

## Evaluation of the Effect of Dietary Virgin Olive Oil on Blood Pressure and Lipid Composition of Serum and Low-Density Lipoprotein in Elderly Type 2 Diabetic Subjects

JAVIER SANCHEZ PERONA,<sup>†</sup> EMILIO MONTERO,<sup>‡</sup> JOSE MARIA SÁNCHEZ-DOMÍNGUEZ,<sup>†</sup>  
 JULIO CAÑIZARES,<sup>§</sup> MARIA GARCIA,<sup>‡</sup> AND VALENTINA RUIZ-GUTIÉRREZ\*<sup>†</sup>

<sup>†</sup>Instituto de la Grasa, Consejo Superior de Investigaciones Científicas, Avenida Padre García Tejero 4, 41012 Sevilla, Spain, <sup>‡</sup>Hospitales Universitarios Virgen del Rocío, 41013 Sevilla, Spain, and <sup>§</sup>Residencia Heliópolis de la Tercera Edad, Junta de Andalucía, 41012 Sevilla, Spain

Dietary virgin olive oil may help to reduce blood pressure in hypertensive individuals, but little is known about the effect on type 2 diabetic patients. For the present study, 17 type 2 diabetic elderly subjects and 23 healthy elderly controls received a diet rich in virgin olive oil for 4 weeks. Blood pressure, biochemical parameters, low-density lipoprotein (LDL), and oxidized LDL lipids and fatty acids were measured. Systolic blood pressure was reduced after virgin olive oil consumption in both controls and diabetic patients. Although the biochemical parameters were not modified, the intervention protected LDL from oxidation and restored the levels of dihomo- $\gamma$ -linolenic acid (20:3, n-6) in serum cholesterol esters and phospholipids of diabetic patients. In conclusion, the present study provides new evidence of the effects of dietary virgin olive oil on blood pressure and LDL oxidation in type 2 diabetics. It is likely that the components responsible for the observed effects are the monounsaturated fatty acids and the presence of antioxidants in the oil, but this needs further investigation.

**KEYWORDS:** Type 2 diabetes; elderly; virgin olive oil; blood pressure; LDL; oxidation

### INTRODUCTION

Type 2 diabetes mellitus is becoming one of the major health risks for elderly people in industrialized countries and is associated with conventional cardiovascular risk factors, including hypertriglyceridemia, low high-density lipoprotein (HDL)-cholesterol levels, and hypertension (1). High blood pressure is, therefore, strongly related to type 2 diabetes because it influences the probability that healthy individuals will develop metabolic syndrome or type 2 diabetes (2). Additionally, diabetic individuals suffering from hypertension have a higher risk of cardiovascular disease (3). Even at systolic pressure levels < 120 mmHg, patients with diabetes have dramatically higher cardiovascular mortality risk than those without diabetes (4). For this reason, recent guidelines agree that the first nutrition priority is to encourage individuals with type 2 diabetes to implement lifestyle strategies that will improve glycemia, dyslipidemia, and blood pressure (1).

Traditionally, the American Diabetes Association recommended low-fat diets (< 30% fat) for diabetic patients, primarily to avoid the increased cardiovascular risk associated with high levels of saturated fats (5). Currently, this recommendation has been refocused toward a more flexible one, allowing consumption of more energy from fat in the form of monounsaturated fatty acids (MUFA), because diets high in carbohydrates may make it

difficult to achieve glycemic control (6). Olive oil, a natural source of MUFA, has shown beneficial effects on glycemic tolerance (7) and cardiovascular parameters (8) in diabetic individuals.

The role of MUFA-rich oils, and in particular olive oil, on blood pressure remains unsolved (9). A possible explanation is that in most studies olive oil was used as a source of MUFA, not considering the minor component composition and, thus, not distinguishing between “virgin” olive oil and “common” olive oil (10, 11). A multicenter, randomized, primary prevention trial of cardiovascular disease, the PREDIMED study, reported a reduction of systolic blood pressure in 772 asymptomatic persons 55–80 years of age at high cardiovascular risk after a 3 month consumption of a Mediterranean-style diet rich in virgin olive oil (VOO) or nuts (12). We have also demonstrated that in normotensive and in hypertensive normocholesterolemic and hypercholesterolemic subjects dietary VOO lowered blood pressure when compared with another MUFA-rich oil, such as high-oleic sunflower oil (HOSO) (13). Moreover, VOO, but not HOSO, also normalized some altered functions of the erythrocyte membrane in hypertensives. It was proposed that some other compounds distinct from oleic acid [18:1 (n-9)] should be responsible for such effects, because the contents of this fatty acid in VOO and HOSO were almost identical. In this regard, we found a similar hypotensive effect in a group of hypertensive elderly individuals on diets rich in VOO presenting a similar composition in minor components, which differed only in their triacylglycerol (TG) molecular species composition, mainly in the presence of

\*Corresponding author (telephone 34-954611550; fax 34-954616790; e-mail valruiz@ig.csic.es).

