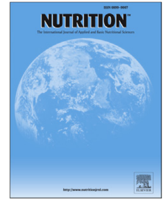
Nutrition 32 (2016) 1254–1267

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com



Basic nutritional investigation

Supplementation with antioxidant-rich extra virgin olive oil prevents hepatic oxidative stress and reduction of desaturation capacity in mice fed a high-fat diet: Effects on fatty acid composition in liver and extrahepatic tissues

Miguel Angel Rincón-Cervera Ph.D. [a], Rodrigo Valenzuela Ph.D. [a,b,*], María Catalina Hernandez-Rodas M.Sc. [b], Macarena Marambio [b], Alejandra Espinosa Ph.D. [c], Susana Mayer [b], Nalda Romero [d], Cynthia Barrera, M.Sc. [b], Alfonso Valenzuela [a], Luis A. Videla M.Sc. [e]

[a] *Lipid Center, Institute of Nutrition and Food Technology, University of Chile, Santiago, Chile*
[b] *Nutrition Department, Faculty of Medicine, University of Chile, Santiago, Chile*
[c] *Medical Technology Department, Faculty of Medicine, University of Chile, Santiago, Chile*
[d] *Faculty of Chemical Sciences and Pharmacy, Department of Food Science and Chemical Technology, University of Chile, Santiago, Chile*
[e] *Molecular and Clinical Pharmacology Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Santiago, Chile*

A R T I C L E I N F O

Article history:
Received 6 February 2016
Accepted 22 April 2016

Keywords:
Extra virgin olive oil
Antioxidants
Oxidative stress
Δ;-5/Δ;-6 desaturase activities
ω-3 long-chain polyunsaturated fatty acid

A B S T R A C T

Objective: The aim of this study was to assess the effect of dietary supplementation with extra virgin olive oil (EVOO) in mice on the reduction of desaturase and antioxidant enzymatic activities in liver, concomitantly with long-chain polyunsaturated fatty acids (LCPUFA) profiles in liver and extrahepatic tissues induced by a high-fat diet (HFD).
Methods: Male mice C57 BL/6 J were fed with a control diet (CD; 10% fat, 20% protein, 70% carbohydrates) or an HFD (60% fat, 20% protein, 20% carbohydrates) for 12 wk. Animals were supplemented with 100 mg/d EVOO with different antioxidant contents (EVOO I, II, and III).
Results: After the intervention, blood and several tissues were analyzed. Dietary supplementation with EVOO with the highest antioxidant content and antioxidant capacity (EVOO III) significantly reduced fat accumulation in liver and the plasmatic metabolic alterations caused by HFD and produced a normalization of oxidative stress–related parameters, desaturase activities, and LCPUFA content in tissues.
Conclusions: Data suggest that dietary supplementation with EVOO III may prevent oxidative stress and reduction of biosynthesis and accretion of ω-3 LCPUFA in the liver of HFD-fed mice.
© 2016 Elsevier Inc. All rights reserved.

Introduction

The long-chain polyunsaturated fatty acids (LCPUFAs) from the ω-3 family, eicosapentaenoic acid (EPA; C20:5 ω-3) and docosahexaenoic acid (DHA; C22:6 ω-3), and from ω-6 family arachidonic acid (AA; C20:4 ω-6), have multiple and relevant functions in the organism [1,2]. EPA plays a role in vascular homeostasis and inflammatory response resolution [3], and DHA is a structural component of nerve cells and is actively involved in brain and visual function development [1]. DHA is also a protective agent of neurons against neurodegenerative damage and other injuries [1]. Furthermore, EPA and DHA have a joint protective effect of cardiovascular health [4], whereas AA plays a role in the immune response and brain physiology [5,6].

Nutritional worldwide guidelines recommend specific intake of ω-3 and n-6 LCPUFA, paying special attention to those population groups with an abnormal physiological status of these fatty acids [7]. In humans and other mammals, EPA and DHA are obtained from the precursor α-linolenic acid (ALA; C18:3 ω-3), whereas AA is obtained from linoleic acid (LA; C18:2 ω-6). Both

This study was supported by grant (11140174) from the initiation FONDECYT (National) Fund for Scientific and Technological Development) to RV. The authors have no conflicts of interest to declare.
* Corresponding author. Tel.: +56 2 978 6014; fax: +56 2 978 6182.
E-mail address: Rvalenzuelab@med.uchile.cl (R. Valenzuela).

http://dx.doi.org/10.1016/j.nut.2016.04.006
0899-9007/© 2016 Elsevier Inc. All rights reserved.

ALA and LA are considered essential fatty acids because they cannot be produced in the human body and therefore must be provided by the diet [8]. LCPUFA biosynthesis takes place mainly in the liver through the activity of elongase and desaturase enzymes [9], which is regulated by hormones and by the final products of enzymatic reactions [10]. Humans and mice with hepatic steatosis and increased systemic and hepatic oxidative stress show a drastic decrease in LCPUFA synthesis and concomitant diminished LCPUFA accretion in different tissues [11,12].

Extra virgin olive oil (EVOO) is a typical food in the Mediterranean diet, and its consumption has been directly associated with protection of cardiovascular health protection and prevention of cancer and neurodegenerative disorders, which support the recommendation for its consumption [13,14]. Oleic acid (OA; C18:1 ω-9) is the main fatty acid found in EVOO, which also contains important bioactive compounds, mainly phenols [15]. To date, >30 phenolic compounds have been identified in EVOO, with much variation in composition and concentration due to diverse factors such as variety, geography, cultivation techniques, maturity of the olive fruit, and processing [16]. Phenols in EVOO constitute a complex mixture including phenolic acids, phenolic alcohols such as hydroxytyrosol and tyrosol, secoiridoids such as oleuropein, lignans, and flavonoids, all of which exhibit antioxidant properties [17]. Such compounds give EVOO healthy properties especially at cardiovascular level [17,18]. Prevention of damage by oxidative stress by EVOO has been extensively studied in tissues and cells, particularly due to the protective action of its natural antioxidants, especially hydroxytyrosol [16,19]. With this background, the aim of this study was to assess the protective effect of EVOO with different antioxidant levels on the following:

- The increase of oxidative stress parameters;
- The decrease of hepatic Δ-5 and Δ-6 desaturase activities; and
- The tissue reduction of ω-3 and ω-6 LCPUFA accretion induced by a high-fat diet (HFD) in mice.

Materials and methods

Ethics statement

Experimental animal protocols and animal procedures complied with the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, NIH Publication 6-23, revised 1985) and were approved by the Bioethics Committee for Research in Animals, Faculty of Medicine, University of Chile (protocols CBA#580 FMUCH and CBA#0630 FMUCH).

Animal preparation and supplementation with EVOO

Weaning male C57 BL/6 J mice weighing 12 to 14 g (Bioterio Central, ICBM, Faculty of Medicine, University of Chile) were randomly assigned to each experimental group and allowed free access to a control diet (CD) or an HFD. The composition of CD (expressed as percent total calories) was 10% fat, 20% protein, and 70% carbohydrate, with a caloric value of 3.85 kcal/g, free of EPA and DHA. The composition of HFD was 60% fat, 20% protein, and 20% carbohydrate, with a caloric value of 5.24 kcal/g, free of EPA and DHA (Research Diet Inc, Rodent Diet, Product data D12450 K and 12492). The fatty acid composition of CD and HFD was previously published [12]. Animals received water ad libitum and were housed on a 12-h light/dark cycle from day 1 to 84 (12 wk).

Three types of EVOO (brands Nabali, Empeltre and Kalamata) provided by Huasco Valley (Atacama, Chile) with different antioxidant contents were used for feeding animals during that period. Supplemented groups received 100 mg/d through oral administration, and the control groups received an isovolumetric amount of saline, thus comprising eight experimental groups: CD (control), CD + EVOO I, CD + EVOO II, CD + EVOO III, HFD, HFD + EVOO I, HFD + EVOO II, and HFD + EVOO III.

Weekly controls of body weight and diet intake were performed during the entire study. At the end of week 12, animals were fasted (6–8 h) and anesthetized with isoflurane, and blood samples were obtained by cardiac puncture for serum aspartate transaminase (AST), alanine transaminase (ALT), glucose, insulin, triacylglycerols (TGs), total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), thiobarbituric acid reactants (TBARs), and antioxidant capacity determination. Blood, liver, heart, adipose tissue, and brain samples were frozen in liquid nitrogen for the determination of fatty acid (FA) profiles.

Measurements of serum parameters and fat content in liver

Serum glucose (mM), cholesterol (mg/100 mL), LDL-C (mg/100 mL), HDL-C (mg/100 mL), and TG levels (mg/dL) were measured using specific diagnostic kits (Wiener Lab, Argentina). A commercial immunoassay kit for mice serum insulin assessment (μU/mL) was used, according to the manufacturer's instructions (Mercodia, Uppsala, Sweden). Insulin resistance was estimated by the homeostasis model assessment method (fasting insulin [μU/mL] × fasting glucose [mM]/22.5) [20]. Serum AST and ALT activities (units/L) were measured using specific diagnostic kits (Biomerieux SA, Marcy l,Etoile, France). Hepatic total fat content (mg/g) was evaluated according to a previously described method [21], and hepatic TG content (mg/g) (Wiener Lab) and hepatic free FA concentration (μM/g) (Cayman Chemical Company, Ann Arbor, MI, USA) were measured using specific kits according to the manufacturer's instructions.

Lipid extraction and fractionation

Quantitative extraction and separation of total lipids from erythrocytes, liver, heart, adipose tissue, and brain was carried out according to a previously described method [22]. Briefly, erythrocytes and tissue samples were homogenized in ice-cold chloroform/methanol (2:1 v/v) containing 0.01% butylated hydroxytoluene in an Ultraturrax homogenizer (Janke & Kunkel, Stufen, Germany). Total lipids from erythrocytes were extracted with chloroform/isopropanol (2:1 v/v). Phospholipids (PLs) from erythrocytes, liver, heart, adipose tissue, and brain were separated from total lipid extracts by thin layer chromatography on silica gel plates (aluminum sheets 20 × 20 cm, silica gel 60 F −254; Merck), using hexane/diethyl ether/acetic acid (80:20:1 v/v/v) as mobile phase. After development and solvent evaporation, lipid spots were visualized by exposing the plates to a Camag UV (250 nm) lamp designed for thin layer chromatography. The solvent system allows the separation of PLs, cholesterol, TGs, and cholesterol esters according to their relative mobility. PL spots were extracted from the silica with chloroform/methanol (2:1 v/v) according to a previously described method [23].

Analysis of total polyphenols, α-tocopherol, antioxidant capacity, and fatty acid profile of EVOO and different tissues

Determination of total polyphenols content in the three tested EVOOs (Nabali, Empeltre, and Kalamata) was assessed according to a previously described method [21], and quantification of α-tocopherol was evaluated according to American Oil Chemistry Society official method [24]. Antioxidant capacity was assessed by oxygen radical antioxidant capacity - fluorescein according to a previously described method [25].

For FA analysis of fatty acid methyl ester (FAME) from the three EVOO (total FA) and erythrocytes, liver, heart, adipose tissue and brain PLs were prepared with boron trifluoride (12% methanolic solution) and sodium hydroxide solution (0.5 N) in methanol, according to a previously described method [26]. Total FA from different EVOO and PLs (all tissues studied) for FAME derivatization were extracted from the silica gel spots with 15 mL of chloroform/methanol/water (10:10:1 v/v/v) and evaporated under a nitrogen stream. Samples were cooled and extracted with 0.5 mL of hexane. FAME were separated and quantified by gas-liquid chromatography in an Agilent Hewlett-Packard equipment (model 7890 A, Palo Alto, CA, USA) using a capillary column (Agilent HP-88, 100 m × 0.250 mm; I.D. 0.25 μm) and a flame ionization detector. The injector temperature was set at 250°C and the flame ionization detector temperature at 300°C. The oven temperature was initially set at 140°C and was programmed to increase temperature until 220°C at a rate of 5°C/min. Hydrogen was used as the carrier gas (35 cm/s flow rate) in the column, and the inlet split ratio was set at 20:1. The identification and quantification of FAME were achieved by comparing the retention times and the peak area values (%) of the unknown samples with those of a commercial lipid standard (Nu-Chek Prep Inc, Elysian MN, USA). C23:0 was used as internal standard (Nu-Chek Prep Inc) and a Hewlett-Packard Chemstation data system was used for processing.

Assays for hepatic and plasma oxidative stress-related parameters

Livers of anesthetized animals were perfused in situ with a cold solution containing 150 mM KCl and 5 mM Tris (pH 7.4) to remove blood and for

glutathione (GSH) and protein carbonylation assessments. Reduced GSH and glutathione disulfide (GSSG) content were assessed with the enzymatic recycling method [27]. Specific kits (Cayman Chemical Company,) were used to measure contents of protein carbonyls, F2-isoprostanes, and TBARs in liver, TBARs in plasma, and the antioxidant capacity of plasma, all according to the manufacturer's instructions.

Determination of hepatic Δ-5 and Δ-6 desaturase activities

Hepatic samples (500 mg) frozen in liquid nitrogen were homogenized in a buffer solution pH 7.9 containing 10 mmol/L HEPES, 1 mmol/l EDTA, 0.6% Nonidet P-40, 150 mmol/l NaCl, and protease inhibitors (1 mmol/l phenylmethylsulfonyl fluoride, 1 μg/mL aprotinin, 1 μg/mL leupeptin, and 1 mmol/l orthovanadate). Hepatic homogenates were centrifuged at 4°C, first at 2000*g* for 30 s, followed by centrifugation of the supernatants at 5000*g* for 5 min, and finally at 100 000*g* for 60 min, to obtain the extracts for the assessment of desaturase activities. Δ-5 Desaturase activity was determined by the amount of dihomo-γ-linolenic acid (DHGLA; C20:3 ω-6) being converted into AA, using albumin-bound FA precursors (LA and DHGLA), whereas Δ-6 desaturase activity was obtained by measuring the amount of γ-linolenic acid (GLA; C18:3 ω-6) produced from LA [28].

Desaturase activity was assayed using 1 mL of incubation medium containing 4 μmol ATP, 0.1 μmol coenzyme-A, 1.28 μmol NADH, 2.42 μmol *N*-acetylcysteine, 0.5 μmol nicotinamide, 5 μmol $MgCl_2$, 62.5 μmol NaF, and 62.5 μmol phosphate buffer pH 7, supplemented with 100 nmol with the respective albumin-bound FA precursor and 1 mg protein of cytosolic extract in a total volume of 100 μL, incubated at 37°C for 30 min with shaking. Δ-5 and Δ-6 desaturase assays were conducted simultaneously. The reaction was stopped by adding 6 mL of a methanol:chloroform mixture (2:1 v/v). Eptadecanoic acid (C17:0; 99%+ pure) was added (20 μg) as internal standard.

