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Fruit and Vegetable Consumption is Inversely Associated with Plasma Saturated Fatty Acids at Baseline in Predimed Plus Trial

*Inés Domínguez-López, María Marhuenda-Muñoz, Anna Tresserra-Rimbau, Álvaro Hernáez, Juan José Moreno, Miguel Ángel Martínez-González, Jordi Salas-Salvadó, Dolores Corella, Montserrat Fitó, José Alfredo Martínez, Ángel M. Alonso-Gómez, Julia Wärnberg, Jesús Vioque, Dora Romaguera, José López-Miranda, M. Rosa Bernal-Lopez, José Lapetra, J. Luís Serra-Majem, Aurora Bueno-Cavanillas, Josep A. Tur, Vicente Martín-Sánchez, Xavier Pintó, Miguel Delgado-Rodríguez, Pilar Matía-Martín, Josep Vidal, Clotilde Vázquez, Lidia Daimiel, Mercè Serra-Mir, Zenaida Vázquez-Ruiz, Stephanie K. Nishi, Jose V. Sorli, Olga Castañer, Itziar Abete, Jessica Vaquero Luna, Rosa Carabaño-Moral, Alberto Asencio, Lara Prohens, Antonio Garcia-Rios, Rosa Casas, Ana Maria Gomez-Perez, José Manuel Santos-Lozano, Cristina Razquin, María Ángeles Martínez, Carmen Saiz, Vanesa Robledo-Pastor, María Angeles Zulet, Itziar Salaverria, Sonia Eguaras, Nancy Babio, Mireia Malcampo, Emilio Ros, Ramon Estruch, M. Carmen López-Sabater, and Rosa M. Lamuela-Raventós**

Scope: Plasma fatty acids (FAs) are associated with the development of cardiovascular diseases and metabolic syndrome. The aim of our study is to assess the relationship between fruit and vegetable (F&V) consumption and plasma FAs and their subtypes.

Methods and Results: Plasma FAs are assessed in a cross-sectional analysis of a subsample of 240 subjects from the PREDIMED-Plus study. Participants are categorized into four groups of fruit, vegetable, and fat intake according to the food frequency questionnaire. Plasma FA analysis is performed using gas chromatography. Associations between FAs and F&V consumption are adjusted for age, sex, physical activity, body mass index (BMI), total energy intake, and alcohol consumption. Plasma saturated FAs are lower in groups with high F&V consumption (-1.20 mg cL^{-1} [95% CI: [-2.22, -0.18], p-value = 0.021), especially when fat intake is high (-1.74 mg cL^{-1} [95% CI: [-3.41, -0.06], p-value = 0.042). Total FAs and n-6 polyunsaturated FAs tend to be lower in high consumers of F&V only in the high-fat intake groups.

Conclusions: F&V consumption is associated with lower plasma saturated FAs when fat intake is high. These findings suggest that F&V consumption may have different associations with plasma FAs depending on their subtype and on the extent of fat intake.

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1. Introduction

The Mediterranean diet is recognized as a healthy dietary pattern proven to provide protection against cardiovascular diseases.[1] This dietary pattern is characterized by high intakes of fruits, vegetables, legumes, nuts, whole grains, and olive oil, some of which make up good sources of healthy fats.[2,3]

Dietary fat is a major energy source, but it can play different roles in health depending on its fatty acid (FA) composition.[4–8] Monounsaturated FAs (MUFAs) and polyunsaturated FAs (PUFAs) have been linked to several health benefits, such as de-

I. Domínguez-López, M. Marhuenda-Muñoz, A. Tresserra-Rimbau, J. J. Moreno, M. C. López-Sabater, R. M. Lamuela-Raventós
Department of Nutrition
Food Science and Gastronomy
School of Pharmacy and Food Sciences and XIA
Institute of Nutrition and Food Safety (INSA-UB)
University of Barcelona
Santa Coloma de Gramenet 08921, Spain
E-mail: lamuela@ub.edu

M. Marhuenda-Muñoz, A. Tresserra-Rimbau, Á. Hernáez, J. J. Moreno, M. Á. Martínez-González, J. Salas-Salvadó, D. Corella, M. Fitó, J. A. Martínez, Á. M. Alonso-Gómez, J. Wärnberg, D. Romaguera, J. López-Miranda, M. R. Bernal-Lopez, J. Lapetra, J. L. Serra-Majem, J. A. Tur, X. Pintó, C. Vázquez, Z. Vázquez-Ruiz, S. K. Nishi, J. V. Sorli, O. Castañer, I. Abete, A. Garcia-Rios, R. Casas, J. M. Santos-Lozano, C. Razquin, C. Saiz, M. A. Zulet, N. Babio, E. Ros, R. Estruch, M. C. López-Sabater, R. M. Lamuela-Raventós
Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y la Nutrición (CIBEROBN)
Instituto de Salud Carlos III
Madrid, Spain

Á. Hernáez
Centre for Fertility and Health
Norwegian Institute of Public Health
Oslo Norway

Á. Hernáez
Blanquerna School of Health Sciences
Universitat Ramon Llull
Barcelona, Spain

M. Á. Martínez-González, Z. Vázquez-Ruiz, C. Razquin, S. Eguaras
Department of Preventive Medicine and Public Health
IDISNA
University of Navarra
Pamplona, Spain

M. Á. Martínez-González
Department of Nutrition
Harvard T.H. Chan School of Public Health
Boston, MA, USA

J. Salas-Salvadó, S. K. Nishi, M. Á. Martínez, N. Babio
Departament de Bioquímica i Biotecnologia
Unitat de Nutrició
Universitat Rovira i Virgili
Reus Spain

J. Salas-Salvadó, S. K. Nishi, M. Á. Martínez, N. Babio
Nutrition Unit
University Hospital of Sant Joan de Reus
Reus Spain

