; 0.19)	0.817	0.927	0.04 (−0.27; 0.34)	0.810	0.927
Palmitic acid	*B (CI) - Model 1*	-0.07 (−0.35; 0.21)	0.628	0.866	0.14 (−0.08; 0.35)	0.203	0.418	0.26 (−0.01; 0.53)	0.062	0.302
	B (CI) - Model 2	0.01 (−0.31; 0.33)	0.937	0.960	0.14 (−0.10; 0.39)	0.244	0.488	0.35 (0.09; 0.61)	0.009	0.173
	B (CI) - Model 3	-0.01 (−0.55; 0.52)	0.957	0.992	0.31 (−0.19; 0.81)	0.220	0.616	0.48 (0.03; 0.93)	0.037	0.259

sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; sE-Esel, soluble E-selectin; sP-Sel, soluble P-selectin; CRP, C-reactive protein; IL6, interleukine-6; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

β, standardized regression coefficient; CI, confidence interval.

Model 1: sex and age. *Model 2*: model 1 + physical activity, smoking habit, educational level, total energy intake and medication (insulin, other glucose lowering drugs, statins, other lipid lowering drugs, ACE inhibitors, other antihypertensive drugs, antiplatelet therapy and NSAIDs). *Model 3:* model 2 + de novo lipogenesis (DNL). $p < 0.05$ were considered significant.

Multiple testing adjustments were conducted by applying the method of Simes (considering 42 comparisons).

circulating inflammatory biomarker after correction for multiple testing.

3.3. Associations between one-year changes in plasma fatty acids and circulating inflammatory biomarkers

Table 3 presents the associations between one-year changes per 1-SD in plasma FAs and circulating inflammatory biomarkers. No relation was observed between changes in PUFAs, MUFAs, EPA, and DHA and changes in the pro-inflammatory molecules. Changes in plasma SFA and palmitic acid concentrations were directly associated with changes in IL-6 levels (0.59 pg/mL [0.28; 0.89], *p*-value = 0.001) and (0.50 pg/mL [0.23; 0.77, *p*-value = 0.001], respectively, per 1-SD increase. These associations were observed before and after the inclusion of DNL in the adjustment model and remained statistically significant after adjustment for multiple testing.

4. Discussion

In this substudy of the PREDIMED trial, we found that plasma SFAs and palmitic acid and their respective changes were associated with higher levels of circulating pro-inflammatory molecules, particularly IL-6. To our knowledge, this is the first study to assess the influence of plasma FAs on inflammatory biomarkers in a Mediterranean population, considering prospectively their respective changes.

It is of interest to examine the composition of plasma FAs, as some of them are involved in the molecular mechanisms of chronic diseases triggered by inflammatory processes, including diabetes and insulin resistance, obesity, CVD, metabolic syndrome, and cancer [25,28–30]. Dietary intake plays a major role in determining the plasma FA profile, and FAs are frequently used as biomarkers of specific food consumption [31]. However, certain FAs may be produced and transformed by endogenous synthesis, especially in individuals with metabolic disorders [26]. In particular, high-carbohydrate diets can stimulate DNL, in which acetyl-CoA carboxylase and FA synthetase catalyze acetyl-CoA and malonyl-CoA derived from glucose or other carbohydrates to generate palmitate [32,33]. In the present study, to subtract the effect of endogenous metabolism, a DNL index was included in the adjustment models, which allowed us to focus on the effects of dietary intake.

Our results show that DNL affected plasma SFA concentrations at baseline, as their association with IL-6 and sE-Sel was no longer significant once this endogenous metabolic pathway was included in the analysis. In contrast, the association between palmitic acid and IL-6 remained unaffected, thus highlighting the dietary origin of this FA. In the longitudinal analysis, SFAs and palmitic acid were strongly associated with IL-6, even when the effect of DNL was accounted for, indicating that endogenous metabolism may not have been involved in their increase.

SFAs have been in the spotlight for a long time due to their implication in several chronic diseases, including CVD or T2D [34–36]. Several mechanisms may explain the relationship between SFAs and inflammatory biomarkers. Previous in vitro studies have shown that SFAs can induce inflammation through the activation of toll-like receptor 4 (TLR-4) receptors and nuclear factor kB, which leads to the up-regulation of inflammatory genes [15,37,38]. Others suggested that SFAs could amplify the inflammatory response due to their processing into ceramides, which activate protein kinase C and MAP kinase [39]. Saravanan et al. also proposed that SFAs could impair the expression of genes related to inflammatory pathways, such as peroxisome proliferator-activated receptor y (PPAR-Y), which would eventually result in reduced insulin sensitivity [40]. Additionally, results in an observational study in humans showed that SFA intake was associated with elevated CRP levels [41]. It is important to consider that dietary surveys are prone to substantial measurement errors, which can be overcome by objective biomarker analyses [42]. In a study on the effect of diet on atherosclerotic disease, Kalogeropoulos et al. obtained different results according to the method used, as dietary SFAs were