To determine the changes in the levels of products or precursors achieved after incubation, lipids were extracted and derivatized to FAME, which were analyzed by gas-liquid chromatographic analysis as described previously [29]. FAME peaks were identified by comparison with a FAME standard mix and quantification was carried out by comparison of each peak area with that of the internal standard. Δ-5 and Δ-6 Desaturase activities, measured as net decrease in DHGLA production and net increase in GLA production, were calculated from gas-liquid chromatography results as differences between baseline values and those obtained after 30 min incubation, and results were expressed as nmol·mg protein·min.

Gene expression assays

Total RNA was isolated from hepatic samples using Trizol (Invitrogen, Paisley, United Kingdom), according to the supplier's protocols. Purified RNA (2 mg) was then treated with DNAasa (DNA free kit; Ambion, Austin, TX, USA) and used to generate first-strand cDNA with M-MLV reverse transcriptase (Invitrogen), using random hexamers (Invitrogen) and dNTP mix (Bioline, London, UK), according to the manufacturer's protocol. Resultant cDNA was amplified with specific primers for mice in a total volume of 10 μL. Gene-specific primer sequences used are shown in Supplementary Table 1. Primers were optimized to yield 95% to 100% of reaction efficiency with polymerase chain reaction products by development in agarose gel to verify the correct amplification length. Real-time polymerase chain reaction was performed in a Strategen M×3000 P System (Agilent Technologies) following the manufacturer's recommendation (Applied Biosystems, Foster City, CA, USA). All the expression levels of target genes under study were normalized by the expression of β-actin as internal control (Applied Biosystems). Fold changes between groups was calculated by the $2^{(-\Delta\Delta Ct)}$ method.

Assessment of sterol regulatory element-binding protein 1 c DNA-binding activity

Nuclear extracts from hepatic tissue (left lobe) were obtained using a commercial extraction kit (Cayman Chemical Company, Item 10011223). Sterol regulatory element-binding protein 1 c (SREBP-1 c) DNA-binding activity was assessed with a commercial enzyme-linked immunosorbent assay kit (Cayman Chemical Company, Item 10010854) and according to the manufacturer's instructions. Values were expressed as percentage of SREBP-1 c DNA-binding activity with respect to a positive control provided by the kit.

Determination of hepatic catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase activities

Hepatic samples were homogenized in three volumes of 30 mM phosphate buffer, pH 7.4, containing EDTA (1 mM) and 250 mmol sucrose. After centrifugation at 500*g* for 10 min, 4°C, one aliquot of the supernatant was used for the determination of both catalase (CAT) and superoxide dismutase (SOD) activities. Another aliquot was centrifuged at 100 000*g* for 60 min at 4°C to carry out glutathione peroxidase (GPX) and glutathione reductase (GR) assays [30]. CAT activity was measured according to a previously described method [31]. Enzyme unit is defined as the amount of enzyme that liberates the half of the oxygen from hydrogen peroxide solution in 100 s at 25°C.

The quantitative assessment of SOD activity was carried out with a commercial assay kit (Cayman Chemical Company) according to the manufacturer's instructions. The sensitivity was 0.044 U mL, and the intra- and interassay coefficients of variations were lower than 16.8% and 13.8%, respectively. GPX activity was determined using hydrogen peroxide as substrate according to a previously described method [32]. The activity of the enzyme was evaluated at 340 nm by measuring the decrease in the absorbance of NADPH. An enzyme unit is defined as the number of μmoles of NADPH oxidized per minute at 20°C.

GR activity was determined by a previously described method [33]. In this case, one enzyme unit is defined as the amount of enzyme that reduces 1 μmol/min of oxidized GSH at pH 6.6 and 25°C.

Hepatic lipolitic and lipogenic enzymatic activities

Acetyl-coenzyme A carboxylase (ACC) activity was determined using a previously described method [34]. Briefly, 1 g of frozen liver was homogenized in 3 volumes of phosphate bicarbonate buffer pH 7.0 (70 mM $KHCO_3$; 85 mM K_2HPO_4; 9 mM KH_2PO_4;1 mM dithiothreitol). The cytosolic fraction was obtained after centrifuging the supernatant at 100 000*g* for 1 h at 4°C. ACC activity was measured using an NADH-linked assay [35]. The assay media (56 mM Tris-HCl pH 8.0; 10 mM $MgCl_2$; 11 mM EDTA; 4 mM ATP; 52 mM $KHCO_3$; 0.75 mg/mL bovine serum albumin; 0.5 mM NADH; 1.4 mM phosphoenolpyruvate) was mixed with 5.6 U/mL pyruvate kinase and 5.6 U/mL lactate dehydrogenase. Baseline was followed at 30°C until a constant slope was reached. For every 2.3 volumes of medium, 1 volume of activated homogenate was added and the reaction was started with acetyl-CoA (0.125 mM final concentration). For enzymatic activation, 1 volume of homogenate was incubated with 1 volume of activation buffer (20 mM citrate; 100 mM Tris-HCl pH 8.0; 1.5 mg/mL bovine serum albumin; 20 mM $MgCl_2$; 20 mM GSH [pH 7.5]) for 15 min at 37°C. Fatty acid synthase (FAS) activity was assessed in hepatic cytosolic fractions by measuring malonyl CoA-dependent NADPH oxidation at 37°C as described previously [36]. Activity of carnitine-palmitoyl transferase-1 (CPT-1) was determined spectrophotometrically using a previous method [37].

Statistical analysis

Statistical analysis was performed with GraphPad Prism 6.0 software (GraphPad Prism Software, Inc. San Diego, CA, USA). Reported values represent the mean ± SEM for each experimental group. Evaluation of normality of data distribution was performed using the Shapiro Wilk test. Assessment of the statistical significance of differences between mean values was performed by two-way analysis of variance and Bonferroni posttest. $P < 0.05$ was considered significant.

Results

Composition of EVOO

EVOO I (brand Nabali) contained 116 mg of total polyphenols and 215 mg α-tocopherol/kg oil, with an antioxidant capacity of 3.378 ± 222 μmoles eq. Trolox/L. EVOO II (brand Empeltre) contained 407 mg total polyphenols and 290 mg α-tocopherol/kg oil and had an antioxidant capacity of 4.841 ± 199 μmoles eq. Trolox/L. EVOO III (brand Kalamata) contained 859 mg total polyphenols and 227 mg α-tocopherol/kg oil, with an antioxidant capacity of 7.156 ± 434 μmoles eq. Trolox/L. The FA composition of the different EVOOs used in the study is shown in Supplementary Table 2.

General and biochemical parameters

Data of general and biochemical parameters from mice fed CD and HFD with and without EVOO supplementation are reported in Table 1. As expected, HFD intake significantly increased body weight and visceral adipose tissue regardless of EVOO supplementation, whereas liver weight was not affected. All studied hepatic parameters, namely, total fat content, TG content, and free FA concentration, were significantly increased in mice fed

Table 1
General and biochemical parameters in control mice and high-fat diet fed mice receiving different EVOO supplementation*

	Groups							
	CD				HFD			
	Saline	EVOO I	EVOO II	EVOO III	Saline	EVOO I	EVOO II	EVOO III
	a	b	c	d	e	f	g	h
General parameters								
Initial body weight (g)	14.7 ± 1.6	14.7 ± 1.5	14.6 ± 1.8	15.0 ± 1.7	14.9 ± 1.3	14.0 ± 1.6	14.5 ± 1.8	14.8 ± 1.7
Final body weight (g)	33.5 ± 2.9[e,f,g,h]	34.5 ± 2.6[e,f,g,h]	36.5 ± 2.8[e,f,g,h]	34.7 ± 3.1[e,f,g,h]	44.5 ± 4.5[a,b,c,d]	42.15 ± 4.3[a,b,c,d]	43.2 ± 3.8[a,b,c,d]	40.6 ± 3.7[a,b,c,d]
Liver weight (g)	1.12 ± 0.3	1.14 ± 0.3	1.19 ± 0.4	1.05 ± 0.2	1.24 ± 0.4	1.25 ± 0.6	1.27 ± 0.4	1.23 ± 0.5
Visceral adipose tissue (g)	1.10 ± 0.2[e,f,g,h]	1.08 ± 0.1[e,f,g,h]	1.13 ± 0.2[e,f,g,h]	1.14 ± 0.2[e,f,g,h]	4.9 ± 1[a,b,c,d]	4.7 ± 0.8[a,b,c,d]	4.8 ± 0.6[a,b,c,d]	4.7 ± 0.7[a,b,c,d]
Liver parameters								
Total fat (mg/g liver)	37.8 ± 3.6[e,f,g,h]	32.5 ± 4.2[e,f,g,h]	33.5 ± 3.6[e,f,g,h]	31.5 ± 3[e,f,g,h]	110.5 ± 9.8[a,b,c,d,h]	98.9 ± 9.7[a,b,c,d]	90.6 ± 8.5[a,b,c,d]	81.5 ± 7.9[a,b,c,d,e]
Triacylglycerols (mg/g liver)	32.6 ± 2.7[e,f,g,h]	30 ± 2.7[e,f,g,h]	31.6 ± 2.9[e,f,g,h]	29.9 ± 2.6[e,f,g,h]	107.2 ± 10.6[a,b,c,d,h]	94.6 ± 8.6[a,b,c,d]	86.4 ± 7.9[a,b,c,d]	76.5 ± 7[a,b,c,d,e]
Free fatty acid (μM/g liver)	268.9 ± 17.4[e,f,g,h]	254.6 ± 25.6[e,f,g,h]	238.9 ± 36.8[e,f,g,h]	241.2 ± 20.1[e,f,g,h]	712.2 ± 30.6[a,b,c,d,h]	684.9 ± 27.9[a,b,c,d]	654.3 ± 22[a,b,c,d]	507.3 ± 26.7[a,b,c,d,e]
Serum parameters								
Triacylglycerols (mg/dL)	130.1 ± 15.8[e,f,g,h]	128.5 ± 12.9[e,f,g,h]	134.2 ± 17.2[e,f,g,h]	125.6 ± 15[e,f,g,h]	184.5 ± 19.2[a,b,c,d]	180.5 ± 17[a,b,c,d]	181.5 ± 16.6[a,b,c,d]	170.4 ± 17.2[a,b,c,d]
Total cholesterol (mg/dL)	72.4 ± 8.2[e,f,g,h]	75.6 ± 9.4[e,f,g,h]	71.3 ± 6.8[e,f,g,h]	78.9 ± 7.5[e,f,g,h]	141.5 ± 22.5[a,b,c,d]	130.1 ± 16.4[a,b,c,d]	125.9 ± 12.3[a,b,c,d]	110.2 ± 10.1[a,b,c,d]
LDL-cholesterol (mg/dL)	47.8 ± 5.1[e,f,g]	48.6.2 ± 4.4[e,f,g]	46.5 ± 5[e,f,g]	40.5 ± 3.1[e,f,g]	94.6 ± 8.6[a,b,c,d,h]	86.5 ± 6.8[a,b,c,d,h]	75.0 ± 6.2[a,b,c,d,h]	50.1 ± 4.8[e,f,g]
HDL-cholesterol (mg/dL)	23.1 ± 3.2[d,e,f,g,h]	25.5 ± 4.5[d,e,f,g,h]	24.1 ± 2.9[d,e,f,g,h]	37.2 ± 3[a,b,c,g,h]	45.2 ± 3.9[d,f,g,h]	42.5 ± 3.3[d,e,g]	49.5 ± 4.2[d,e,f]	58.6 ± 6.2[a,b,c,d,e]
Insulin resistance								
Fasting glucose (mg/dL)	120.4 ± 15.3[e,f,g,h]	125.6 ± 12.5[e,f,g,h]	119.5 ± 10.4[e,f,g,h]	122.7 ± 16.8[e,f,g,h]	245.5 ± 29.7[a,b,c,d]	239.5 ± 20.1[a,b,c,d]	230.5 ± 15.6[a,b,c,d]	221.4 ± 17.5[a,b,c,d]
Fasting insulin (units/mL)	5.35 ± 0.8[e,f,g,h]	5.22 ± 0.9[e,f,g,h]	5.10 ± 0.8[e,f,g,h]	5.30 ± 0.6[e,f,g,h]	17.8 ± 1.9[a,b,c,d,f,g,h]	13.5 ± 1.1[a,b,c,d,e]	12.6 ± 1.4[a,b,c,d,e]	11.9 ± 0.9[a,b,c,d,e]
HOMA	1.22 ± 0.2[e,f,g,h]	1.19 ± 0.1[e,f,g,h]	1.21 ± 0.1[e,f,g,h]	1.18 ± 0.2[e,f,g,h]	8.70 ± 1.2[a,b,c,d]	7.55 ± 0.8[a,b,c,d]	7.51 ± 0.7[a,b,c,d]	6.85 ± 0.8[a,b,c,d]
Serum transaminases								
AST (U/L)	145.2 ± 15.6	143.8 ± 12.6	150.6 ± 18.5	140.1 ± 11.5	168.6 ± 19.5	155.3 ± 15.6	150.6 ± 13.3	152.6 ± 10.2
ALT (U/L)	70.5 ± 7.5	68.9 ± 5.8	71.2 ± 9.5	70.2 ± 7.3	81.5 ± 10.5	76.5 ± 9.5	80.5 ± 6.5	74.5 ± 9.5

ALT, aspartate transaminase; AST, alanine transaminase; CD, control diet; EVOO, extra virgin olive oil; HDL, high-density lipoprotein; HFD, high-fat diet; HOMA, homeostasis model assessment method; LDL, low-density lipoprotein

* Values represent means ± SEM for 10 to 12 mice per experimental group. Significant differences between the groups are indicated by the superscript letter identifying each group (two-way analysis of variance and Bonferroni post-test; $P < 0.05$).

HFD compared with CD (Table 1). EVOO supplementation had no effect within CD groups, whereas total fat, TGs, and free FA concentrations were reduced by 26.2%, 28.6%, and 28.8% by EVOO III containing the highest polyphenol content, respectively, when compared with HFD + saline group (Table 1). Despite the beneficial effects of EVOO supplementation in HFD-fed mice, values were still higher (~2- to 2.5-fold) compared with the levels observed for CD groups (Table 1).