J. Salas-Salvadó, S. K. Nishi, M. Á. Martínez, N. Babio
Institut d'Investigació Sanitària Pere Virgili (IISPV)
Reus, Spain

D. Corella, J. V. Sorli, C. Saiz
Department of Preventive Medicine
University of Valencia
Valencia, Spain

M. Fitó, O. Castañer, M. Malcampo
Unit of Cardiovascular Risk and Nutrition
Institut Hospital del Mar de Investigaciones Médicas Municipal d'Investigació Médica (IMIM)
Barcelona, Spain

J. A. Martínez, I. Abete, M. A. Zulet
Department of Nutrition
Food Sciences, and Physiology
Center for Nutrition Research
University of Navarra
Pamplona Spain

J. A. Martínez, V. Martín-Sánchez, M. Delgado-Rodríguez, L. Daimiel, I. Abete, M. A. Zulet
Nutritional Control of the Epigenome Group
IMDEA Food
CEI UAM + CSIC, Madrid, Spain

Á. M. Alonso-Gómez, J. V. Luna, I. Salaverria
Cardiovascular, Respiratory and Metabolic Area
Bioaraba Health Research Institute
Vitoria-Gasteiz, Spain

Á. M. Alonso-Gómez, J. V. Luna
Osakidetza Basque Health Service
Araba University Hospital
University of the Basque Country UPV/EHU
Vitoria-Gasteiz Spain

J. Wärnberg
Department of Nursing
Institute of Biomedical Research in Malaga (IBIMA)
University of Málaga
Málaga Spain

J. Vioque
CIBER de Epidemiología y Salud Pública (CIBERESP)
Instituto de Salud Carlos III
Madrid Spain

J. Vioque, A. Bueno-Cavanillas
Instituto de Investigación Sanitaria y Biomédica de Alicante (ISABIAL-UMH)
Alicante, Spain

D. Romaguera, L. Prohens
Health Research Institute of the Balearic Islands (IdISBa)
Palma de Mallorca
Spain

J. López-Miranda, A. Garcia-Rios
Department of Internal Medicine
Maimonides Biomedical Research Institute of Cordoba (IMIBIC)
Reina Sofia University Hospital
University of Cordoba
Cordoba, Spain

M. R. Bernal-Lopez, A. M. Gomez-Perez
Department of Internal Medicine
Instituto de Investigación Biomédica de Malaga (IBIMA)
Regional University Hospital of Malaga

J. Lapetra, J. M. Santos-Lozano
Department of Family Medicine
Research Unit
Distrito Sanitario Atención Primaria Sevilla
Sevilla, Spain

J. L. Serra-Majem
Research Institute of Biomedical and Health Sciences (IUIBS)
Canarian Health Service
Las Palmas de Gran Canaria
University of Las Palmas de Gran Canaria & Centro Hospitalario Universitario Insular Materno Infantil (CHUIMI)
Spain

A. Bueno-Cavanillas
Department of Preventive Medicine and Public Health
University of Granada
Granada, Spain

crease in metabolic syndrome incidence[9] and improvement in cardiovascular status.[10,11] Special attention has been paid to n-3 PUFAs due to their anti-inflammatory properties.[12,13] Conversely, in the past decades, diets rich in saturated FAs (SFAs) were associated with higher cardiometabolic and type 2 diabetes risk.[14–16] Overall, replacing saturated fat with unsaturated fat has been shown to be an effective strategy to prevent coronary heart disease[17,18] and reduce overall cardiovascular risk.[14,19,20]

Absorption of dietary fat takes place in the duodenum and proximal jejunum, where emulsified triglycerides arrive in the form of lipid droplets, are hydrolyzed by pancreatic lipases to form FAs and monoglycerides, and the lipolytic products are solubilized by bile acids into mixed micelles. The micelles deliver FAs and monoglycerides to the lipid bilayer of enterocytes, and these molecules are absorbed by simple diffusion, to be reconstituted into triglycerides in the cytosol.[21] Additionally, there are other minority mechanisms involved in FA uptake, mainly long-chain FAs, based on protein transporters.[22]fhd

Various dietary components modulate intestinal fat absorption, including fiber, proteins, carbohydrates, and cholesterol.[21] In particular, fiber is known to significantly reduce nutrient bioavailability,[23] and there is evidence that it can decrease postprandial triglyceride concentrations when consumed in a mixed meal.[24] Fruits and vegetables (F&Vs) are rich sources of fiber; therefore, these foods may affect dietary lipid bioavailability.[25] However, no large studies have investigated whether F&V consumption may affect the plasma concentrations of the different FA classes.

The purpose of this study was to evaluate the association of F&V consumption with plasma concentrations of total FAs and their subtypes in an older Mediterranean population with metabolic syndrome.

2. Experimental Section

2.1. Study Design

This study was a cross-sectional analysis of baseline data from the PREDIMED-Plus study, an ongoing 6-year multicenter, randomized, parallel-group clinical trial designed to assess the effect of an energy-restricted Mediterranean diet, combined with physical activity and behavioral support, on hard cardiovascular events. A total of 6874 participants were recruited and randomized into the trial in 23 Spanish centers from September 2013 to December 2016.[26]

Eligible participants were males (aged 55–75 years) and females (aged 60–75 years) with overweight (body mass index [BMI] ≥25) or obesity (BMI ≥30) who met at least three metabolic syndrome criteria according to the International Diabetes Federation and the American Heart Association and National Heart, Lung, and Blood Institute.[27] The participant selection and the description of the study sample was described previously.[28] Details on the study protocol could be found at http://predimedplus.com.