**Table 1.** Fatty Acid Composition of Sunflower Oil (SO, Baseline) and Virgin Olive Oil (VOO)<sup>a</sup>

	mg/100 mg	
	SO	VOO
16:0	6.4 ± 0.4	10.9 ± 1.8*
16:1 (n-7)	0.1 ± 0.0	1.1 ± 0.3*
18:0	1.6 ± 0.3	1.8 ± 1.0
18:1 (n-9)	25.1 ± 1.4	74.9 ± 0.6*
18:1 (n-7)	1.6 ± 0.3	3.5 ± 0.1*
18:2 (n-6)	64.5 ± 1.2	5.1 ± 0.4*
18:3 (n-3)	nd	0.6 ± 0.2
18:3 (n-6)	0.9 ± 0.1	1.1 ± 0.3
20:1 (n-9)	0.4 ± 0.1	0.2 ± 0.1
22:0	nd	0.8 ± 0.1
SFA	8.0 ± 0.6	13.5 ± 2.1
MUFA	27.2 ± 1.2	79.7 ± 1.7
PUFA	65.8 ± 0.9	6.8 ± 0.6

<sup>a</sup>\*,  $p < 0.01$  vs SO; nd, not detected;  $n = 3$ .

oleic acid as triolein (trioleoyl-glycerol) or as dioleoyl-linoleoyl-glycerol (14). Although both VOO-rich diets were capable of reducing systolic and diastolic blood pressures after 4 weeks, only one of them caused a significant reduction in serum lipids and lipoprotein levels. This evidence points toward fatty acids as responsible for the effects of dietary VOO on serum lipid levels and minor components as responsible for the hypotensive effects of VOO.

For the moment, there is very scarce information on the effects of dietary olive oil on blood pressure in type 2 diabetes (6). Therefore, the present study was designed to evaluate the effect of VOO on blood pressure and serum lipid composition of type 2 diabetic elderly patients. These results could in part explain some of the benefits of VOO consumption, as part of the Mediterranean diet, in regard to the development of cardiac complications of diabetic patients.

## MATERIALS AND METHODS

**Subjects and Diets.** This study was performed at the Residencia Heliópolis (Junta de Andalucía, Seville, Spain), a residential home for the elderly where the diet of all the participants was controlled. All subjects gave their written informed consent to participate in the study, and the protocol was previously approved by the Institutional Committee for Research on Humans (Virgen del Rocío University Hospital, Seville, Spain). During the study the participants first consumed a diet enriched in sunflower oil (baseline, SO) and then, for 4 weeks, a diet rich in VOO of the Hojiblanca cultivar (*Olea europaea* var. Hojiblanca). SO was chosen as baseline dietary oil as it was habitually consumed at the residence. Before the study, the health officers recorded the regular dietary intake of the participants over 4 consecutive weeks using 24 h recall and food frequency questionnaires. The energy consumption and nutrient intake of the subjects were calculated and approved by a dietitian. Diets were revised weekly and adjusted so that 30% of their energy was obtained from fats, 55% from carbohydrates, and 15% from proteins. The diets for each experimental group and period were analyzed in triplicate to determine the fat content and that of other nutrients. The energy consumption was approximately 1800 kcal/day in both experimental groups. The fatty acid and minor component compositions of SO and VOO are shown in **Tables 1** and **2**, respectively.

The elderly subjects included in this study were type 2 diabetic patients and normoglycemic control subjects. The diabetic group consisted of 17 subjects (3 males/14 females) with a mean age of  $81.8 \pm 6.9$  years, who were treated with sulfonyl urea, biguanides, acarbose, insulin, and insulin analogues. The control group consisted of 23 subjects (6 males/22 females) with a mean age of  $83.7 \pm 7.1$  years. The medical histories of all the

**Table 2.** Minor Components of Sunflower Oil (SO, Baseline) and Virgin Olive Oil (VOO)<sup>a</sup>

	mg/kg	
	SO	VOO
sterols	1503 ± 54	1558 ± 33
tocopherols	406 ± 25	207 ± 48*
erythrodiol + uvaol	nd	20.98 ± 1.07
waxes	30.3 ± 4.8	83.5 ± 7.9*
squalene	nd	6621 ± 235
polyphenols	nd	232.1 ± 18.6
tyrosol	nd	92.0 ± 1.4
hydroxytyrosol	nd	132.8 ± 4.2

<sup>a</sup>\*,  $p < 0.01$  vs SO; nd, not detected;  $n = 3$ .

participants were reviewed before recruitment into the study, and a physical examination and a clinical biochemical analysis were performed to exclude possible secondary causes of diabetes. None of the subjects in the study had hypothyroidism, and no history of alcohol abuse or cigarette smoking was found.

**Blood Pressure Measurements.** Blood pressure measurements were performed in the morning, after an overnight fast, at the right brachial artery in seated participants using a mercury-gauge sphyngomanometer. The measurements were recorded by the same nurse at the residential home at the beginning, middle, and end of each experimental period. At each visit three blood pressure measurements were recorded, and the average was used to determine eligibility.