Regarding serum parameters (TGs, total cholesterol, LDL-C, and HDL-C), a large increase was observed for HFD compared with CD group (Table 1). It can be seen that supplementation with EVOO III raised HDL-C levels in both CD (61% higher in CD + EVOO III than CD + saline) and HFD mice (29.6% higher in HFD + EVOO III than in HFD + saline). EVOO III also influenced serum HDL/LDL ratios, as shown by enhancements from 0.48 in CD + saline and HFD + saline groups to 0.92 in CD + EVOO III and 1.17 for HFD + EVOO III, an effect that was not elicited by EVOO I or EVOO II (from Table 1). The assessment of insulin resistance parameters revealed that HFD mice exhibited significantly higher fasting glucose, fating insulin, and homeostasis model assessment method values than those in CD groups (Table 1). Furthermore, no significant differences were found within CD and HFD groups, with the exception of the serum levels of fasting insulin in HFD + saline mice that were significantly higher than HFD groups supplemented with EVOO (I, II, and III). In all experimental groups studied, serum AST and ALT levels were comparable.

Fatty acid composition of phospholipids from liver, erythrocyte, heart, adipose tissue, and brain

FA profiles of hepatic PLs from CD and HFD groups are shown in Table 2. The amount of palmitic acid (C16:0), the major FA found within the eight experimental groups, showed no significant differences except for HFD + saline, where it was significantly higher. EVOO supplementation in HFD restored C16:0 levels to the values found in CD.

Concerning OA, another major FA found in liver PLs, all figures were comparable except CD + saline, whose value was significantly lower compared with the other seven groups. EVOO supplementation had no effect on OA levels in HFD-fed animals. Some PUFAs from ω-3 family comprising ALA, stearidonic acid (C18:4 ω-3), eicosatrienoic acid (C20:4 ω-3), EPA, and docosapentaenoic acid (ω:5 ω-3) were found at lower percentages in HFD than in CD groups, although after EVOO III supplementation,

Table 2
Fatty acid composition of liver phospholipids obtained from control mice and HFD-fed mice receiving different EVOO supplementation*

Fatty acid	Fatty acid composition (g/100 g FAME)							
	Groups							
	Control diet				HFD			
	Saline	EVOO I	EVOO II	EVOO III	Saline	EVOO I	EVOO II	EVOO III
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
C16:0	35.4 ± 3.3^{e}	32.1 ± 2.7^{e}	32.9 ± 2.7^{e}	31.5 ± 2.8^{e}	44.8 ± 3.5^{a,b,c,d,f,g,h}	35.4 ± 2.9^{e}	33.3 ± 2.6^{e}	34.1 ± 2.7^{e}
C 18:1 ω-9	23.8 ± 1.9^{b,c,d,e,f,g,h}	30.4 ± 2.5^{a}	31.1 ± 2.2^{a}	31.5 ± 2^{a}	28.3 ± 2.3^{a}	28.4 ± 2.1^{a}	29.4 ± 2.6^{a}	30.2 ± 3^{a}
C18:2 ω-6 (LA)	12.9 ± 1.2	11.8 ± 1.1	11.6 ± 1.3	11.5 ± 0.9	12.0 ± 1.3	14.0 ± 1.9	11.7 ± 1.5	12.4 ± 1.6
C18:3 ω-6	1.02 ± 0.1	0.95 ± 0.05	1.04 ± 0.1	0.97 ± 0.04	1.02 ± 0.04	1.03 ± 0.1	1.07 ± 0.1	0.97 ± 0.05
C18:3 ω-3 (ALA)	1.16 ± 0.1^{e,f,g}	1.10 ± 0.05^{e,f,g}	1.13 ± 0.06^{e,f,g}	1.14 ± 0.05^{e,f,g}	0.83 ± 0.04^{a,b,c,d,h}	0.92 ± 0.03^{a,b,c,d,h}	0.98 ± 0.04^{a,b,c,d,h}	1.08 ± 0.1^{e,f,g}
C18:4 ω-3	0.25 ± 0.04^{e,f,g}	0.26 ± 0.03^{e,f,g}	0.25 ± 0.05^{e,f,g}	0.24 ± 0.04^{e,f,g}	0.11 ± 0.04^{a,b,c,d,h}	0.14 ± 0.04^{a,b,c,d,h}	0.16 ± 0.03^{a,b,c,d}	0.22 ± 0.04^{e,f,g}
C20:3 ω-6	0.23 ± 0.04^{e,f}	0.20 ± 0.03^{e,f}	0.21 ± 0.03^{e,f}	0.22 ± 0.05^{e,f}	0.14 ± 0.02^{a,b,c,d,h}	0.16 ± 0.02^{a,h}	0.19 ± 0.04	0.24 ± 0.04^{e,f}
C20:4 ω-6 (AA)	11.5 ± 0.8^{e,f}	11.3 ± 0.5^{e,f}	10.8 ± 0.6^{e,f}	11.2 ± 0.4^{e,f}	6.64 ± 0.5^{a,b,c,d,g,h}	7.14 ± 0.4^{a,b,c,d,g,h}	8.66 ± 0.3^{a,b,c,d,e,f,h}	10.5 ± 0.9^{e,f,g}
C20:4 ω-3	0.15 ± 0.05^{e,f}	0.15 ± 0.03^{e,f}	0.14 ± 0.02^{e,f}	0.14 ± 0.4^{e,f}	0.05 ± 0.01^{a,b,c,d,h}	0.06 ± 0.02^{a,b,c,d}	0.08 ± 0.03^{a,b,c,d,h}	0.11 ± 0.02^{e,f}
C20:5 ω-3 (EPA)	1.05 ± 0.1^{e,f,g}	1.11 ± 0.04^{e,f,g}	1.03 ± 0.1^{e,f,g}	1.09 ± 0.1^{e,f,g}	0.38 ± 0.1^{a,b,c,d,g,h}	0.45 ± 0.1^{a,b,c,d,g}	0.74 ± 0.2^{a,b,c,d,e,f,h}	0.99 ± 0.1^{e,f,g}
C22:5 ω-6 (DPA ω-6)	0.08 ± 0.02^{e}	0.07 ± 0.02^{e}	0.09 ± 0.03^{e}	0.08 ± 0.01^{e}	0.03 ± 0.01^{a,b,c,d,h}	0.04 ± 0.01^{a,b,c,d,h}	0.06 ± 0.02^{e}	0.08 ± 0.01^{e}
C22:5 ω-3 (DPA ω-3)	0.10 ± 0.01^{e,f}	0.09 ± 0.01^{e,f}	0.11 ± 0.02^{e,f}	0.09 ± 0.01^{e,f}	0.05 ± 0.01^{a,b,c,d,h}	0.06 ± 0.1^{a,b,c,d,h}	0.07 ± 0.03	0.09 ± 0.01^{e,f}
C22:6 ω-3 (DHA)	4.10 ± 0.3^{e}	4.17 ± 0.2^{e}	4.14 ± 0.2^{e}	4.20 ± 0.2^{e}	2.18 ± 0.2^{a,b,c,d,f,g,h}	3.80 ± 0.4^{e}	4.05 ± 0.3^{e}	4.11 ± 0.4^{e}
Total SFA	37.9 ± 3.4^{e}	33.7 ± 2.4^{e}	34.2 ± 3.1^{e}	33.8 ± 2.9^{e}	47.1 ± 3.8^{a,b,c,d,f,g,h}	38.6 ± 3.3^{e}	35.4 ± 3.5^{e}	36.8 ± 3.2^{e}
Total MUFA	27.1 ± 2.4	32.4 ± 2	33.1 ± 2.9	32.4 ± 3.1	29.3 ± 2.9	32.6 ± 2.6	32.4 ± 2.8	33.1 ± 3.1
Total PUFA	35.0 ± 3.1^{e}	33.9 ± 2.6^{e}	32.7 ± 2.8^{e}	33.8 ± 2.7^{e}	23.6 ± 2.1^{a,b,c,d,g,h}	28.8 ± 2^{e}	32.2 ± 3^{e}	30.1 ± 2.8^{e}
Total LCPUFA	17.2 ± 1.2^{e,f,g}	17.1 ± 1.4^{e,f,g}	16.9 ± 1.1^{e,f,g}	17.3 ± 0.9^{e,f,g}	9.50 ± 0.7^{a,b,c}	11.8 ± 0.8^{a,b,c}	13.9 ± 0.9^{a,b,c}	16.3 ± 1.4^{e,f,g}
Total ω-6 LCPUFA	11.7 ± 0.8^{e,f,g}	12.6 ± 1^{e,f,g}	11.2 ± 0.9^{e,f,g}	11.8 ± 1^{e,f,g}	6.83 ± 0.4^{a,b,c}	7.40 ± 0.6^{a,b,c}	8.95 ± 0.6^{a,b,c}	10.8 ± 1.1^{e,f,g}
Total ω-3 LCPUFA	5.50 ± 0.2^{e,f}	5.52 ± 0.3^{e,f}	5.70 ± 0.2^{e,f}	5.50 ± 0.3^{e,f}	2.67 ± 0.3^{a,b,c,d,f,g,h}	4.40 ± 0.4^{a,b,c,d,e,h}	4.95 ± 0.4^{e,c}	5.50 ± 0.2^{e,f}
ω-6/ω-3 LCPUFA ratio	2.13 ± 0.2^{e,f,g}	2.29 ± 0.3^{e,f,g}	1.97 ± 0.3^{e,f,g}	2.15 ± 0.2^{e,f,g}	1.40 ± 0.05^{a,b,c,d,f,g,h}	1.70 ± 0.04^{a,b,c,d,e,g,h}	1.81 ± 0.05^{a,b,c,d,e,f,h}	1.97 ± 0.04^{e,f,g}

AA, arachidonic acid; ALA, α-linolenic acid; ANOVA, analysis of variance; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; EVOO, extra virgin olive oil; FAME, fatty acid methyl ester; HFD, high-fat diet; LA, linolenic acid; LCPUFA, polyunsaturated fatty acid; long-chain MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid

* Values are expressed as g fatty acid per 100 g FAME and represent the mean ± SEM for n = 10 to 12 mice per experimental group. Significant differences between the groups are indicated by the letter identifying each group (two-way ANOVA and Bonferroni posttest; $P < 0.05$). SFAs correspond to C14:0, C16:0 and C18:0. MUFAs correspond to C14:1 ω-7, C16:1 ω-7, and C18:1 ω-9. PUFAs correspond to C18:2 ω-6, C18:3 ω-3, C20:4 ω-6, C20:5 ω-3, C22:5 ω-3, and C22:6 ω-3; ω-6 LCPUFA are C20:4 ω-6 and C22:5 ω-3; ω-3 LCPUFA are C20:5 ω-3, C22:5 ω-3, and C22:6 ω-3; ω-6/ω-3 LCPUFA ratio: C20:4 ω-6/(C20:5 ω-3 + C22:5 ω-3 + C22:6 ω-3).

values were restored. The same trend was observed for some ω-6 LCPUFA such as AA and ω-6 DPA (C22:5 ω-6), and for total ω-6 and ω-3 LCPUFA and ω-6/ω-3 LCPUFA ratio. However, no significant differences within the eight experimental groups were found for LA, GLA, and total monounsaturated fatty acids (MUFAs).

EVOO supplementation had no effect on DHA amounts within CD groups, and although HFD reduced significantly the DHA level, it was restored after EVOO (I, II, and III) intake in all cases. Differences in total saturated fatty acids (SFA) and total PUFA were only observed for HFD + saline, with higher and lower levels respectively than for the other seven experimental groups, showing that although HFD causes an imbalance between SFA and PUFA groups, EVOO intake is able to restore these proportions in all cases.

FA profiles of erythrocyte PLs from CD and HFD groups are shown in Table 3. No significant difference was found for any FA or FA class within the four CD groups. However, HFD caused modifications in most FA that were normalized in some cases after EVOO supplementation. For C16:0, the main FA found in all groups, the highest value was shown in HFD + saline (45.2% of total FA), which decreased to 38.7 % in HFD + EVOO III. Levels of OA, LA, ALA, and AA were comparable in all experimental conditions. Concerning EPA, significant lower values were found in HFD groups supplemented with saline, EVOO I, and EVOO II; however, EPA levels in HFD + EVOO III mice were comparable to those found in CD groups (Table 3). This trend was also observed for ω-6 DPA, ω-3 DPA, DHA, and total ω-3 PUFA. HFD intake increased the level of total SFA but EVOO II and III supplementation restored the level to those found in CD. Total MUFA and total PUFA percentages were not affected by HFD. This diet increased the ω-6/ω-3 LCPUFA ratio, the highest value found for HFD + saline, although it a decrease after EVOO supplementation was observed, being the value restored with HFD + EVOO III.

FA profiles of heart tissue PLs from CD and HFD groups are shown in Table 4. No FA was modified by EVOO supplementation in CD groups. Concerning HFD, ALA, EPA, ω-6 DPA, ω-3 DPA, total PUFA, total LCPUFA, and total ω-3 LCPUFA percentages were lowered, but values were restored to CD figures after EVOO III supplementation. The LCPUFA ω-6/ω-3 ratio was higher in HFD than in CD, but it was restored after EVOO III supplementation. For the other FAs, no changes were observed between CD and HFD.

Table 5 shows the FA profiles of adipose tissue PLs from CD and HFD groups. No significant differences were found within CD groups in any case. Higher values were found for C16:0 in HFD compared with CD, and although EVOO supplementation had a reducing effect, values were not restored to CD level in any case. In contrast, the higher percentages found after HFD intake for SFA and ω-6/ω-3 LCPUFA ratio were restored to CD levels after supplementation with EVOO II and III. For OA, LA, and total MUFA percentages, no differences were observed among the eight experimental groups. In the case of ALA, EPA, ω-6 DPA, ω-3 DPA, DHA, total PUFA, total LCPUFA, and total ω-3 LCPUFA, lower figures were found in HFD compared with CD; EVOO increased such percentages to values comparable to CD figures, however, levels of AA and ω-6 LCPUFA were not restored.