2.2. Ethics Statement

The study was conducted according to the ethical standards' guidelines of the Declaration of Helsinki and all procedures in-

J. A. Tur
Research Group on Community Nutrition & Oxidative Stress
University of Balearic Islands-IUNICS, and IDISBA, Palma de Mallorca
Spain

V. Martín-Sánchez
Institute of Biomedicine (IBIOMED)
University of León
León, Spain

X. Pintó
Lipids and Vascular Risk Unit, Internal Medicine
Hospitalet de Llobregat
Hospital Universitario de Bellvitge
Barcelona Spain

M. Delgado-Rodríguez
Division of Preventive Medicine
Faculty of Medicine
University of Jaén
Jaén, Spain

P. Matía-Martín
Department of Endocrinology and Nutrition
Instituto de Investigación Sanitaria Hospital Clínico San Carlos (IdISSC)
Madrid, Spain

J. Vidal
CIBER Diabetes y Enfermedades Metabólicas (CIBERDEM)
Instituto de Salud Carlos III (ISCIII)
Madrid, Spain

J. Vidal
Department of Endocrinology
Institut d'Investigacions Biomédiques August Pi Sunyer (IDIBAPS)
Hospital Clinic
University of Barcelona
Barcelona, Spain

C. Vázquez
Department of Endocrinology and Nutrition
Hospital Fundación Jimenez Díaz
Instituto de Investigaciones Biomédicas IISFJD
University Autonoma
Madrid, Spain

M. Serra-Mir, E. Ros
Lipid Clinic, Endocrinology and Nutrition Service
Institut d'Investigacions Biomèdiques August Pi Sunyer (IDIBAPS)
Hospital Clínic
Barcelona, Spain

R. Carabaño-Moral
Distrito de Atención Primaria Costa del Sol
Servicio Andaluz de Salud
Unidad de Gestión Clínica Arroyo de la Miel
Benalmádena Spain

A. Asencio
Centro Atención Primaria de Mutxamel de FISABIO
Alicante, Spain

R. Casas, R. Estruch
Internal Medicine Department
Institut d'Investigacions Biomèdiques August Pi Sunyer (IDIBAPS)
Hospital Clinic
University of Barcelona
Barcelona, Spain

V. Robledo-Pastor
Atenció Primària Barcelona Ciutat
Institut Català de la Salut (ICS)
Barcelona, Spain

S. Eguaras
Primary Care Services
Servicio Navarro de Salud (Osasunbidea)
Pamplona, Spain

volving human participants and patients were approved by the Institutional Review Boards of the participating centers. The clinical trial was registered in the ISRCTN of London, England with the number 89898870 on July 24, 2014. Written informed consent was obtained from all participants.

2.3. Sample Selection

Dietary intake was assessed using the validated, semi-quantitative 143-item PREDIMED-Plus food frequency questionnaire (https://www.predimedplus.com/wp-content/uploads/2020/07/FFQ-PREDIMED-Plus.pdf).[28] A total of 240 participants with extreme values of F&V and fat intake were randomly selected and classified into the following groups: 1) low F&V consumption (first decile) and low-moderate fat (first quartile) intake (reference); 2) low F&V consumption (first decile) and high fat intake (fourth quartile); 3) high F&V consumption (tenth decile) and low-moderate fat intake (first quartile); and 4) high F&V consumption (tenth decile) and high fat intake (fourth quartile).

2.4. Covariates

Trained personnel collected baseline data on age; sex; prevalence of diabetes, hypercholesterolemia, and hypertension; BMI; and smoking habit as previously described.[28] From the validated food frequency questionnaire, consumption of alcohol (g day^{-1}) and energy intake (kcal day^{-1}) were also estimated. We estimated the overall quality of diet based on the 17-item energy-reduced Mediterranean diet score.[29]

2.5. Sample Size Calculation

This study was performed in a sub-cohort of 240 participants that had been previously selected for another work of our group.[30] As the design and sample size was suitable for this study, we decided to use the same sub-cohort.

2.6. Derivatization and Analysis of Total Plasma Fatty Acids

2.6.1. Reagents and Standards

Sodium methylate, boron trifluoride in methanol (14% w/v) and *n*-hexane were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium chloride was purchased from Panreac Quimica SLU (Barcelona, Spain) and anhydrous sodium sulphate from Scharlab (Barcelona, Spain).

Tridecanoic acid methyl ester (C13:0), used as internal standard, was purchased from Sigma-Aldrich. Supelco 37 component FAME mix and PUFA No.2 (Animal source), used for peak identification, were purchased from Merck (Darmstadt, Germany). Standards were stored in powder form at –20 °C and protected from light.

2.6.2. Sample Preparation

Plasma samples used for FA analyses were stored at −80 °C, prior to analysis. Plasma FAs were determined by fast gas chromatography with a previous derivatization to their corresponding FAMEs.[31] Briefly, 20 µL of the internal standard were added to 100 µL of plasma sample. One milliliter of sodium methylate (0.5% w/v) was directly added and heated to 100 °C for 15 min. After cooling, samples were esterified with 1 mL of boron trifluoride-methanol reagent, also at 100 °C, for 15 min. Once the tubes were cooled, FAMEs were isolated by adding 500 µL of n-hexane. After shaking for 1 min, 1 mL of a saturated sodium chloride solution was added. Finally, the tubes were centrifuged for 10 min at 3000 rpm. After drying with anhydrous sodium sulphate, the clear n-hexane top layer was transferred into an automatic injector vial equipped with a volume adapter of 300 µL.

2.6.3. Gas Chromatographic Conditions

Fast GC analyses were performed on a Shimadzu GC-2010 Gas Chromatograph (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a Shimadzu AOC-20i Autoinjector. Separation of FAMEs was carried out on a capillary column (40 m × 0.18 mm I.D. x 0.1 µm film thickness), coated with an RTX-2330 stationary phase of 10% cyanopropyl phenyl—90% byscyanopropyl polysiloxane from Restek (Bellefonte, USA).

