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# 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

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ABSTRACT

*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.


## 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

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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