In Table 6, the FA profiles of brain PLs from CD and HFD groups are reported. EVOO supplementation did not produce any modification of the FA profile within CD groups. C16:0 and total SFA percentages were increased after HFD intake, but EVOO supplementation decreased them, and finally they were restored

Table 3
Fatty acid composition of erythrocyte phospholipids obtained from control mice and HFD-fed mice receiving different EVOO supplementation*

Fatty acid	Fatty acid composition (g/100 g FAME)							
	Groups							
	Control diet				HFD			
	Saline	EVOO I	EVOO II	EVOO III	Saline	EVOO I	EVOO II	EVOO III
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
C16:0	35.8 ± 2.4^{e,f,}	32.1 ± 2.2^{e,f,g,h}	34 ± 3.1^{e,f}	34.2 ± 2.1^{e,f,g,h}	45.2 ± 3.1^{a,b,c,d}	43.1 ± 3.5^{a,b,c,d}	40.1 ± 3.2^{b}	38.7 ± 2.8^{b}
C 18:1 ω-9	22.5 ± 1.9	24.3 ± 2	25.2 ± 2.4	27.3 ± 2.5	20.1 ± 1.7	21.7 ± 1.7	22.5 ± 2.2	25.6 ± 2.6
C18:2 ω-6 (LA)	11.8 ± 1.1	10.4 ± 1.5	10.5 ± 0.9	11.6 ± 1.1	10.5 ± 0.8	11.1 ± 0.9	10.2 ± 1.5	10.1 ± 1.4
C18:3 ω-3 (ALA)	1.16 ± 0.05	1.14 ± 0.1	1.10 ± 0.2	1.32 ± 0.2	0.96 ± 0.1	1.05 ± 0.05	1.09 ± 0.04	1.12 ± 0.05
C20:4 ω-6 (AA)	13.6 ± 1.2	14.1 ± 1.5	12.5 ± 1.8	12.0 ± 1.6	13.2 ± 1.4	12.8 ± 0.9	13.4 ± 0.8	11.4 ± 1.5
C20:5 ω-3 (EPA)	2.02 ± 0.4^{e,f,g}	1.94 ± 0.3^{e,f,g}	2.01 ± 0.2^{e,f,g}	1.95 ± 0.1^{e,f,g}	0.75 ± 0.05^{a,b,c,d,f,g,h}	0.97 ± 0.04^{a,b,c,d,e,g,h}	1.35 ± 0.2^{a,b,c,d,e,f,h}	1.85 ± 0.2^{e,f,g}
C22:5 ω-6 (DPAω-6)	0.15 ± 0.03^{e,f,g,h}	0.14 ± 0.04^{e,f}	0.15 ± 0.04^{e,f}	0.16 ± 0.05^{e,f}	0.05 ± 0.01^{a,b,c,d,g,h}	0.07 ± 0.01^{a,b,c,d,h}	0.09 ± 0.02^{a}	0.13 ± 0.04^{e,f}
C22:5 ω-6 (DPAω-3)	0.84 ± 0.05^{e,f,g}	0.80 ± 0.04^{e,f,g}	0.81 ± 0.05^{e,f,g}	0.85 ± 0.1^{e,f,g}	0.03 ± 0.01^{a,b,c,d,f,g,h}	0.11 ± 0.02^{a,b,c,d,e,g,h}	0.32 ± 0.04^{a,b,c,d,e,f,h}	0.75 ± 0.1^{e,f,g}
C22:6 ω-3 (DHA)	4.10 ± 0.5^{e,f,g}	4.04 ± 0.6^{e,f,g}	4.12 ± 0.6^{e,f,g}	4.14 ± 0.5^{e,f,g}	2.10 ± 0.04^{a,b,c,d,g,h}	2.15 ± 0.06^{a,b,c,d,g,h}	2.82 ± 0.2^{a,b,c,d,e,f}	3.72 ± 0.6^{e,f}
Total SFA	39.5 ± 3.1^{e,f}	37.8 ± 2.9^{e,f}	38.9 ± 3^{e,f}	37.2 ± 3.2^{e,f}	48.4 ± 3.3^{a,b,c,d}	46.5 ± 3.4^{a,b,c,d}	44.4 ± 2.9	42.1 ± 2.7
Total MUFA	26.2 ± 2.2	28.9 ± 2.5^{e}	28.7 ± 2.3^{e}	29.4 ± 2.9^{e}	23.5 ± 2.2^{b,c,d}	24.6 ± 2.4	25.6 ± 2.4	28.4 ± 2.6
Total PUFA	34.3 ± 3.3	33.3 ± 2.8	32.4 ± 3	33.4 ± 3.2	28.1 ± 2.6	28.9 ± 2.3	30 ± 3.5	29.5 ± 2.8
Total LCPUFA	20.9 ± 1.7^{e,f}	21.2 ± 2^{e,f}	19.7 ± 1.6^{e,f}	19.3 ± 2.1^{e,f}	16.3 ± 0.7^{a,b,c,d}	16.2 ± 0.5^{a,b,c,d}	18.3 ± 1.4	18.1 ± 1.5
Total ω-6 LCPUFA	13.9 ± 0.6^{h}	14.3 ± 0.8^{h}	12.8 ± 0.9	12.3 ± 0.7	13.4 ± 0.4^{h}	13.0 ± 0.3^{h}	13.6 ± 1.1	11.6 ± 0.5^{a,b,e,f}
Total ω-3 LCPUFA	7.00 ± 0.05^{e,f,g}	6.90 ± 0.1^{e,f,g}	6.90 ± 0.2^{e,f,g}	7.00 ± 0.05^{e,f,g}	2.90 ± 0.1^{a,b,c,d,g,h}	3.20 ± 0.1^{a,b,c,d,g,h}	4.70 ± 0.3^{a,b,c,d,e,f,h}	6.50 ± 0.3^{e,f,g}
ω-6/ω-3 LCPUFA ratio	2.00 ± 0.03^{e,f,g}	2.10 ± 0.05^{e,f,g}	1.90 ± 0.1^{e,f,g}	1.80 ± 0.02^{e,f,g}	4.60 ± 0.2^{a,b,c,d,g,h}	4.10 ± 0.2^{a,b,c,d,g,h}	2.90 ± 0.05^{a,b,c,d,e,f,h}	1.80 ± 0.05^{e,f,g}

AA, arachidonic acid; ALA, α-linolenic acid; ANOVA, analysis of variance; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; EVOO, extra virgin olive oil; FAME, fatty acid methyl ester; HFD, high-fat diet; LA, linolenic acid; LCPUFA, polyunsaturated fatty acid; long-chain MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid

* Values are expressed as g fatty acid/100 g FAME and represent the mean ± SEM for N = 10 to 12 mice per experimental group. Significant differences between the groups are indicated by the letter identifying each group (two-way ANOVA and Bonferroni posttest; $P < 0.05$). Identification of saturated and unsaturated fatty acids and their relationships are shown in Table 2.

Table 4
Fatty acid composition of heart tissue phospholipids obtained from control mice and HFD-fed mice receiving different EVOO supplementation*

Fatty acid	Fatty acid composition (g/100 g FAME)							
	Groups							
	Control diet				HFD			
	Saline	EVOO I	EVOO II	EVOO III	Saline	EVOO I	EVOO II	EVOO III
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
C16:0	34.5 ± 3.2	35.8 ± 3.1	34.9 ± 2.9	34.3 ± 3.4	42.1 ± 3.8	41.2 ± 3.5	40.1 ± 3.7	36.4 ± 2.9
C18:1 ω-9	22.5 ± 2.3	24.5 ± 2.1	24.2 ± 2.4	21.6 ± 2	21.7 ± 1.9	23.8 ± 2.8	25.6 ± 2.3	23.8 ± 2.7
C18:2 ω-6 (LA)	13.5 ± 1.9[g]	11.7 ± 1.7	11.9 ± 1.9	12.4 ± 1.6	11.6 ± 1.8	10.3 ± 1.5	9.85 ± 1.2[a]	11.5 ± 1.1
C18:3 ω-3 (ALA)	1.25 ± 0.07[e,f,g]	1.21 ± 0.1[e,f,g]	1.24 ± 0.09[e,f,g]	1.28 ± 0.1[e,f,g]	0.85 ± 0.05[a,b,c,dg,h]	0.88 ± 0.1[a,b,c,dg,h]	1.05 ± 0.04[a,b,c,d,e,f,h]	1.19 ± 0.05[e,f,g]
C20:4 ω-6 (AA)	11.2 ± 1.3	10.9 ± 1.4	11.7 ± 1.3	11.0 ± 1	11.0 ± 1.5	11.3 ± 1.1	10.8 ± 0.9	10.7 ± 0.8
C20:5 ω-3 (EPA)	2.75 ± 0.2[e,f,g]	2.58 ± 0.3[e,f,g]	2.41 ± 0 .2[e,f,g]	2.64 ± 0.3[e,f,g]	0.98 ± 0.1[a,b,c,d,f,g,h]	1.25 ± 0.05[a,b,c,d,e,g,h]	1.82 ± 0.1[a,b,c,d,e,f,h]	2.46 ± 0.3[e,f,g]
C22:5 ω-6 (DPAω-6)	0.74 ± 0.1[e,f,g]	0.72 ± 0.05[e,f]	0.70 ± 0.05[e,f]	0.73 ± 0.1[e,f]	0.40 ± 0.04[a,b,c,d,g,h]	0.45 ± 0.03[a,b,c,d,g,h]	0.62 ± 0.05[a,e,f]	0.70 ± 0.05[e,f]
C22:5 v-6 (DPAω-3)	0.63 ± 0.1[e,f,g]	0.60 ± 0.05[e,f]	0.62 ± 0.01[e,f]	0.63 ± 0.05[e,f]	0.35 ± 0.05[a,b,c,d,g,h]	0.47 ± 0.1[a,b,c,d,g,h]	0.56 ± 0.04[a,e,f]	0.55 ± 0.1[e,f]
C22:6 ω-3 (DHA)	5.05 ± 0.3[e,f,g]	4.78 ± 0.4[e,f,g]	4.84 ± 0.3[e,f,g]	5.00 ± 0.5[e,f,g]	2.25 ± 0.05[a,b,c,d,g,h]	2.79 ± 0.04[a,b,c,d,g,h]	3.48 ± 0.2[a,b,c,d,e,f]	4.15 ± 0.5[e,f]
Total SFA	39.1 ± 3.1	38.7 ± 3.4	37.6 ± 3.5	38.9 ± 3.5	46.9 ± 4.1	44.5 ± 3.8	42.2 ± 3.6	40.1 ± 3
Total MUFA	25.1 ± 2.2	27.8 ± 2.5	27.1 ± 2.5	26.2 ± 2.6	24.9 ± 2.2	27.3 ± 2.5	28.9 ± 2.5	27.5 ± 2.6
Total PUFA	35.8 ± 3.8[e,f,g]	33.5 ± 2.9[e,f,g]	35.3 ± 3.3[e,f,g]	34.9 ± 3.1[e,f,g]	28.2 ± 2.5[a,b,c,d]	28.2 ± 2.3[a,b,c,d]	28.9 ± 2[a,b,c,d]	32.4 ± 2.4
Total LCPUFA	20.6 ± 1.9[e,f]	19.9 ± 2[e,f]	21.0 ± 1.7[e,f]	20.2 ± 1.6[e,f]	15.2 ± 0.6[a,b,c,d]	16.5 ± 1.7[a,b,c,d]	17.4 ± 1.5	18.9 ± 1.6[e]
Total ω-6 LCPUFA	12.0 ± 1.1	11.8 ± 0.9	12.9 ± 1.4	11.9 ± 0.7	11.6 ± 0.8	11.9 ± 1.1	11.6 ± 1.1	11.6 ± 0.8
Total ω-3 LCPUFA	8.60 ± 0.7[e,f,g]	8.10 ± 0.7[e,f,g]	8.30 ± 0.4[e,f,g]	8.30 ± 0.6[e,f,g]	3.60 ± 0.3[a,b,c,d,g,h]	4.60 ± 0.3[a,b,c,d,g,h]	5.80 ± 0.3[a,b,c,d,e,f,h]	7.30 ± 0.5[e,f,g]
ω-6/ω-3 LCPUFA ratio	1.40 ± 0.1[e,f,g]	1.50 ± 0.05[e,f,g]	1.55 ± 0.1[e,f,g]	1.43 ± 0.1[e,f,g]	3.22 ± 0.4[a,b,c,d,g,h]	2.59 ± 0.1[a,b,c,d,g,h]	2.00 ± 0.04[a,e]	1.59 ± 0.2[e,f,g]

AA, arachidonic acid; ALA, α-linolenic acid; ANOVA, analysis of variance; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; EVOO, extra virgin olive oil; FAME, fatty acid methyl ester; HFD, high-fat diet; LA, linolenic acid; LCPUFA, polyunsaturated fatty acid; long-chain MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid

* Values are expressed as g fatty acid/100 g FAME and represent the mean ± SEM for N = 10 to 12 mice per experimental group. Significant differences between the groups are indicated by the letter identifying each group (two-way ANOVA and Bonferroni posttest; $P < 0.05$). Identification of saturated and unsaturated fatty acids and their relationships are shown in Table 2.

to CD levels with EVOO III. For ω-6 DPA, higher levels found for HFD + saline were reduced after EVOO supplementation, without reaching CD values. Additionally, ALA, AA, ω-3 DPA, DHA, total PUFA, total LCPUFA, total ω-6 LCPUFA, and total ω-3 LCPUFA levels for HFD were lower than for CD, and restored with EVOO III. There were no significant changes among the eight groups studied for OA, LA, total MUFA, or the ω-6/ω-3 LCPUFA ratio.