Operating conditions were as follows: the split-splitness injector was used in split mode with a split ratio of 1:50. The injection volume of the sample was 1 µL. The injector and detector temperatures were kept at 250 °C and 300 °C, respectively. The temperature program was as follows: initial temperature 110 °C, increased at 52 °C min^{-1} to 195 °C and held at this temperature for 6 min, and then increased at 25 °C min^{-1} until 230 °C and held for 6.5 min (total run time: 16.03 min). Hydrogen was used as the carrier gas, at a constant pressure of 26 psi that refers to a linear velocity of 40 cm s^{-1} at 110 °C. Data acquisition and processing were performed with the Shimadzu-Chemstation software for GC systems. Results were presented as plasma FA concentrations (mg cL^{-1}).

2.7. Statistical Analyses

The data were analyzed using the available complete PREDIMED-Plus database, dated August 10, 2017.

Baseline characteristics of the participants were presented as means ± standard deviations (SD) for continuous variables and percentages for categorical variables. To determine possible between-group differences in baseline characteristics, we used one-way ANOVA for continuous variables and χ^2-test for categorical variables. Total and specific subtypes of fat intake were expressed as median (interquartile range). For continuous variables, significant differences between groups were analyzed using Bonferroni post-hoc test for one-way ANOVA.

Nine participants who reported extreme total energy intakes were excluded from the analysis (>3500 Kcal day^{-1} for females and >4000 Kcal day^{-1} for males).[32]

Multivariable adjusted linear regression models were used to assess differences between groups and plasma FAs. Three different adjustment models were applied. Model 1 was minimally adjusted for age (continuous) and sex. Model 2 was additionally adjusted for physical activity (quartiles) and BMI (continuous).

Table 1. Participant characteristics according to dietary F&V consumption and fat intake.

Characteristics	All	Low F&V		High F&V		*p*-value
		Low-moderate fat	Very high fat	Low-moderate fat	Very high fat	
No. of participants	228	57	58	60	53	
Age (y)	66.1 ± 4.4	65.9 ± 4.52	66.3 ± 3.61	65.8 ± 5.07	66.2 ± 4.25	0.936
Females, n (%)	106 (46.1)	26 (44.1)	26 (44.8)	32 (53.3)	22 (41.5)	0.604
Type-2 diabetes mellitus, n (%)	53 (23.2)	10 (17.5)	11 (19)	21 (35.0)	11 (20.8)	0.157
Hypercholesterolemia, n (%)	154 (67.5)	39 (68.4)	42 (72.4)	40 (66.7)	34 (64.2)	0.792
Hypertension, n (%)	198 (86.4)	48 (84.2)	53 (91.4)	53 (88.3)	44 (83.0)	0.496
BMI (kg/m^2)	32.7 ± 3.53	32.1 ± 3.03	32.7 ± 3.64	32.4 ± 3.76	33.6 ± 3.53	0.115
Current smoker, n (%)	37 (16.2)	8 (14.0)	10 (17.2)	6 (10.0)	13 (24.5)	0.200
Leisure-time physical activity, MET (min/week)	2499 ± 2406	1649 ± 1855^{a}	2276 ± 1890^{a,b}	3064 ± 3248^{b}	3019 ± 2368^{b}	0.003
Total cholesterol (mg/dL)	193.2 ± 2.8	194.6 ± 35.7^{a,b}	209.09 ± 36.6^{b}	188.91 ± 38.6^{a}	192.6 ± 31.3^{a,b}	0.014
LDL (mg/dL)	128.1 ± 8.3	135.0 ± 124.2	142.3 ± 122.2	115.9 ± 35.5	134.6 ± 125.7	0.613
HDL (mg/dL)	47.5 ± 0.9	49.2 ± 9.1	48.8 ± 13.3	48.7 ± 11.9	47.4 ± 11.6	0.867
VLDL (mg/dL)	29.3 ± 1.1	28.0 ± 12.7^{a,b}	35.5 ± 20.7^{b}	25.8 ± 14.0^{a}	30.3 ± 14.6^{a,b}	0.015
AST (U/L)	23.01 ± 0.6	23.1 ± 11.3	23.7 ± 8.6	22.7 ± 4.9	23.0 ± 5.4	0.933
ALT (U/L)	26.5 ± 1.0	25.6 ± 12.9	27.4 ± 15.0	24.5 ± 7.6	27.8 ± 10.0	0.429
GGT (U/L)	35.6 ± 2.1	38.6 ± 30.2	41.3 ± 31.0	31.2 ± 19.2	29.8 ± 15.9	0.053
ALP (U/L)	77.0 ± 2.6	76.5 ± 28.5	80.2 ± 43.3	71.9 ± 22.7	70.0 ± 16.6	0.383

F&V, fruit and vegetable; BMI, body mass index; MET, metabolic equivalent of task; LDL, low density lipoprotein; HDL, high density lipoprotein; VLDL, very low density lipoprotein; AST, aspartate aminotransferase; ALT (alanine transaminase); GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; Values are percentages for categorical variables and means ± SD for continuous variables; *p*-values were calculated by analysis of variance–one factor was used for continuous variables and the χ^2-test for categorical variables, $p < 0.05$ considered significant; Different lower-case letters indicate a significant difference among groups.

Model 3 was additionally adjusted for total energy intake (continuous) and alcohol consumption (categorized as low-moderate and high). To accommodate the use of some categorical variables, we estimated parameters using weighted least squares with robust standard errors.

Statistical analyses were performed for individual FAs and for their subtypes of FAs according to their saturation degree and series: SFAs (C14:0, C16:0, and C18:0), MUFAs (C16:1 n-9, C16:1 n-7, C18:1 n-9, C20:1 n-9, and C24:1 n-9), PUFAs (C18:2 n-6, C18:3 n-6, C18:3 n-3, C20:2 n-6, C20:3 n-6, C20:4 n-6, C20:5 n-3, C22:4 n-6, C22:5 n-6, C22:5 n-3 and C22:6 n-3), n-6 PUFAs (C18:2 n-6, C18:3 n-6, C20:2 n-6, C20:3 n-6, C20:4 n-6, C22:4 n-6, and C22:5 n-6), and n-3 PUFAs (C18:3 n-3, C20:5 n-3, C22:5 n-3, and C22:6 n-3). The differences between groups were expressed as mean changes (95% confidence intervals, CI).