Table 3
Multivariable-adjusted associations between 1-year changes in plasma fatty acids and changes in circulating inflammatory biomarkers (per 1-SD change in FA).

		sICAM-1, ng/mL *n = 68*	*p*-value	Adjusted *p*-value[d]	sVCAM-1, ng/mL *n = 78*	*p*-value	Adjusted *p*-value[d]	sE-Sel, ng/cL *n = 72*	*p*-value	Adjusted *p*-value[d]
n-3 PUFA	*B (CI) - Model 1*	< 0.01 (−0.25; 0.25)	0.999	0.999	0.14 (−0.09; 0.38)	0.231	0.999	-0.03 (−0.26; 0.21)	0.822	0.999
	B (CI) - Model 2	0.03 (−0.20; 0.27)	0.770	0.969	0.16 (−0.04; 0.35)	0.119	0.969	0.01 (−0.23; 0.25)	0.903	0.969
n-6 PUFA	*B (CI) - Model 1*	0.03 (−0.23; 0.30)	0.812	0.999	0.05 (−0.21; 0.31)	0.693	0.999	< −0.01 (−0.27; 0.27)	0.998	0.999
	B (CI) - Model 2	< 0.01 (−0.28; 0.28)	0.983	0.983	-0.04 (−0.30; 0.23)	0.795	0.969	0.04 (−0.21; 0.29)	0.740	0.969
MUFA	*B (CI) - Model 1*	-0.03 (−0.29; 0.22)	0.805	0.999	-0.12 (0.37; 0.13)	0.355	0.999	-0.09 (−0.35; 0.18)	0.524	0.999
	B (CI) - Model 2	-0.03 (−0.29; 0.24)	0.851	0.969	-0.03 (−0.29; 0.24)	0.844	0.969	-0.15 (−0.38; 0.08)	0.203	0.969
SFA	*B (CI) - Model 1*	-0.04 (−0.29; 0.22)	0.765	0.999	0.05 (−0.19; 0.29)	0.654	0.999	0.12 (−0.12; 0.34)	0.318	0.999
	B (CI) - Model 2	-0.04 (−0.35; 0.27)	0.810	0.969	0.04 (−0.18; 0.25)	0.748	0.969	0.14 (−0.08; 0.36)	0.223	0.969
	B (CI) - Model 3	0.06 (−0.25; 0.36)	0.705	0.966	0.05 (−0.21; 0.31)	0.703	0.966	0.17 (−0.09; 0.44)	0.189	0.966
EPA	*B (CI) - Model 1*	0.02 (−0.24; 0.27)	0.901	0.999	0.10 (−0.14; 0.35)	0.400	0.999	< −0.01 (−0.25; 0.25)	0.995	0.999
	B (CI) - Model 2	0.02 (−0.27; 0.31)	0.892	0.969	0.18 (−0.07; 0.43)	0.148	0.969	0.01 (−0.25; 0.28)	0.920	0.969
DHA	*B (CI) - Model 1*	-0.08 (−0.34; 0.18)	0.530	0.999	0.13 (−0.11; 0.36)	0.287	0.999	-0.01 (−0.25; 0.23)	0.943	0.999
	B (CI) - Model 2	-0.06 (−0.33; 0.22)	0.685	0.969	0.11 (−0.13; 0.34)	0.366	0.969	0.05 (−0.20; 0.30)	0.687	0.969
Palmitic acid	*B (CI) - Model 1*	-0.02 (−0.27; 0.23)	0.888	0.999	0.02 (−0.22; 0.26)	0.847	0.999	0.13 (−0.11; 0.37)	0.287	0.999
	B (CI) - Model 2	-0.01 (−0.28; 0.26)	0.946	0.969	0.01 (−0.22; 0.24)	0.935	0.969	0.13 (−0.12; 0.37)	0.309	0.969
	B (CI) - Model 3	0.01 (−0.23; 0.40)	0.603	0.966	0.01 (−0.27; 0.29)	0.933	0.966	0.16 (−0.11; 0.42)	0.232	0.966