Table 5
Fatty acid composition of adipose tissue phospholipids obtained from control mice and HFD-fed mice receiving different EVOO supplementation*

Fatty acid	Fatty acid composition (g/100 g FAME)							
	Groups							
	Control diet				HFD			
	Saline	EVOO I	EVOO II	EVOO III	Saline	EVOO I	EVOO II	EVOO III
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
C16:0	37.1 ± 2.9[e,f,g,h]	35.6 ± 2.5[e,f,g,h]	39.8 ± 2.6[e,f,g,h]	37.4 ± 3.1[e,f,g,h]	54.4 ± 2.3[a,b,c,d,g,h]	52.3 ± 4[a,b,c,d,g,h]	47.3 ± 3.9[a,b,c,d,e,f]	43.3 ± 3.6[a,b,c,d,e,f]
C18:1 ω-9	27.8 ± 2.5	31.5 ± 2.8	31.8 ± 2.7	31.2 ± 2.9	22.4 ± 1.8	25.9 ± 2.6	27.8 ± 2.8	29.6 ± 2.4
C18:2 ω-6 (LA)	9.86 ± 1.2	9.55 ± 1.1	7.84 ± 1.5	8.95 ± 0.9	8.12 ± 1.3	7.95 ± 1.4	8.25 ± 0.8	8.01 ± 1.1
C18:3 ω-3 (ALA)	1.79 ± 0.4[e,f,g]	1.85 ± 0.5[e,f,g]	1.95 ± 0.4[e,f,g]	1.83 ± 0.6[e,f,g]	0.51 ± 0.05[a,b,c,d,f,g,h]	0.70 ± 0.1[a,b,c,d,e,g,h]	0.95 ± 0.05[a,b,c,d,e,f,h]	1.64 ± 0.3[e,f,g]
C20:4 ω-6 (AA)	8.81 ± 0.4[e,f,g,h]	8.96 ± 0.3[e,f,g,h]	7.90 ± 0.5[e,f,g,h]	8.51 ± 0.6[e,f,g,h]	5.18 ± 0.2[a,b,c,d,g,h]	5.47 ± 0.3[a,b,c,d,g,h]	6.42 ± 0.4[a,b,c,d,e,f]	6.98 ± 0.5[a,b,c,d,e,f]
C20:5 ω-3 (EPA)	1.05 ± 0.07[e,f,g]	1.11 ± 0.05[e,f,g]	0.99 ± 0.1[e,f,g]	1.16 ± 0.1[e,f,g]	0.32 ± 0.03[a,b,c,d,f,g,h]	0.45 ± 0.02[a,b,c,d,e,f,h]	0.88 ± 0.05[a,b,c,d,e,f,h]	0.97 ± 0.1[e,f,g]
C22:5 ω-6 (DPAω-6)	0.10 ± 0.01[e,f,g]	0.09 ± 0.02[e,f,g]	0.11 ± 0.1[e,f,g]	0.10 ± 0.05[e,f,g]	0.05 ± 0.02[a,b,c,d,h]	0.06 ± 0.01[a,b,c,d,h]	0.08 ± 0.02[a,b,c,d,h]	0.10 ± 0.05[e,f,g]
C22:5 v-6 (DPAω-3)	0.09 ± 0.02[e,f]	0.08 ± 0.01[e,f]	0.07 ± 0.02[e,f]	0.08 ± 0.02[e,f]	0.03 ± 0.01[a,b,c,d,g,h]	0.05 ± 0.01[a,b,c,d,h]	0.07 ± 0.02[e]	0.09 ± 0.02[e,f]
C22:6 ω-3 (DHA)	2.58 ± 0.2[e,f,g]	2.71 ± 0.1[e,f,g]	2.48 ± 0.2[e,f,g]	2.85 ± 0.1[e,f,g]	0.82 ± 0.1[a,b,c,d,g,h]	1.15 ± 0.05[a,b,c,d,g,h]	1.51 ± 0.1[a,b,c,d,e,f,g]	2.18 ± 0.2[e,f,g]
Total SFA	42.5 ± 3.4[e,f]	40.6 ± 3.3[e,f]	43.4 ± 3.8[e,f]	41.9 ± 4[e,f]	58.1 ± 5.5[a,b,c,d,h]	54.7 ± 5.1[a,b,c,d]	50.7 ± 4.5	46.3 ± 4.7[e,f]
Total MUFA	31.8 ± 2.9	33.6 ± 3.3	34.5 ± 3.2	33.6 ± 2.8	26.7 ± 2.2	28.9 ± 2.0	30.3 ± 2.7	32.7 ± 2.8
Total PUFA	25.7 ± 2.4[e,f,g]	25.8 ± 2.7[e,f,g]	22.1 ± 1.9[e,f,g]	24.5 ± 2.1[e,f,g]	15.2 ± 1.7[a,b,c,d,h]	16.4 ± 1.2[a,b,c,d,h]	19 ± 1.6[a,b,c,d]	21.0 ± 1.8[e,f]
Total LCPUFA	12.9 ± 1.1[e,f,g]	13.3 ± 1.2[e,f,g]	11.7 ± 0.8[e,f,g]	13 ± 1.3[e,f,g]	6.50 ± 0.6[a,b,c,d,h]	7.29 ± 1[a,b,c,d,h]	9.10 ± 1.6	10.5 ± 1[e,f]
Total ω-6 LCPUFA	9.10 ± 0.8[e,f,g,h]	9.11 ± 0.6[e,f,g,h]	8.05 ± 0.7[e,f,g,h]	8.74 ± 0.7[e,f,g,h]	5.30 ± 0.4[a,b,c,d,h]	5.60 ± 0.6[a,b,c,d,h]	6.68 ± 1.4[a,b,c,d,h]	7.20 ± 0.7[a,b,c,d,e,f]
Total ω-3 LCPUFA	3.80 ± 0.3[e,f,g]	4.19 ± 0.4[e,f,g]	3.65 ± 0.3[e,f,g]	4.26 ± 0.5[e,f,g]	1.20 ± 0.1[a,b,c,d,g]	1.69 ± 0.2[a,b,c,d,g]	2.42 ± 0.6[a,b,c,d,e,f]	3.30 ± 0.4[e,f,g]
ω-6/ω-3 LCPUFA ratio	2.40 ± 0.2[e,f]	2.17 ± 0.3[e,f]	2.21 ± 0.2[e,f]	2.10 ± 0.3[e,f]	4.42 ± 0.5[a,b,c,d,g,h]	3.31 ± 0.4[a,b,c,d,g,h]	2.76 ± 0.3[e,f]	2.20 ± 0.2[e,f]

AA, arachidonic acid; ALA, α-linolenic acid; ANOVA, analysis of variance; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; EVOO, extra virgin olive oil; FAME, fatty acid methyl ester; HFD, high-fat diet; LA, linolenic acid; LCPUFA, polyunsaturated fatty acid; long-chain MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid

* Values are expressed as g fatty acid/100 g FAME and represent the mean ± SEM for N = 10 to 12 mice per experimental group. Significant differences between the groups are indicated by the letter identifying each group (two-way ANOVA and Bonferroni posttest; $P < 0.05$). Identification of saturated and unsaturated fatty acids and their relationships are shown in Table 2.

Table 6
Fatty acid composition of brain phospholipids obtained from control mice and HFD-fed mice receiving different EVOO supplementation*

Fatty acid	Fatty acid composition (g/100 g FAME)							
	Groups							
	Control diet				HFD			
	Saline	EVOO I	EVOO II	EVOO III	Saline	EVOO I	EVOO II	EVOO III
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
C16:0	$37.9 \pm 3.3^{e,f,g}$	$36.5 \pm 3^{e,f,g}$	$35.9 \pm 2.9^{e,f,g,h}$	$35.7 \pm 2.8^{e,f,g,h}$	$47.1 \pm 3.6^{a,b,c,d}$	$46.4 \pm 4.1^{a,b,c,d}$	$45.8 \pm 3.8^{a,b,c,d}$	$42.5 \pm 3.2^{c,d}$
C18:1 ω-9	22.3 ± 1.8	23.5 ± 2.1	24.2 ± 2.4^{e}	23.4 ± 2.6	19.1 ± 1.9^{c}	20.3 ± 1.6	21.5 ± 1.9	22.1 ± 1.7
C18:2 ω-6 (LA)	3.78 ± 0.4	4.02 ± 0.3	3.92 ± 0.2	4.05 ± 0.3	3.89 ± 0.4	3.95 ± 0.3	4.01 ± 0.2	3.95 ± 0.4
C18:3 ω-3 (ALA)	$1.18 \pm 0.3^{e,f}$	$1.15 \pm 0.2^{e,f}$	$1.19 \pm 0.2^{e,f}$	$1.12 \pm 0.2^{e,f}$	$0.61 \pm 0.05^{a,b,c,d,f,g,h}$	$0.75 \pm 0.04^{a,b,c,d,e,g,h}$	$0.84 \pm 0.06^{a,b,c,d,e,f,h}$	$0.96 \pm 0.05^{e,f,g}$
C20:4 ω-6 (AA)	$16.4 \pm 1.4^{e,f,g}$	$16.1 \pm 1.1^{e,f,g}$	$15.8 \pm 1.7^{e,f,g}$	$16.8 \pm 1.8^{e,f,g}$	$11.5 \pm 0.8^{a,b,c,d,h}$	$12.5 \pm 1.4^{a,b,c,d,h}$	$12.8 \pm 1.8^{a,b,c,d,h}$	$14.5 \pm 1.8^{e,f,g}$
C20:5 ω-3 (EPA)	$0.48 \pm 0.04^{e,f,g}$	$0.50 \pm 0.03^{e,f,g,h}$	$0.52 \pm 0.04^{e,f,g,h}$	$0.56 \pm 0.05^{e,f,g,h}$	$0.22 \pm 0.03^{a,b,c,d,f,g,h}$	$0.31 \pm 0.02^{a,b,c,d,e,g,h}$	$0.41 \pm 0.03^{b,c,d}$	$0.44 \pm 0.02^{b,c,d,e,f}$
C22:5 ω-6 (DPAω-6)	$0.24 \pm 0.02^{e,f,g,h}$	$0.26 \pm 0.01^{e,f,g,h}$	$0.30 \pm 0.03^{e,f,g,h}$	$0.29 \pm 0.04^{e,f,g,h}$	$0.91 \pm 0.04^{a,b,c,d,f,g,h}$	$0.78 \pm 0.04^{a,b,c,d,e,h}$	$0.70 \pm 0.04^{a,b,c,d,e,h}$	$0.60 \pm 0.03^{a,b,c,d,e,f,g}$
C22:5 v-6 (DPAω-3)	$0.51 \pm 0.03^{e,f,g}$	$0.46 \pm 0.02^{e,f,g}$	$0.55 \pm 0.04^{e,f,g}$	$0.52 \pm 0.05^{e,f,g}$	$0.24 \pm 0.03^{a,b,c,d,f,g,h}$	$0.33 \pm 0.02^{a,b,c,d,e,g,h}$	$0.41 \pm 0.03^{a,b,c,d,e,f,h}$	$0.49 \pm 0.04^{e,f,g}$
C22:6 ω-3 (DHA)	$10.5 \pm 1.1^{e,f,g}$	$10.3 \pm 0.9^{e,f,g}$	$10.8 \pm 0.8^{e,f,g}$	$10.3 \pm 1^{e,f,g}$	$7.05 \pm 0.4^{a,b,c,d,h}$	$7.28 \pm 0.5^{a,b,c,d,h}$	$7.82 \pm 0.4^{a,b,c,d,h}$	$9.92 \pm 0.7^{e,f,g}$
Total SFA	$41.5 \pm 3.4^{e,f}$	$40.9 \pm 3.8^{e,f,g}$	$40.5 \pm 3.6^{e,f,g}$	$39.8 \pm 3.4^{e,f,g}$	$51.4 \pm 3.8^{a,b,c,d,h}$	$50.2 \pm 3.7^{a,b,c,d,h}$	$48.6 \pm 3.3^{b,c,d}$	44.2 ± 3.5
Total MUFA	24.2 ± 2.2	25.6 ± 2.8	26.3 ± 2.9	25.7 ± 2.6	21.9 ± 2.3	22.4 ± 1.7	23.7 ± 2.1	24.3 ± 1.9
Total PUFA	$34.3 \pm 3.1^{e,f,g}$	$33.5 \pm 2.9^{e,f,g}$	$33.2 \pm 3.4^{e,f,g}$	$34.5 \pm 3.2^{e,f,g}$	$26.7 \pm 2.4^{a,b,c,d}$	$27.4 \pm 2.6^{a,b,c,d}$	$27.7 \pm 2.5^{a,b,c,d}$	31.5 ± 3
Total LCPUFA	$28.6 \pm 2.2^{e,f,g}$	$27.9 \pm 2.3^{e,f,g}$	$27.7 \pm 2.5^{e,f,g}$	$28.8 \pm 2.4^{e,f,g}$	$20.8 \pm 1.9^{a,b,c,d,h}$	$22.2 \pm 2^{a,b,c,d}$	$22.4 \pm 1.7^{a,b,c,d}$	26.1 ± 2.1^{e}
Total ω-6 LCPUFA	$16.7 \pm 1.3^{e,f,g}$	$16.5 \pm 1.2^{e,f,g}$	$15.6 \pm 1.4^{e,f,g}$	$17.2 \pm 1.4^{e,f,g}$	$12.5 \pm 1.1^{a,b,c,d}$	$13.4 \pm 1.3^{a,b,c,d}$	$13.7 \pm 1.5^{a,b,c,d}$	15.2 ± 1.4^{e}
Total ω-3 LCPUFA	$11.9 \pm 0.8^{e,f,g}$	$11.4 \pm 0.7^{e,f,g}$	$12.1 \pm 0.9^{e,f,g}$	$11.6 \pm 0.7^{e,f,g}$	$8.30 \pm 0.6^{a,b,c,d,h}$	$8.00 \pm 0.5^{a,b,c,d,h}$	$8.70 \pm 0.6^{a,b,c,d,h}$	$10.9 \pm 0.6^{e,f,g}$
ω-6/ω-3 LCPUFA ratio	1.40 ± 0.3	1.48 ± 0.4	1.29 ± 0.3	1.48 ± 0.5	1.51 ± 0.4	1.68 ± 0.3	1.57 ± 0.4	1.39 ± 0.3

AA, arachidonic acid; ALA, α-linolenic acid; ANOVA, analysis of variance; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; EVOO, extra virgin olive oil; FAME, fatty acid methyl ester; HFD, high-fat diet; LA, linolenic acid; LCPUFA, polyunsaturated fatty acid; long-chain MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid

* Values are expressed as g fatty acid/100 g FAME and represent the mean ± SEM for N = 10 to 12 mice per experimental group. Significant differences between the groups are indicated by the letter identifying each group (two-way ANOVA and Bonferroni posttest; $P < 0.05$). Identification of saturated and unsaturated fatty acids and their relationships are shown in Table 2.