Statistical analyses were performed using Stata 16.0 (StataCorp LP, Tx. USA). Results were considered statistically significant when *p*-values were <0.05 for bilateral contrast.

3. Results

After excluding nine participants with implausible energy intakes, 228 were included in the present analysis (three samples were unavailable for analysis). The four groups were well balanced, as there were no significant differences between age, sex and cardiovascular risk factors. BMI was also comparable in the four groups, considering that 70% of participants had obesity and the rest had overweight. By study design, there was a wide prevalence of other cardiovascular risk factors, such as hypertension, hypercholesterolemia, diabetes, and smoking. Participants with lower consumption of F&V and fat intake were less physically active than those who consumed more F&V. Regarding biochemical data, low density lipoprotein (LDL), high density lipoprotein (HDL) and the liver enzymes alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transpeptidase (GGT) and alkaline phosphatase (ALP) did not differ between groups. Nevertheless, the high fat intake and low F&V consumption group presented higher concentrations of total cholesterol and very low density lipoprotein (VLDL) than those with low fat intake and high F&V consumption (**Table 1**).

The average consumption of F&V in the first decile was 289 g day^{-1}, whereas in the tenth decile it was 1295 g day^{-1}. As for fat intake, the first quartile had an average intake of 67 g day^{-1} and the fourth quartile 141 g day^{-1}. Groups with low-moderate fat intake were comparable in total fat, SFA, MUFA and PUFA intake. The groups categorized as high fat intake were also comparable in total fat, SFA and MUFA intake, but they differed in PUFA intake (**Table 2**). Inter-group differences in food and nutrient intake are detailed in Table S1, Supporting Information.

3.1. Total Fatty Acid Concentrations in Plasma

The predominant FA subtypes in plasma were PUFAs, followed by MUFAs and SFAs. The main FAs in the SFA, MUFA and PUFA groups were palmitic acid (16:0), oleic acid (C18:1 n-9) and linoleic acid (C18:2 n-6), respectively.

Table 2. Types of fat intake according to F&V consumption and fat intake.

	Low F&V		High F&V		*p*-Value
	Low-moderate fat	Very high fat	Low-moderate fat	Very high fat	
Total fat (g day^{-1})	64.9 ± 15.6[a]	140 ± 14.6[b]	69.7 ± 12.6[a]	142 ± 16.9[b]	<0.001
SFA (g day^{-1})	17.7 (4.5)[a]	36.9 (7.2)[b]	17.8 (4.7)[a]	34.5 (7.1)[b]	<0.001
MUFA (g day^{-1})	32.4 (8.8)[a]	72.8 (11.0)[b]	33.7 (7.8)[a]	75.5 (12.4)[b]	<0.001
PUFA (g day^{-1})	9.9 (3.6)[a]	22.5 (6.1)[b]	12.2 (4.0)[a]	25.3 (7.8)[c]	<0.001
F&V	277 ± 69.1[a]	300 ± 52.7[a]	1265 ± 299[b]	1328 ± 270[b]	<0.00

F&V, fruit and vegetable; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; Values are expressed as median (interquartile range); *p*-values were calculated by analysis of variance–one factor, $p < 0.05$ considered significant, after adjustment for multiple comparisons with the Bonferroni method; Different lower-case letters indicate a significant difference among groups.

3.2. High F&V Versus Low F&V Consumption

Table 3 discloses plasma FA concentrations according to F&V consumption and total dietary fat intake. Individuals with high F&V consumption tended to present with lower plasma total FA concentrations and significantly lower concentrations of SFAs (-1.20 mg cL^{-1} [95% CI: -2.22, -0.18], *p*-value = 0.021), including palmitic acid (-0.92 mg cL^{-1} [-1.71, -0.13], *p*-value = 0.023), compared with those consuming low amounts of F&V. No differences were observed between the low and high F&V consumption groups for MUFAs, total PUFAs and n-3 PUFAs. Accordingly, oleic and linoleic acid concentrations were similar. The specific group of n-6 PUFAs tended to be lower, but their major representative linoleic acid, showed no difference with high consumption of F&V.

3.3. High F&V Versus Low F&V Consumption Within the Low-moderate Fat Intake Group

The comparison between high and low F&V consumption groups when the intake of fat was low-moderate showed no differences in the plasma concentrations of total FAs, SFAs, MUFAs nor PUFAs (n-6 and n-3 series), or the individual FAs palmitic, oleic, linoleic and alpha-linolenic (**Table 3**).

3.4. High F&V versus Low F&V Consumption Within the High Fat Intake Group

Concerning the high fat intake group, individuals with higher consumption of F&V tended to present with lower concentrations of total FAs than those with low F&V consumption. However, different patterns were observed when focusing on each subtype of FA. High F&V consumption was associated with significantly lower concentrations of total SFA and a clear trend towards lower concentrations of palmitic acid in comparison with low F&V consumption (-1.74 mg cL^{-1} [-3.41, -0.06] and -1.29 mg cL^{-1} [-2.59; 0.01], *p*-values = 0.042 and 0.052, respectively). As for MUFA concentrations, no differences were found between the low and high F&V consumption groups. Likewise, oleic acid did not differ depending on F&V consumption. On the other hand, plasma PUFA concentrations tended to be lower when the consumption of F&V was high, although no significant statistical difference was observed. Separate analyses of PUFAs subclasses showed that n-6 FA concentrations were significantly lower when F&V consumption was high (-1.81 mg cL^{-1} [-3.50, -0.12], *p*-value = 0.036), whereas n-3 PUFAs remained unaffected. When considering the essential FAs linoleic and alpha-linolenic, no differences were found between the two groups (**Table 3**).