		sP-Sel, ng/cL *n = 64*	*p*-value	Adjusted *p*-value[d]	CRP, μg/mL *n = 72*	*p*-value	Adjusted *p*-value[d]	IL-6, pg/mL *n = 54*	*p*-value	Adjusted *p*-value[d]
n-3 PUFA	*B (CI) - Model 1*	0.08 (−0.20; 0.36)	0.560	0.999	< 0.01 (−0.24; 0.24)	0.998	0.999	-0.06 (−0.34; 0.23)	0.684	0.999
	B (CI) - Model 2	0.09 (0.23; 0.42)	0.567	0.969	-0.02 (−0.34; 0.29)	0.875	0.969	-0.08 (−0.30; 0.14)	0.451	0.969
n-6 PUFA	*B (CI) - Model 1*	0.07 (−0.18; 0.33)	0.568	0.999	-0.02 (−0.27; 0.23)	0.873	0.999	-0.11 (−0.44; 0.23)	0.527	0.999
	B (CI) - Model 2	0.02 (−0.25; 0.28)	0.909	0.969	0.07 (−0.29; 0.43)	0.694	0.969	-0.15 (−0.43; 0.13)	0.280	0.969
MUFA	*B (CI) - Model 1*	-0.17 (−0.43; 0.09)	0.192	0.999	0.01 (−0.25; 0.27)	0.929	0.999	-0.12 (−0.47; 0.22)	0.478	0.999
	B (CI) - Model 2	-0.11 (−0.44; 0.21)	0.484	0.969	-0.10 (−0.53; 0.32)	0.682	0.969	-0.10 (−0.34; 0.14)	0.401	0.969
SFA	*B (CI) - Model 1*	0.06 (−0.18; 0.30)	0.623	0.999	0.02 (−0.20; 0.25)	0.842	0.999	0.41 (0.14; 0.69)	0.004	0.168
	B (CI) - Model 2	0.08 (−0.15; 0.31)	0.485	0.969	0.01 (−0.22; 0.24)	0.927	0.969	0.48 (0.22; 0.75)	0.001	0.021
	B (CI) - Model 3	0.14 (−0.11; 0.39)	0.271	0.966	0.01 (−0.24; 0.25)	0.966	0.966	0.59 (0.28; 0.89)	0.001	0.021
EPA	*B (CI) - Model 1*	0.10 (−0.19; 0.39)	0.503	0.999	0.02 (−0.22; 0.27)	0.845	0.999	-0.10 (−0.39; 0.18)	0.477	0.999
	B (CI) - Model 2	0.23 (−0.07; 0.53)	0.129	0.969	-0.04 (−0.33; 0.25)	0.785	0.969	-0.14 (−0.43; 0.15)	0.344	0.969
DHA	*B (CI) - Model 1*	-0.04 (−0.31; 0.23)	0.773	0.999	0.05 (−0.20; 0.29)	0.706	0.999	< 0.01 (−0.29; 0.30)	0.984	0.999
	B (CI) - Model 2	-0.07 (−0.35; 0.21)	0.623	0.969	0.03 (−0.23; 0.30)	0.805	0.969		0.907	0.969

(*continued on next page*)

Table 3 (*continued*)

								-0.02 (−0.31; 0.27)		
Palmitic acid	*β (CI) - Model 1*	0.04 (−0.20; 0.29)	0.713	0.999	0.07 (−0.16; 0.29)	0.555	0.999	0.38 (0.09; 0.67)	0.012	0.252
	β (CI) - Model 2	0.04 (−0.21; 0.28)	0.770	0.969	0.06 (−0.20; 0.31)	0.634	0.969	0.50 (0.23; 0.78)	0.001	0.021
	β (CI) - Model 3	0.10 (−0.15; 0.36)	0.421	0.966	0.05(−0.20; 0.29)	0.700	0.966	0.64 (0.31; 0.98)	0.001	0.021

sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; sE-Esel, soluble E-selectin; sP-Sel, soluble P-selectin; CRP, C-reactive protein; IL6, interleukine-6; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

β, standardized regression coefficient; CI, confidence interval.

Model 1: sex and age. *Model 2*: model 1 + physical activity, smoking habit, educational level, total energy intake and medication (insulin, other glucose lowering drugs, statins, other lipid lowering drugs, ACE inhibitors, other antihypertensive drugs, antiplatelet therapy and NSAIDs) and intervention group. *Model 3:* model 2 + de novo lipogenesis (DNL). $p < 0.05$ were considered significant.

Multiple testing adjustments were conducted by applying the method of Simes (considering 42 comparisons).

associated with IL-6 whereas plasma SFAs were also correlated with CRP [43].