Hepatic and plasma oxidative stress-related parameters

Hepatic parameters of oxidative stress in mice from CD and HFD groups are shown in Figures 1 and 2. With the exception of GSSG (Fig. 1B), all parameters were significantly modified by HFD, whereas EVOO supplementation showed no effects within CD groups (Figs. 1A–F and 2A–F). Liver antioxidant parameters GSH (Fig. 1A), total GSH equivalents (Fig. 1C), and GSH/GSSG ratios (Fig. 1D) were significantly reduced in HFD + saline, and progressively increased after EVOO supplementation to reach values comparable to CD + EVOO III. The content of the pro-oxidant parameters protein carbonyls (Fig. 1E), F2-isoprotanes (Fig. 1F), and TBARs (Fig. 2A) were enhanced ($P < 0.05$) after HFD intake, which returned toward values after EVOO III supplementation. Estimation of parameters indicative of oxidative stress revealed that liver protein carbonyls/total GSH (Fig. 2B), F2-isoprostanes/total GSH (Fig. 2C), and TABARs/total GSH (Fig. 2D) ratios showed no differences within CD groups but a significant increase in the HFD groups, the latter effect being diminished by EVOO III supplementation. EVOO caused a significant decrease in hepatic TBARs/total GSH ratio in CD, independent of the antioxidant content (Fig. 2D). In agreement with data obtained in hepatic tissue, HFD significantly increased the oxidative stress status in plasma compared with CD values, as shown by the increased in TBARs levels (Fig. 2E) and diminution in the antioxidant capacity (Fig. 2F), changes that were normalized by EVOO III.

Desaturase activities and mRNA expression

No significant differences were found for either Δ-5 and Δ-6 desaturase activities among CD groups regardless of EVOO supplementation (Fig. 3A, B), showing similar values for both enzymes. In HFD + saline group, activities were sharply reduced, but EVOO supplementation increased them especially with EVOO III, with values being restored to those found in CD. Hepatic mRNA levels for Δ-5 and Δ-6 desaturase in CD with EVOO supplementation groups were comparable; however, HFD caused a significant increase that was normalized after EVOO intake, reaching CD levels after EVOO III supplementation.

SREBP-1 c expression and DNA-binding activity

HFD caused hepatic overexpression of the prolipogenic transcription factor SREBP-1 c (Fig. 3E) and in its DNA binding capacity (Fig. 3F) compared with CD animals, which is associated with the enhanced levels of liver total fat, TGs, and free FAs (Table 1). Both hepatic SREBP-1 c mRNA expression and DNA binding activity were decreased after EVOO supplementation, although CD levels were not attained in any case (Fig. 3E, F).

Activity of antioxidant enzymes

No significant changes were observed in the activities of SOD, CAT, GPX, and GR within CD groups, although a sharp activity decrease was observed after HFD intake (Fig. 4A–D), suggesting

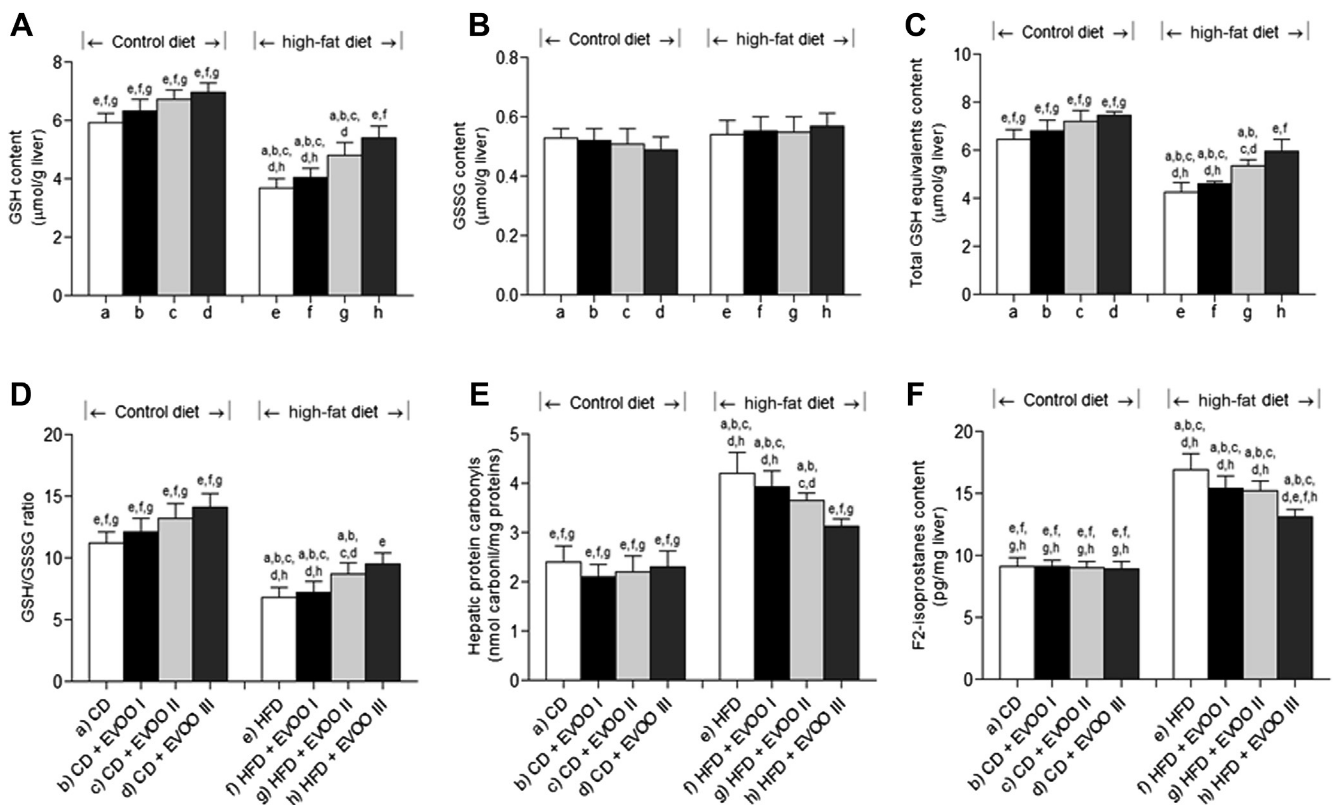


Fig. 1. Oxidative stress-related parameters from control mice and HFD-fed animals receiving different EVOO supplementation. (A) GSH; (B) GSSG; (C) total GSH equivalents (GSH + 2 GSSG); (D) GSH/GSSG ratio; (E) protein carbonyls; (F) F2-isoprostanes. Values represent means ± SEM for 10 to 12 mice per experimental group. Significant differences between the groups are indicated by the letter identifying each group (two-way ANOVA and Bonferroni post-test; $P < 0.05$). Differential EVOO supplementation: EVOO I, 166 mg PP + 215 mf α- TP/kg oil (PP + α-TP = 331 mg/kg oil); EVOO II, 407 mg PP + 290 mg α-TP/kg oil (PP + α-TP = 697 mg/kg); EVOO III, 859 mg PP + 227 mg α-TP/kg oil (PP + α-TP = 1086 mg/kg). ANOVA, analysis of variance; CD, control diet; EVOO, extra virgin olive oil; GSH, glutathione; GSSG, glutathione disulfide; HFD, high-fat diet; PP, polyphenols; α-TP, α-tocopherol.

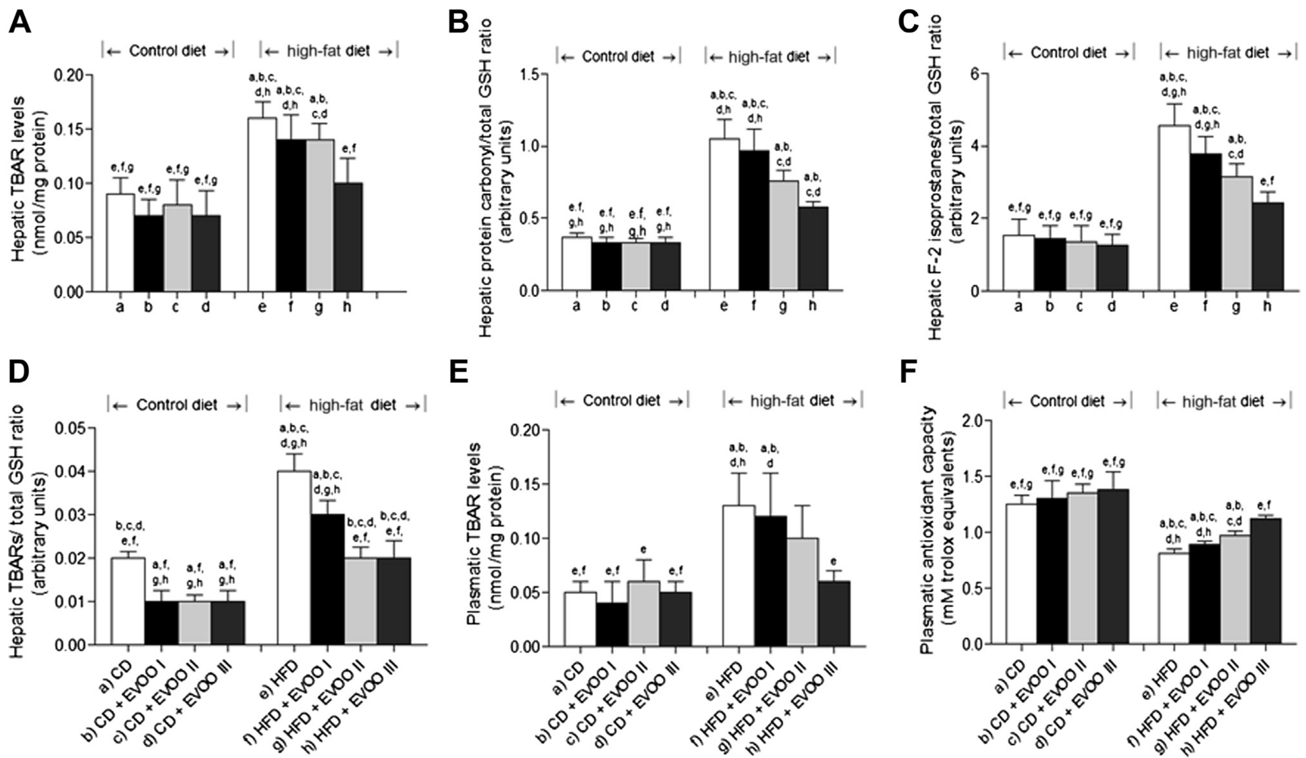


Fig. 2. Oxidative stress-related parameters from control mice and HFD-fed animals receiving different EVOO supplementation. (A) hepatic TBARs; (B) protein carbonyl/GSH ratio; (C) F2-isoprostanes/GSH ratio; (D) hepatic TBARs/total GSH ratio; (E) plasma TBARs; (F) plasma antioxidant capacity. Values represent means ± SEM for 10 to 12 mice per experimental group. Significant differences between the groups are indicated by the letter identifying each group (two-way ANOVA and Bonferroni post-test). For differential EVOO supplementation see legend to Fig. 1. ANOVA, analysis of variance; CD, control diet; EVOO, extra virgin olive oil; GSH, glutathione; HFD, high-fat diet; TBARS, thiobarbituric acid reactants.

that HFD leads to an impaired antioxidant status in the liver. However, EVOO supplementation progressively enhanced the activity of the enzymes in HFD groups to reach CD values after EVOO III supplementation (Fig. 4A–D).

Activity of hepatic lipogenic and lipolytic enzymes

Liver activities of the lipogenic enzymes ACC and FAS and that of the lipolytic enzyme CPT-1 for CD and HFD groups are shown in Figure 4E–G. No enzymatic activity modification was observed within the four CD groups. HFD induced a large increase in ACC and FAS activity and a decrease in that of CPT-1, whereas EVOO supplementation contributes to regularize these changes without attaining CD levels.

Discussion

In agreement with previous studies in experimental animals fed HFDs [38,39], C57 BL/6 J mice fed an HFD providing 60% of energy mainly as saturated fat (6.5 g of palmitic acid/100 g of diet) for 12 wk developed hyperlipidemia and hepatic steatosis in association with liver and plasma oxidative stress enhancement and insulin resistance, without progression into the steatohepatitis phase. HFD-induced liver oxidative stress is evidenced by a significant diminution in the content of total GSH equivalents and the GSH/GSSG ratio, with enhanced levels of the pro-oxidant indicators protein carbonyls, F2-isopostanes, and TBARs, and reduction in the activity of antioxidant enzymes. This redox imbalance is related to the fact that HFD provides excess SFAs, especially, palmitic acid, a condition that triggers mitochondrial FA oxidation and reactive oxygen species production, a process known as lipotoxicity [40–42]. The excess of free SFAs in hepatic tissue would reflect a major imbalance in the redox state of nutritional origin [43,44], which also is observed in obese individuals with hepatic steatosis [45]. As a central pathogenic mechanism, oxidative stress may trigger secondary alterations that are relevant in the development of hepatic steatosis, including the following:

1. ω-3 LCPUFA depletion, a feature that is associated with a higher lipid peroxidation extent of these FAs and/or lower synthesis of ω-3 LCPUFAs due to the reduction in the activity of Δ-5 and Δ-6 desaturase enzymes;
2. Endoplasmic reticulum stress development [40];
3. Insulin resistance.