Table S2, Supporting Information includes the comparisons of all the individual FAs quantified.

4. Discussion

In this sub-analysis of the PREDIMED-Plus trial, the consumption of F&V was significantly associated with lower plasma SFA concentrations in individuals with high fat intake, but not in those with low-moderate fat intake. To the best of our knowledge, the current study is the first to show the different relationships between F&V consumption and plasma FAs according to their subtypes.

Plasma FAs have been widely used to assess the influence of diet on plasma lipids, as several studies have reported that most plasma FAs reflect dietary intake.[33–38] Recently, Furtado et al. (2019) performed a detailed comparison between dietary FA intake and the corresponding FA proportions in plasma lipid fractions, demonstrating that FAs in total plasma perform reasonably well as biomarkers that reflect long-term dietary intake of this class of macronutrients.[39]

The PREDIMED-Plus trial was conducted in a Spanish Mediterranean population, which stands out for its sizable consumption of fruits, vegetables, and sources of healthy fats.[2] According to the World Health Organization (WHO), the recommended range for PUFA intake is 6–11% of total energy intake, whereas SFA should not exceed 10%.[40] Mean PUFA and SFA intakes in the participants of this sub-study were 6.3% and 9.8% of total energy, respectively; thus, aligning with the WHO guidelines. Regarding F&V, participants reported a mean consumption of 792 g day^{-1}, which surpasses the WHO recommendation of minimum 600 g day^{-1}.[41]

F&V consumption has been related to a lower risk of cardiovascular diseases.[42] In fact, improving the lipid profile is a potential mechanism of action involved in the cardioprotective effect of F&V.[43–45] In this sense, our study shows that the groups with higher consumption of F&V tended to present with lower total FA concentrations in plasma. Interestingly, this relationship was not homogeneous for all FA classes. SFAs and their principal repre-

Table 3. Multivariable-adjusted differences between extreme deciles of F&V consumption for total plasma FAs, specific subtypes and individual FAs (mg cL^{-1}), by categories of total fat intake.

		High F&V vs. Low F&V	*p*-value	High F&V vs. Low F&V (low-moderate fat)	*p*-value	High F&V vs. Low F&V (high fat)	*p*-value
TOTAL FAs	Mean (mg cL^{-1})	44.10 vs. 46.98		43.03 vs. 45.21		45.31 vs. 48.72	
	ß [CI]-model 1	−2.82 [-5.92; 0.28]	0.075	−2.19 [-6.68; 2.30]	0.335	−3.51 [-7.87; 0.85]	0.113
	ß [CI]-model 2	−2.77 [-5.91; 0.36]	0.082	−2.77 [-7.23; 1.67]	0.219	−3.09 [-7.52; 1.35]	0.170
	ß [CI]-model 3	−3.00 [-6.19; 0.19]	0.065	−2.28 [-7.54; 2.97]	0.391	−4.52 [-9.68; 0.65]	0.086
SFAs	Mean (mg cL^{-1})	12.61 vs. 13.79		12.36 vs. 13.21		12.89 vs. 14.36	
	ß [CI]-model 1	−1.15 [-2.14; -0.16]	0.023	−0.81 [-2.23; 0.61]	0.264	−1.50 [-2.89; -0.11]	0.034
	ß [CI]-model 2	−1.16 [-2.18; -0.15]	0.025	−1.04 [-2.51; 0.42]	0.160	−1.41 [-2.83; 0.01]	0.052
	ß [CI]-model 3	−1.20 [-2.22; -0.18]	0.021	−1.12 [-2.90; 0.66]	0.215	−1.74 [-3.41; -0.06]	0.042
MUFAs	Mean (mg cL^{-1})	13.61 vs. 14.73		13.25 vs. 14.45		14.02 vs. 15.01	
	ß [CI]-model 1	−1.07 [-2.35; 0.21]	0.102	−1.13 [-3.01; 0.76]	0.239	−1.06 [-2.88; 0.75]	0.248
	ß [CI]-model 2	−1.06 [-2.35; 0.23]	0.107	−1.35 [-3.24; 0.55]	0.161	−0.97 [-2.76; 0.82]	0.287
	ß [CI]-model 3	−0.93 [-2.22; 0.36]	0.156	−1.22 [-3.47; 1.03]	0.285	−1.07 [-3.20; 1.06]	0.321
PUFAs	Mean (mg cL^{-1})	17.75 vs. 18.33		17.29 vs. 17.43		18.27 vs. 19.22	
	ß [CI]-model 1	−0.59 [-1.67; 0.48]	0.278	−0.26 [-1.75; 1.24]	0.735	−0.94 [-2.48; 0.59]	0.226
	ß [CI]-model 2	−0.55 [-1.63; 0.54]	0.321	−0.38 [-1.77; 1.00]	0.585	−0.71 [-2.30; 0.89]	0.382
	ß [CI]-model 3	−0.86 [-2.00; 0.29]	0.143	0.06 [-1.48; 1.61]	0.934	−1.70 [-3.57; 0.17]	0.075
n-6 PUFAs	Mean (mg cL^{-1})	16.10 vs. 16.78		15.69 vs. 15.92		16.56 vs. 17.62	
	ß [CI]-model 1	−0.68 [-1.68; 0.30]	0.172	−0.33 [1.70; 1.04]	0.635	−1.06 [-2.46; 0.34]	0.136
	ß [CI]-model 2	−0.63 [-1.63; 0.37]	0.214	−0.41 [-1.69; 0.87]	0.526	−0.83 [-2.27; 0.60]	0.253
	ß [CI]-model 3	−0.95 [-2.01; 0.12]	0.081	0.02 [-1.40; 1.44]	0.976	−1.81 [-3.50; -0.12]	0.036
n-3 PUFAs	Mean (mg cL^{-1})	1.65 vs. 1.56		1.60 vs. 1.51		1.71 vs. 1.60	
	ß [CI]-model 1	0.09 [-0.06; 0.25]	0.239	0.07 [-0.14; 0.29]	0.498	0.12 [-0.13; 0.36]	0.349
	ß [CI]-model 2	0.08 [-0.07; 0.24]	0.285	0.03 [-0.17; 0.23]	0.772	0.13 [-0.15; 0.40]	0.361
	ß [CI]-model 3	0.09 [-0.06; 0.24]	0.243	0.04 [-0.17; 0.25]	0.686	0.11 [-0.22; 0.43]	0.507
Palmitic acid (C16:0)	Mean (mg cL^{-1})	9.36 vs. 10.26		9.18 vs. 9.79		9.56 vs. 10.73	
	ß [CI]-model 1	−0.88 [-1.64; -0.12]	0.024	−0.57 [-1.66; 0.53]	0.307	−1.20 [-2.28; -0.12]	0.029
	ß [CI]-model 2	−0.90 [-1.69; -0.11]	0.026	−0.76 [-1.89; 0.38]	0.191	−1.14 [-2.24; -0.04]	0.043
	ß [CI]-model 3	−0.92 [-1.71; -0.13]	0.023	−0.87 [-2.25; 0.51]	0.215	−1.29 [-2.59; 0.01]	0.052
Oleic acid (C18:1 n-9)	Mean (mg cL^{-1})	12.43 vs. 13.30		12.01 vs. 12.87		12.90 vs. 13.72	
	ß [CI]-model 1	−0.82 [-1.95; 0.31]	0.156	−0.76 [-2.35; 0.82]	0.341	−0.88 [-2.55; 0.78]	0.295
	ß [CI]-model 2	−0.82 [-1.97; 0.32]	0.158	−0.96 [-2.56; 0.63]	0.234	−0.79 [-2.43; 0.85]	0.341
	ß [CI]-model 3	−0.78 [-1.93; 0.38]	0.186	−0.90 [-2.82; 1.02]	0.355	−0.85 [-2.78; 1.08]	0.384
Linoleic acid (C18:2 n-6)	Mean (mg cL^{-1})	11.94 vs. 12.20		11.49 vs. 11.41		12.44 vs. 12.97	
	ß [CI]-model 1	−0.27 [-1.05; 0.51]	0.500	−0.02 [-1.11; 1.08]	0.978	−0.53 [-1.59; 0.53]	0.325
	ß [CI]-model 2	−0.23 [-1.02; 0.56]	0.567	−0.09 [-1.11; 0.94]	0.867	−0.37 [-1.45; 0.72]	0.505
	ß [CI]-model 3	−0.54 [-1.38; 0.31]	0.210	0.20 [-0.95; 1.35]	0.729	−0.99 [-2.31; 0.32]	0.137
Alpha-linolenic acid (C18:3 n-3)	Mean (mg cL^{-1})	0.14 vs. 0.13		0.13 vs. 0.13		0.16 vs. 0.15	
	ß [CI]-model 1	0.01 [-0.02; 0.03]	0.531	<0.01 [-0.03; 0.04]	0.864	0.01 [-0.03; 0.05]	0.522
	ß [CI]-model 2	0.01 [-0.01; 0.03]	0.484	←0.01 [-0.03; 0.03]	0.803	0.02 [-0.02; 0.06]	0.310
	ß [CI]-model 3	0.01 [-0.02; 0.03]	0.654	−0.01 [-0.05; 0.02]	0.401	0.03 [-0.02; 0.06]	0.210