In the present study, a significant relation was found between SFAs and IL-6, in accordance with previous results, and this pro-inflammatory effect was reflected in the association between their respective changes observed after one year of nutritional intervention. However, as shown by Santaren et al., individual SFAs have different effects on inflammatory biomarkers, palmitic acid standing out for its detrimental impact [44]. The unhealthy effects of palmitic acid have been linked to CVD, obesity, T2D and even cancer [45]. Regarding its role in inflammatory pathways, palmitic acid has been reported to induce pro-inflammatory cytokine expression by macrophages that is mediated by its binding to TLR-4 [46,47]. Our results showed that palmitic acid was consistently associated with higher concentrations of IL-6, both at baseline and when their respective changes were assessed. This consistency, together with the adjustment for potential endogenous sources, reinforces the support for a causal effect related to dietary intake. This relationship is of particular interest as IL-6 is involved in multiple pathological processes related to chronic inflammation [48]. *In vitro* studies have shown that palmitic acid stimulates IL-6 production in different biological tissues [49–51]. It increases the production of reactive species and decreases oxidative capacity, and that induces mitochondrial dysfunction that finally leads to insulin resistance [52]. The triggered inflammatory response is in part mediated by IL-6, as palmitic acid is responsible for the upregulation of IL-6 mRNA [53]. All these molecular mechanisms, isolated or combined, may explain the concomitant changes of palmitic acid and IL-6. In addition, IL-6 secretion is induced by palmitate in combination with lipopolysaccharide (LPS), a pro-inflammatory component of gram-negative bacteria [54]. In one of the few clinical studies assessing this relationship, Voon et al. reported that inflammatory biomarkers were unaltered by a high palmitic acid diet; their participants, however, were administered commercial fats without analysis of individual FAs, and therefore the FA content was not standardized [55]. In contrast, Mu et al. reported a positive association between palmitic acid (measured in red blood cell phospholipids) and IL-6 in consistency with our results [56]. Overall, studies that measured fatty acids in biological samples consistently support the hypothesis that SFAs and palmitic acid stimulate inflammatory responses mediated by IL-6.

The main strength of the current study is that we performed a prospective analysis of plasma FAs and circulating inflammatory molecules, which reflected participant status in a more reliable way than information provided by self-reported questionnaires assessing dietary intakes of these FAs. The main limitations were the small sample size and that participants were older subjects at high CVD risk, which may limit the generalization of the results. However, generalizability should be based on biological grounds and not only in "representativeness" from a statistical, survey-like point of view [57].

In conclusion, plasma SFAs, particularly palmitic acid, were directly associated with circulating IL-6 both at baseline and when assessing their changes after one year of nutritional intervention. These findings suggest that SFA intake promotes inflammatory processes mediated by IL-6.

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Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the Institutional Review Board of the 11 participating centers. The study was registered with the International Standard Randomized Controlled Trial Number (ISRCTN) 35739639.

CRediT authorship contribution statement

Domínguez-López: Formal analysis, Writing – original draft. **C. Arancibia-Riveros**: Formal analysis, Writing – original draft. **R. Casas**: Formal analysis, Writing – review & editing. **A. Tresserra-Rimbau**: Writing – review & editing. **C. Razquin**: Writing – review & editing. **M. A. Martínez-González**: Writing – review & editing. **F.B. Hu**: Writing – review & editing. **E. Ros**: Writing – review & editing. **M. Fitó**: Writing – review & editing. **R. Estruch**: Conceptualization, Writing – review & editing. **M.C. López-Sabater**: Conceptualization, Writing – review & editing. **R.M. Lamuela-Raventós**: Supervision, Writing – review & editing.

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Conflict of interest statement

E.R. reports grants, personal fees, non-financial support and other from the California Walnut Commission while the study was carried out; grants, personal fees, non-financial support and other from Alexion; and personal fees and other from Amarin, outside the submitted work. R.M. L.-R. reports personal fees from Cerveceros de España, personal fees, and other from Adventia, Wine in Moderation, Ecoveritas S.A., outside the submitted work. R.E. reports grants from the Fundación Dieta Mediterránea (Spain), and Cerveza y Salud (Spain), and personal fees for given lectures from Brewers of Europe (Belgium), the Fundación Cerveza y Salud (Spain), Pernaud-Ricard (Mexico), Instituto Cervantes (Alburquerque, USA), Instituto Cervantes (Milan, Italy), Instituto Cervantes (Tokyo, Japan), Lilly Laboratories (Spain), and the Wine and Culinary International Forum (Spain), as well as non-financial support for the organization of a National Congress on Nutrition and feeding trials with products from Grand Fountain and Uriach Laboratories (Spain).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2022.113028.

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