The prolipogenic state induced in the liver by HFD may be contributed by the following factors:

1. Palmitic acid-dependent upregulation of the mRNA expression and DNA binding of transcription factor SREBP-1 c with consequent ACC and FAS induction, thus promoting de novo FA synthesis [40,41,46]. This mechanism can be favored by the ω-3 LCPUFA depletion observed, considering that ω-3 LCPUFA normally inhibit the proteolytic processing of nascent SREBP-1 c, with reduction of nuclear SREBP-1 c levels [47].
2. The alteration in the hepatic synthesis of ω-3 LCPUFAs and their depletion may be related to the diminution in the activity of Δ-5 and Δ-6 desaturases found, the transcription of

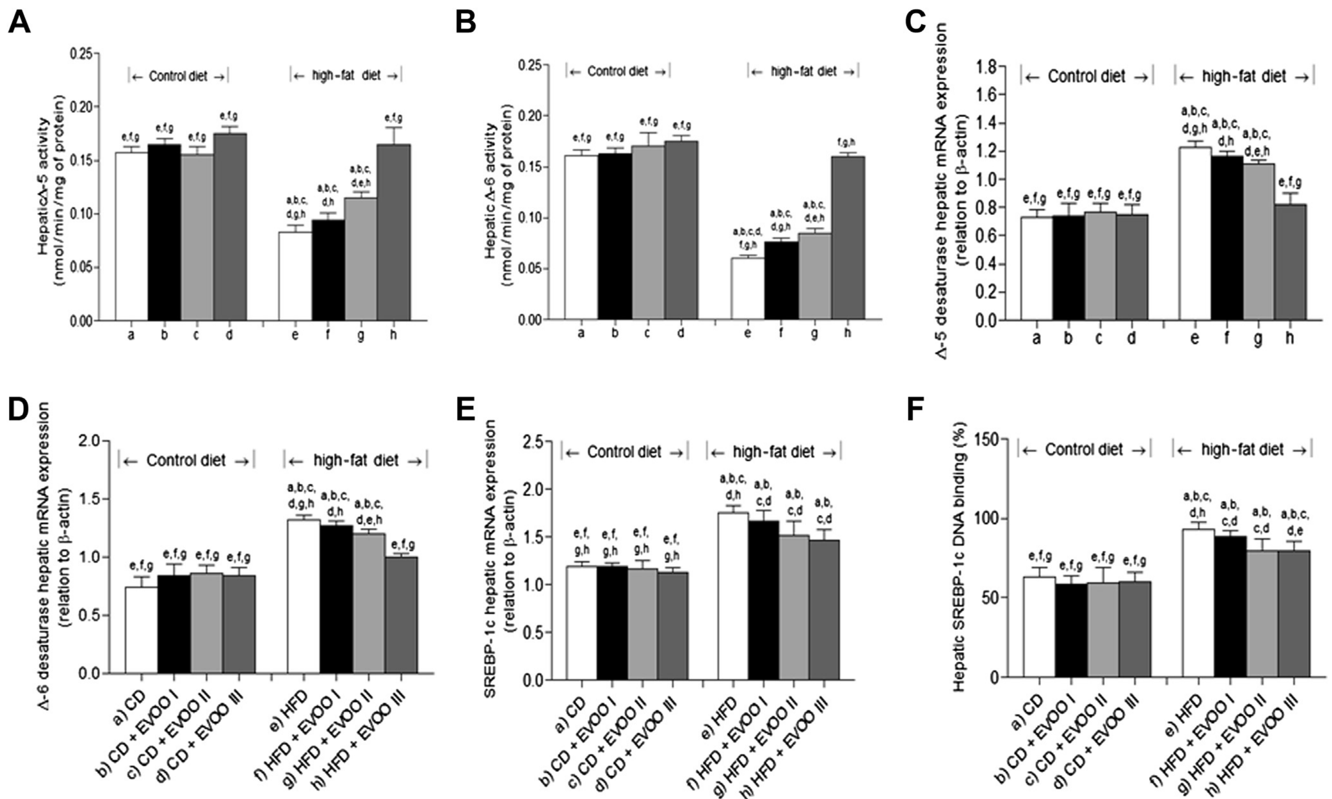


Fig. 3. Hepatic desaturase activities and expression and hepatic SREBP-1 c expression and DNA-binding activity from control mice and HFD-fed animals receiving different EVOO supplementation. (A) Δ-5 desaturase activity; (B) Δ-6 desaturase activity; (C) Δ-5 desaturase mRNA expression; (D) Δ-6 desaturase mRNA expression; (E) SREBP-1 c mRNA expression; (F) SREBP-1 c DNA binding activity. Values represent means ± SEM for 10 to 12 mice per experimental group. Significant differences between the groups are indicated by the letter identifying each group (two-way ANOVA and Bonferroni post-test; $P < 0.05$). For differential EVOO supplementation see legend to Fig. 1. ANOVA, analysis of variance; EVOO, extra virgin olive oil; HFD, high-fat diet.

which may be elevated as a compensatory mechanism. This finding requires further investigation in the insulin resistance model use, considering that insulin has a direct effect on the expression of these enzymes [10]. Loss of desaturase activity is associated with the induction of oxidative stress, which triggers free radical-mediated protein carbonylation that can lead to protein misfolding and proteasomal degradation [48]. Interestingly, increased levels of reactive oxygen species–induced unfolded proteins cause endoplasmic reticulum stress that triggers the proteolytic cleavage of SREBP-1 c [49], a process also stimulated by high saturated fat diets [50], thus reinforcing the lipogenic mechanisms discussed in the first factor.

3. Insulin resistance triggering peripheral lipolysis with enhanced fluxes of FAs and glycerol to the liver to support lipogenesis [43]. These observations establish an interrelationship between oxidative stress, endoplasmic reticulum stress, and insulin resistance leading to hepatic steatosis, which are related to a derangement in the synthesis and availability ω-3 LCPUFA in the liver and extrahepatic tissues [11,12] and SREBP-1 c upregulation.

Dietary supplementation with EVOO III, having the highest content of α-tocopherol and polyphenols among the three assayed oils, produced either normalization or attenuation of the harmful effects of HFD, particularly those preventing oxidative stress or conserving tissue levels and biosynthetic capacity of n-3 LCPUFA. In this regard, EVOO is considered an important dietary component in the prevention and/or treatment of hepatic steatosis [51] that is capable of improving postprandial glucose levels and the response to insulin [52]. These effects are related to its content of OA and antioxidants, bioactive substances that would regulate different cyto-protective mechanisms against an overload of energy, such as a high intake of SFA, fructose, or sucrose [16,53].

In rats fed a prolipogenic diet (deficient in choline and methionine) EVOO prevents hepatic TG accumulation [54], establishing that increasing hepatic antioxidant defenses would be one of the main cytoprotective mechanisms of action of EVOO [16,53,55]. In the present study, only EVOO III was able to prevent hepatic and systemic oxidative stress induced by HFD, with concomitant normalization of the expression and the activity of Δ-5 and Δ-6 desaturases. These effects were not elicited by the other two EVOO types employed, which had a lower content of antioxidants and antioxidant capacity. Therefore, it is suggested that the antioxidant effect generated by the dietary supplementation with the antioxidant-rich EVOO III may positively influence the intracellular redox state [16], which is reflected in the normalization of the activity of hepatic antioxidant enzymes.

Among the polyphenols present in EVOO, hydroxytyrosol is probably the molecule having the greatest ability to regulate the intracellular redox state by increasing both the expression and the activity of antioxidant enzymes, particularly SOD, CAT, GPX, and GR [56,57]. Additionally, hydroxytyrosol modulates the expression of genes linked to the metabolism of xenobiotics in a positive way through activation of transcription factor Nrf2 [58].

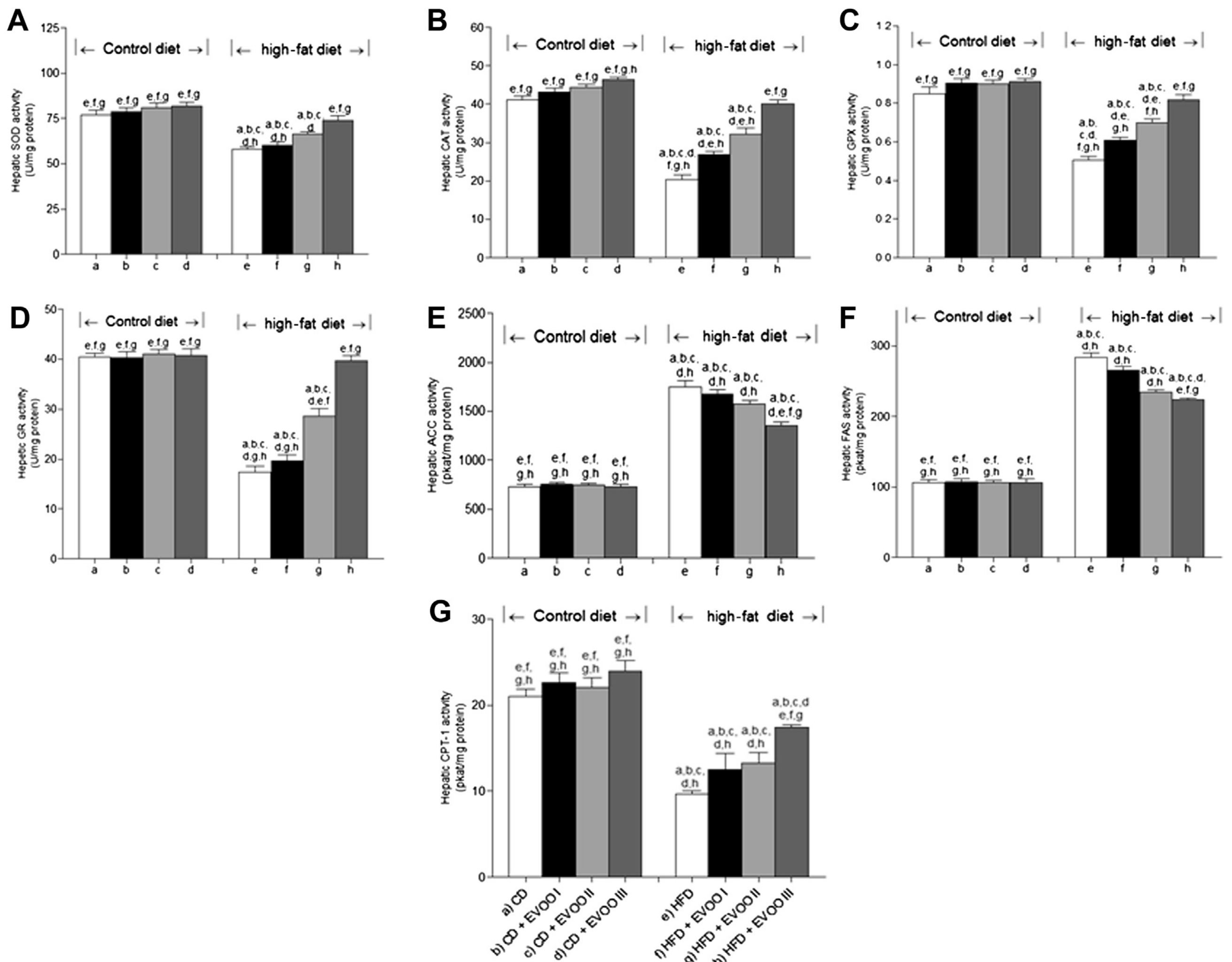


Fig. 4. Hepatic activity of antioxidant enzymes and lipolytic and lipogenic enzymes from control mice and HFD-fed animals receiving different EVOO supplementation. (A) SOD; (B) CAT; (C) GPX; (D) GR; (E) ACC; (F) FAS; (G) CPT-1. Values represent means ± SEM for 10 to 12 mice per experimental group. Significant differences between the groups are indicated by the letter identifying each group (two-way ANOVA and Bonferroni post-test; $P < 0.05$). For differential EVOO supplementation see legend to Fig. 1. ACC, acetyl CoA carboxylase; ANOVA, analysis of variance; EVOO, extra virgin olive oil; FAS, fatty acid synthase; HFD, high-fat diet; CAT, catalase; CPT, carnitine-palmitoyl transferase; GPX, glutathione peroxidase; GR, glutathione reductase; SOD, superoxide dismutase.

HFD generated an important reduction of ω-3 LCPUFA tissue levels, which was normalized by dietary supplementation with EVOO III in various tissues studied. In this regard, HFD and the subsequent induced oxidative stress directly affected hepatic synthesis and accretion of ω-3 LCPUFA in extrahepatic tissues such as erythrocytes, heart, adipose tissue, and brain [12], effects that were prevented by antioxidant-rich EVOO III. In this context, the hepatic synthesis of ω-3 LCPUFAs and their consequent storage (e.g., in adipose tissue) and active transport of these FAs to other tissues (e.g., brain) allows maintaining physiological levels of ω-3 LCPUFA [59]. In brain, HFD produced a significant 33% reduction in the levels of DHA compared with CD, which was prevented by EVOO III. Therefore, by preventing oxidative stress generated by HFD it was possible to maintain hepatic ω-3 LCPUFA synthesis, the flow of these FAs to systemic circulation, and their deposition in other tissues, particularly DHA into the brain. In this regard, brain ω-3 LCPUFA metabolism is quite complex [60].

Rats fed diets with very low DHA content increased up to 100-fold the brain DHA synthesis from ALA [61], whereas feeding with diets rich in ALA showed a significant increase in brain DHA levels but not EPA levels [29]. The low brain content of EPA may be due to a limited transport from the systemic circulation, primarily from hepatic origin, and a marginal brain synthesis [62]. Brain EPA is quickly β-oxidized, elongated, and desaturated to ω-3 DPA and then to DHA, but is not stored in brain phospholipids [62].

A remarkable aspect observed in all the studied tissues is the reduction in ω-3 LCPUFA levels by the HFD, particularly EPA and DHA, which may be ascribed to a reduced synthesis from the precursor due to low desaturase activity and/or increased metabolic utilization. This has been particularly shown in hepatic and brain tissues [12,63]. Cellular stress, particularly oxidative stress, increases the synthesis of eicosanoids from EPA and docosanoids from DHA as a way to prevent cell damage [63]. EVOO III, which prevents oxidative stress induced by HFD, is able to maintain tissue levels of ω-3 LCPUFA in different tissues, which is important in the regulation of energy metabolism and whole-cell physiology [64]. In this context, ω-3 LCPUFAs activate peroxisome-proliferator receptor-α (PPAR-α) leading to the

stimulation of mitochondrial FA oxidation [65], which constitutes a major aspect related to the antilipogenic effects of ω-3 LCPUFA [66]. In the tissues studied, especially in the liver, HFD induced a significant increase in the ω-6/ω-3 ratios in addition to the reduction in the levels of ω-3 LCPUFAs, a LCPUFA unbalance directly related to the development of hepatic steatosis [43]. EVOO III was the supplement able to preserve ω-3 LCPUFA tissue levels attenuating the hepatic prolipogenic state induced by HFD.

Conclusion

Dietary supplementation with EVOO rich in antioxidants (α-tocopherol and polyphenols; EVOO III) mitigated the deleterious effects induced by HFD in mice in association with reduction in the oxidative stress status, maintenance of the synthesis of ω-3 LCPUFA, and prevention of tissue depletion of these FAs. These effects were not observed with the other EVOO types (EVOO I and EVOO II) containing lower levels of antioxidants, thus establishing a threshold for the antioxidant content of EVOOs to attain beneficial effects. Data presented also demonstrate the importance of dietary interventions that consider supplementation with EVOO, particularly its antioxidant potential [15,16,53]. Recently, it has been shown that administration of low doses of ω-3 LCPUFA (EPA + DHA) and EVOO rich in antioxidants prevent hepatic steatosis in mice fed HFD [67], an observation that reinforces the importance of dietary interventions addressing oxidative stress prevention and ω-3 LCPUFA tissue-level preservation [12,38,43].

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.nut.2016.04.006.