F&V, fruit and vegetable; FA, fatty acid; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; ß, difference between groups; CI, confidence interval; Model 1—adjusted for age and sex. Model 2—Model 1 additionally adjusted for physical activity, and BMI. Model 3—Model 2 additionally adjusted for total energy intake and alcohol consumption. *p*-values < 0.05 were considered significant.

sentative, palmitic acid, disclosed lower concentrations when the consumption of F&V was higher. MUFAs and PUFAs remained unaffected, however, as well as their main representatives oleic and alpha-linoleic acid. Taken together, these results suggest that the consumption of F&V is inversely associated with SFAs but it is not related to other FA subtypes. Growing evidence reports the relationship between SFA and harmful health outcomes, such as coronary heart disease or inflammation.[46,47] Palmitic acid,

which is widely used in the food industry, is thought to be involved in the development of obesity, diabetes, cancer and even in increased mortality risk.[48,49] Therefore, F&V consumption may contribute to a healthier FA profile which might lead to an improvement of overall health.

Whisner et al. (2019) conducted a randomized controlled trial with a similar design that combined low-fat and high-fat meals with and without dietary fiber and found different effects on endothelial function according to the amount of fat and fiber consumed.[50] Similarly, Djuric et al. (2006) found different effects on plasma micronutrients of high F&V consumption depending on the intake of fat.[51] Indeed, in the present stratified analysis we observed that the association of F&V consumption with plasma SFA concentrations depends on the amount of fat intake. On one hand, the low-moderate fat intake group did not show any association between plasma FA concentrations and F&V consumption. Only when fat intake was high, we observed lower total FA concentrations with high F&V consumption. As explained above, plasma SFA concentrations were significantly lower when F&V consumption was higher, supporting the notion that this subgroup of FAs is affected by F&V to a higher extent than other FA classes. Palmitic acid also showed an inverse association when fat intake was high, although not significant, probably due to the reduction in the sample size with the statistical analysis when comparing only individuals with high fat intake. These results show that FA concentrations are associated with F&V consumption in different magnitudes depending on the amount of total dietary fat, with lower SFAs associated with a high fat intake with high consumption of F&V, but no difference when fat intake was lower. It is remarkable that F&V are specifically related to plasma FAs when fat intake is high, as population with high dietary fat intake is exposed to a greater risk of developing obesity and related diseases.[52] Therefore, when assessing the influence of F&V over fat intake it is important to consider this aspect.