References

[1] Janssen CI, Kiliaan AJ. Long-chain polyunsaturated fatty acids from genesis to senescence: the influence of LCPUFA on neural development, aging, and neurodegeneration. Prog Lipid Res 2014;53:1–17.
[2] Maehre HK, Jensen IJ, Elvevoll EO, Eilertsen KE. ω-3 Fatty acids and cardiovascular diseases: effects, mechanisms and dietary relevance. Int J Mol Sci 2015;16:22636–61.
[3] Doi M, Nosaka K, Miyoshi T, Iwamoto M, Kajiya M, Okawa K, et al. Early eicosapentaenoic acid treatment after percutaneous coronary intervention reduces acute inflammatory responses and ventricular arrhythmias in patients with acute myocardial infarction: a randomized, controlled study. Int J Cardiol 2014;176:577–82.
[4] Mozaffarian D, Wu JH. Fatty acids and cardiovascular health: are effects of EPA and DHA shared or complementary? J Nutr 2012;142:614S–25S.
[5] Lee LK, Bryant KJ, Bouveret R, Lei PW, Duff AP, Harrop SJ, et al. Selective inhibition of human group IIA-secreted phospholipase A2 signaling reveals arachidonic acid metabolism is associated with colocalization of hGIIA to vimentin in rheumatoid synoviocytes. J Biol Chem 2013;288:15269–79.
[6] van Goor SA, Dijck-Brouwer DA, Erwich JJ, Schaafsma A, Hadders-Algra M. The influence of supplemental docosahexaenoic and arachidonic acids during pregnancy and lactation on neurodevelopment at eighteen months. Prostaglandins Leukot Essent Fatty Acids 2011;84:139–46.
[7] Fats and fatty acids in human nutrition. Report of an expert consultation. FAO Food Nutr Pap 2010;91:1–166.
[8] Hansen A, Wiese H, Boelsche A, Haggard M, Adam D, Davis H. Role of linolenic acid in infant nutrition: clinical and chemical study of 428 infants fed on milk mixtures varying in kind and amount of fat. Pediatrics 1963;31:171–92.
[9] Nakamura MT, Nara TY. Structure, function, and dietary regulation of delta6, delta5, and delta9 desaturases. Annu Rev Nutr 2004;24:345–76.
[10] Das UN. A defect in the activity of Delta6 and Delta5 desaturases may be a factor predisposing to the development of insulin resistance syndrome. Prostaglandins Leukot Essent Fatty Acids 2005;72:343–50.
[11] Araya J, Rodrigo R, Pettinelli P, Araya AV, Poniachik J, Videla LA. Decreased liver fatty acid delta-6 and delta-5 desaturase activity in obese patients. Obesity 2010;18:1460–3.
[12] Valenzuela R, Barrera C, Espinosa A, Llanos P, Orellana P, Videla LA. Reduction in the desaturation capacity of the liver in mice subjected to high fat diet: relation to LCPUFA depletion in liver and extrahepatic tissues. Prostaglandins Leukot Essent Fatty Acids 2015;98:7–14.
[13] Toledo E, Salas-Salvadó J, Donat-Vargas C, Buil-Cosiales P, Estruch R, Ros E, et al. Mediterranean diet and invasive breast cancer risk among women at high cardiovascular risk in the PREDIMED trial: a randomized clinical trial. JAMA Intern Med 2015;14:1–9.
[14] Qosa H, Mohamed LA, Batarseh YS, Alqahtani S, Ibrahim B, LeVine H 3rd, et al. Extra-virgin olive oil attenuates amyloid-β and tau pathologies in the brains of TgSwDI mice. J Nutr Biochem 2015;26:1479–90.
[15] Cicerale S, Conlan XA, Barnett NW, Sinclair AJ, Keast RS. Influence of heat on biological activity and concentration of oleocanthal—a natural anti-inflammatory agent in virgin olive oil. J Agric Food Chem 2009;57:1326–30.
[16] Cicerale S, Lucas LJ, Keast RS. Antimicrobial, antioxidant and anti-inflammatory phenolic activities in extra virgin olive oil. Curr Opin Biotechnol 2012;23:129–35.
[17] Scoditti E, Capurso C, Capurso A, Massaro M. Vascular effects of the Mediterranean diet-part II: role of omega-3 fatty acids and olive oil polyphenols. Vascul Pharmacol 2014;63:127–34.
[18] Storniolo CE, Roselló-Catafau J, Pintó X, Mitjavila MT, Moreno JJ. Polyphenol fraction of extra virgin olive oil protects against endothelial dysfunction induced by high glucose and free fatty acids through modulation of nitric oxide and endothelin-1. Redox Biol 2014;2:971–7.
[19] Carrasco-Pancorbo A, Cerretani L, Bendini A, Segura-Carretero A, Del Carlo M, Gallina-Toschi T, et al. Evaluation of the antioxidant capacity on individual phenolic compounds in virgin olive oil. J Agric Food Chem 2005;53:8918–25.
[20] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
[21] Mateos R, Espartero JL, Trujillo M, Ríos JJ, León Camacho M, Alcudia F, et al. Determination of phenols, flavones, and lignans in virgin olive oils by solid-phase extraction and high-performance liquid chromatography with diode array ultraviolet detection. J Agric Food Chem 2001;49:2185–92.
[22] Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911–7.
[23] Ruiz-Gutierrez V, Cert A, Rios JJ. Determination of phospholipid fatty acid and triacylglycerol composition of rat caecal mucosa. J Chromatogr 1992;575:1–6.
[24] American Oil Chemists' Society. Official methods and recommended practices of the American Oil Chemists' Society. Champaign, IL: American Oil Chemists' Society; 1993.
[25] Prior RL, Hoang H, Gu L, Wu X, Bacchiocca M, Howard L, et al. Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC(FL))) of plasma and other biological and food samples. J Agric Food Chem 2003;51:3273–9.
[26] Morrison WR, Smith LM. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride—methanol. J Lipid Res 1964;5:600–8.
[27] Rahman I, Kode A, Biswas SK. Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. Nat Protoc 2006;1:3159–65.
[28] Su HM, Brenna JT. Simultaneous measurement of desaturase activities using stable isotope tracers or a nontracer method. Anal Biochem 1998;261:43–50.
[29] Valenzuela R, Barrera C, González-Astorga M, Sanhueza J, Valenzuela A. Alpha linolenic acid from Rosa canina, sacha inchi and chia oils may increase ALA accretion and its conversion into n-3 LCPUFA in diverse tissues of the rat. Food Funct 2014;5:1564–72.
[30] Chow CK, Reddy K, Tappel AL. Effect of dietary vitamin E on the activities of the glutathione peroxidase system in rat tissues. J Nutr 1973;103:618–24.
[31] Lúck H. In: Bergmeyer HU, editor. Methods of enzymatic assays. New York, NY: Academic Press; 1965. p. 885–94.
[32] Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967;70:158–69.
[33] Bergmeyer HU, Horn HD. Gluthathione reductase. In: Bergmeyer HU, editor. Methods of enzymatic assays. New York, NY: Academic Press; 1965. p. 875–9.
[34] Zimmermann R, Haemmerle G, Wagner EM, Strauss JG, Kratky D, Zechner R. Decreased fatty acid esterification compensates for the reduced lipolytic activity in hormone-sensitive lipase-deficient white adipose tissue. J Lipid Res 2003;44:2089–99.
[35] Cohen AM, Briller S, Shafrir E. Effect of long term sucrose feeding on the activity of some enzymes regulating glycolysis, lipogenesis and gluconeogenesis in rat liver and adipose tissue. Biochim Biophys Acta 1972;279:129–38.
[36] Halestrap AP, Denton RM. Insulin and the regulation of adipose tissue acetyl-coenzyme A carboxylase. Biochem J 1973;132:509–17.
[37] Karlic H, Lohninger S, Koeck T, Lohninger A. Dietary l-carnitine stimulates carnitine acyltransferases in the liver of aged rats. J Histochem Cytochem 2002;50:205–12.

[38] Valenzuela R, Espinosa A, González-Mañán D, D'Espessailles A, Fernández V, Videla LA, et al. N-3 long-chain polyunsaturated fatty acid supplementation significantly reduces liver oxidative stress in high fat induced steatosis. PLoS One 2012;7:E46400.
[39] Wiedemann MS, Wueest S, Item F, Schoenle EJ, Konrad D. Adipose tissue inflammation contributes to short-term high-fat diet-induced hepatic insulin resistance. Am J Physiol Endocrinol Metab 2013;305:E388–95.
[40] Nissar AU, Sharma L, Tasduq SA. Palmitic acid induced lipotoxicity is associated with altered lipid metabolism, enhanced CYP450 2 E1 and intracellular calcium mediated ER stress in human hepatoma cells. Toxicol Res 2015;4:1344–58.
[41] Win S, Than TA, Le BH, García-Ruiz C, Fernandez-Checa JC, Kaplowitz N. Sab dependence of JNK mediated inhibition of mitochondrial respiration in palmitic acid induced hepatocyte lipotoxicity. J Hepatol 2015;62:1367–74.
[42] Satapati S, Sunny NE, Kucejova B, Fu X, He TT, Méndez-Lucas A, et al. Elevated TCA cycle function in the pathology of diet-induced hepatic insulin resistance and fatty liver. J Lipid Res 2012;53:1080–92.
[43] Valenzuela R, Videla LA. The importance of the long-chain polyunsaturated fatty acid n-6/n-3 ratio in development of non-alcoholic fatty liver associated with obesity. Food Funct 2011;2:644–8.
[44] Sies H, Stahl W, Sevanian A. Nutritional, dietary and postprandial oxidative stress. J Nutr 2005;135:969–72.
[45] Videla LA, Rodrigo R, Orellana M, Fernández V, Tapia G, Quiñones L, et al. Oxidative stress-related parameters in the liver of non-alcoholic fatty liver disease patients. Clin Sci 2004;106:261–8.
[46] Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest 2002;109:1125–31.
[47] Deng X, Dong Q, Bridges D, Raghow R, Park EA, Elam MB. Docosahexaenoic acid inhibits proteolytic processing of sterol regulatory element-binding potein-1 c via activation of AMP-activated kinase. Biochim Biophys Acta 2015;1851:1521–9.
[48] Shang F, Taylor A. Ubiquitin-proteasome pathway and cellular responses to oxidative stress. Free Radic Biol Med 2011;51:5–16.
[49] Kammoun HL, Chabanon H, Hainault I, Luquet S, Magnan C, Koike T, et al. GRP78 expression inhibits insulin and ER stress-induced SREBP-1 c activation and reduces hepatic steatosis in mice. J Clin Invest 2009;119:1201–15.
[50] Gentile CL, Fyye MA, Pagliassotti MJ. Fatty acids and the endoplasmic reticulum in nonalcoholic fatty liver disease. Biofactors 2011;37:8–16.
[51] Priore P, Cavallo A, Gnoni A, Damiano F, Gnoni GV, Siculella L. Modulation of hepatic lipid metabolism by olive oil and its phenols in nonalcoholic fatty liver disease. IUBMB Life 2015;67:9–17.
[52] Jemai H, El Feki A, Sayadi S. Antidiabetic and antioxidant effects of hydroxytyrosol and oleuropein from olive leaves in alloxan-diabetic rats. J Agric Food Chem 2009;57:8798–804.
[53] Cicerale S, Conlan XA, Sinclair AJ, Keast RS. Chemistry and health of olive oil phenolics. Crit Rev Food Sci Nutr 2009;49:218–36.
[54] Hussein O, Grosovski M, Lasri E, Svalb S, Ravid U, Assy N. Monounsaturated fat decreases hepatic lipid content in non-alcoholic fatty liver disease in rats. World J Gastroenterol 2007;13:361–8.
[55] Nakbi A, Tayeb W, Dabbou S, Chargui I, Issaoui M, Zakhama A, et al. Hypolipidimic and antioxidant activities of virgin olive oil and its fractions in 2,4-diclorophenoxyacetic acid-treated rats. Nutrition 2012;28:81–91.
[56] Merra E, Calzaretti G, Bobba A, Storelli MM, Casalino E. Antioxidant role of hydroxytyrosol on oxidative stress in cadmium-intoxicated rats: different effect in spleen and testes. Drug Chem Toxicol 2014;37:420–6.
[57] Vilaplana-Pérez C, Auñón D, García-Flores LA, Gil-Izquierdo A. Hydroxytyrosol and potential uses in cardiovascular diseases, cancer, and AIDS. Front Nutr 2014;27:1–18.
[58] Zou X, Feng Z, Li Y, Wang Y, Wertz K, Weber P, et al. Stimulation of GSH synthesis to prevent oxidative stress-induced apoptosis by hydroxytyrosol in human retinal pigment epithelial cells: activation of Nrf2 and JNK-p62/SQSTM1 pathways. J Nutr Biochem 2012;23:994–1006.
[59] Domenichiello AF, Kitson AP, Bazinet RP. Is docosahexaenoic acid synthesis from α-linolenic acid sufficient to supply the adult brain? Prog Lipid Res 2015;59:54–66.
[60] Chen CT, Kitson AP, Hopperton KE, Domenichiello AF, Trépanier MO, Lin LE, et al. Plasma non-esterified docosahexaenoic acid is the major pool supplying the brain. Sci Rep 2015;5:15791.
[61] Domenichiello AF, Chen CT, Trepanier MO, Stavro PM, Bazinet RP. Whole body synthesis rates of DHA from α-linolenic acid are greater than brain DHA accretion and uptake rates in adult rats. J Lipid Res 2014;55:62–74.
[62] Bazinet RP, Layé S. Polyunsaturated fatty acids and their metabolites in brain function and disease. Nat Rev Neurosci 2014;15:771–85.
[63] Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. Nature 2014;510:92–101.
[64] Nakamura MT, Yudell BE, Loor JJ. Regulation of energy metabolism by long-chain fatty acids. Prog Lipid Res 2014;53:124–44.
[65] Zúñiga J, Cancino M, Medina F, Varela P, Vargas R, Tapia G, et al. N-3 PUFA supplementation triggers PPAR-α activation and PPAR-α/NF-κB interaction: anti-inflammatory implications in liver ischemia-reperfusion injury. PLoS One 2011;6:E28502.
[66] Tapia G, Valenzuela R, Espinosa A, Romanque P, Dossi C, Gonzalez-Mañán D, et al. N-3 long-chain PUFA supplementation prevents high fat diet induced mouse liver steatosis and inflammation in relation to PPAR-α upregulation and NF-κB DNA binding abrogation. Mol Nutr Food Res 2014;58:1333–41.
[67] Valenzuela R, Espinosa A, Llanos P, Hernandez-Rodas MC, Barrera C, Vergara D, et al. Anti-steatotic effects of an n-3 LCPUFA and extra virgin olive oil mixture in the liver of mice subjected to high-fat diet. Food Funct 2016;7:140–50.