Unexpectedly, plasma PUFA concentrations tended to be lower when F&V consumption was high, even though this association was not significant, and further analyses showed that this tendency was only ascribable to n-6 PUFAs. Interestingly, we did not observe any difference for linoleic or alpha-linolenic acid, both essential FAs that cannot be synthesized endogenously. Other n-6 PUFAs, such as gamma-linolenic acid (C18:3 n-6) or arachidonic acid (C20:4 n-6), for which significant lower concentrations were associated with high F&V consumption, mainly come from endogenous metabolism rather than dietary intake, as they are rare in foods.[53,54] Thus, inter-individual metabolic variability might play a major role in plasma PUFA concentrations and the differences found in this study may not be attributed only to F&V consumption.

These associations between F&V consumption and plasma FAs might be explained by the well-known high content of fiber of these foods. Fiber is known to interfere in nutrients absorption[25]; thus, it may play an important role in modifying the uptake of FAs according to their characteristics and the amount of fat intake.

The main strength of this study is that it involved participants at baseline of the PREDIMED-Plus study; therefore, the results reflect real-life conditions. We performed a comprehensive analysis of plasma FAs and their subtypes that allowed us to detect different patterns depending on the degree of saturation and species of each FA. Our study also has limitations. Causality could not be determined due to the cross-sectional design. Second, culinary techniques or co-intake of different nutrients could not be determined from the food frequency questionnaires. Finally, the concentrations of some FAs may be related to endogenous metabolism besides dietary intake.

In conclusion, higher F&V consumption was associated with lower plasma concentrations of SFAs and with a tendency to lower total FAs in plasma when intake of fat was high. The findings from the current study support the idea that F&V consumption may influence plasma FAs differently depending on their subtype, as it was associated with differences in plasma SFAs to a higher extent than with any other FA group. Further randomized controlled trials are needed to confirm the reported results and definitively establish the role of fiber and F&V consumption in modulating plasma FA concentrations, as well as their implications in cardiovascular health.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

J.S.-S. reported receiving research support from the Instituto de Salud Carlos III, Ministerio de Educación y Ciencia, Departament de Salut Pública de la Generalitat de Catalunya, the European Commission, the USA National Institutes of Health; receiving consulting fees or travel expenses from Danone, California Walnut Commission, Eroski Foundation, Instituto Danone, Nestle, and Abbott Laboratories, receiving nonfinancial support from Hojiblanca, Patrimonio Comunal Olivarero, the California Walnut Commission, Almond Board of California, La Morella Nuts, Pistachio Growers and Borges S.A; serving on the board of and receiving grant support through his institution from the International Nut and Dried Foundation and the Eroski Foundation; and grants and personal fees from Instituto Danone; Serving in the Board of Danone Institute International. D.C. reported receiving grants from Instituto de Salud Carlos III. R.E. reported receiving grants from Instituto de Salud Carlos III, Fundación Dieta Meditarránea and Cerveza y Salud and olive oil for the trial from Fundación Patrimonio Comunal Olivarero and personal fees from Brewers of Europe, Fundación Cerveza y Salud, Interprofesional del Aceite de Oliva, Instituto Cervantes in Albuquerque, Milano and Tokyo, Pernod Ricard, Fundación Dieta Mediterránea (Spain), Wine and Culinary International Forum and Lilly Laboratories; non-financial support from Sociedad Española de Nutrición and Fundación Bosch y Gimpera; and grants from Uriach Laboratories. E.R. reports grants, personal fees, non-financial support and other from California Walnut Commission, during the conduct of the study; grants, personal fees, non-financial support and other from Alexion; personal fees, non-financial support and other from Ferrer International and Danone, 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, other from Ecoveritas, S.A., outside the submitted work. S.K.N. reported being a volunteer member of the group Plant-Based Canada. The rest of the authors have declared that no competing interests exist. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Author Contributions

I.D.-L. and M.M.-M. contributed equally to the work. R.M.L.-R., Á.H. and M.M.-M. conceptualized this study; I.D.-L. performed the investigation and the formal analysis. I.D.-L. and M.M.-M. wrote the original draft. A.T.-R, Á.H., J.J.M., M.Á.M.-G., J.S.-S., D.C., M.F., J.A.M., Á.M.A.-G., J.W., J.V. (Jesús Vioque), D.R., J.L.-M., M.R.B.-L., J.L., J.L.S.-M., A.B.-C., J.A.T., V.M.-S., X.P., M.D.-R., P.M.-M., J.V. (Josep Vidal), C.V., L.D., M.S.-M., Z.V.-R., S.K.N., J.V.S., O.C., I.A., J.V.-L., R.C.-M., A.A., L.P., A.G.-R., R.C., A.M.G.-P., J.M.S.-L., C.R., M.A.M., C.S., V.R.-P., M.A.Z., I.S., S.E., N.B., M.M., E.R., R.E., M.C.L.-S., and R.M.L.-R. reviewed and edited the manuscript. All authors have read and approved the final manuscript.

Data Availability Statement

There are restrictions on the availability of data for the PREDIMED-Plus trial, due to the signed consent agreements around data sharing, which only allow access to external researchers for studies following the project purposes. Requestors wishing to access the PREDIMED-Plus trial data used in this study can make a request to the PREDIMED-Plus trial Steering Committee chair: jordi.salas@urv.cat. The request will then be passed to members of the PREDIMED-Plus Steering Committee for deliberation. Clinical Trial Registry number and website where it was obtained (if applicable).

The PREDIMED-Plus study was registered at the ISRCTN of London, England: 89898870.

Keywords

dietary fats, Mediterranean diet, MUFA, PREDIMED-Plus, PUFA

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