**Biochemical Parameters.** Venous blood was obtained in fasting status after an overnight period, at the beginning and end of each period of the study. Blood was collected in Vacutainer tubes, and serum was obtained by centrifugation at 1500 rpm during 30 min at 4 °C. Insulin was measured by immunoassay (Abbott Laboratories, Maidenhead, U.K.) and glucose using an enzymatic colorimetric slide assay (Amersham, U.K.). HOMA2 software (<http://www.dtu.ox.ac.uk/homa>) was used for the calculation of insulin resistance (HOMA2-IR). Serum total and HDL-cholesterol and TG concentrations were measured by conventional enzymatic methods. LDL-cholesterol concentration was calculated according to the Friedewald equation.

**Isolation and Oxidation of LDL.** LDL was isolated by density gradient ultracentrifugation from 4 mL of serum (40000 rpm, 18 h, 15 °C). Ultracentrifugation was performed using an SW 41 Ti rotor in a Beckman L8-70 M preparative ultracentrifuge (Beckman Instruments, Inc., Palo Alto, CA). Lipid peroxidation was assayed by incubating LDL (1 mg of protein) in 0.05 M phosphate buffer, pH 7.4, at 37 °C, final volume = 2 mL. The reaction was started by the addition of ascorbate (final concentration = 0.4 mM). Phosphate buffer was contaminated with sufficient iron (25 mM) to provide the necessary ferrous or ferric iron for lipid peroxidation.

**Lipid Extraction and Separation from Serum and LDL.** Total lipids were extracted from total serum and LDL following the method of Folch et al. (15), using 2,6-di-*tert*-butyl-*p*-cresol as antioxidant. An aliquot of LDL lipids was collected for the analysis of lipid classes by HPLC as described below and was stored until use at -80 °C. Lipids from serum were separated by thin layer chromatography (TLC) on silica gel 60 plates (Kieselgel 60 F254, Merck) using an elution system of hexane/diethyl ether/acetic acid (80:20:1, v/v/v) (Merck KGaA, Darmstadt, Germany). Bands corresponding to cholesteryl esters (CE), TG, diacylglycerols (DG), free cholesterol, and phospholipids (PL) were separated according to their polarity, giving different retardation factor (Rf) values. CE and PL were identified using standards (cholesteryl oleate and phosphatidylcholine), scraped from the silica, recovered with hexane and chloroform/methanol (1:1), respectively, and stored at -20 °C until use.

Ten microliters of LDL lipids, dissolved in chloroform/methanol (2:1, v/v), were injected into a 2690 Alliance liquid chromatograph (Waters, Co., Milford, MA), controlled by computer (Millennium System, Waters Co.) and provided with a Lichrosphere column (250 × 4.6 mm, 5 μm particle size, Merck KGaA). The liquid chromatograph was coupled

**Table 3.** Anthropometric Parameters and Serum Biochemical Levels<sup>a</sup>

	controls		diabetic patients	
	baseline	VOO	baseline	VOO
weight (kg)	63.7 ± 12.7 a	65.1 ± 17.3 a	71.3 ± 15.9 a	64.1 ± 7.8 a
BMI (kg/m <sup>2</sup> )	28.3 ± 3.4 a	27.9 ± 5.3 a	31.0 ± 7.1 a	29.0 ± 4.1 a
WHR	0.91 ± 0.09 a	0.91 ± 0.09 a	0.90 ± 0.07 a	0.91 ± 0.08 a
systolic pressure (mmHg)	143.1 ± 18.3 a	132.9 ± 10.1 b	143.8 ± 22.5 b	136.9 ± 16.8 ab
diastolic pressure (mmHg)	73.8 ± 10.5 a	71.4 ± 7.4 a	70.2 ± 9.2 a	71.5 ± 8.2 a
glucose (mg/dL)	101.1 ± 28.1 a	97.4 ± 14.1 a	180.9 ± 68.5 b	168.8 ± 85.4 b
insulin (mU/mL)	8.1 ± 5.9 a	6.9 ± 3.8 a	10.0 ± 6.3 a	8.8 ± 4.7 a
HOMA2-IR	2.02 ± 0.41 a	1.66 ± 0.13 a	4.46 ± 1.06 b	3.66 ± 0.99 b
creatinine (mg/dL)	0.97 ± 0.18 a	1.00 ± 0.16 a	0.96 ± 0.15 a	1.06 ± 0.29 a
uric acid (mg/dL)	5.0 ± 1.5 a	5.0 ± 1.2 a	4.3 ± 1.6 a	4.7 ± 1.9 a
triacylglycerols (mg/dL)	77.6 ± 30 a	92.7 ± 34.8 a	111.9 ± 40.6 b	107.2 ± 39.1 b
cholesterol (mg/dL)	186.2 ± 38.1 a	188.4 ± 43.1 a	203.2 ± 38.4 a	201.1 ± 54.2 a
LDL-cholesterol (mg/dL)	113.0 ± 35.0 a	124.0 ± 38.0 a	125.0 ± 33.0 a	130.0 ± 54.2 a
HDL-cholesterol (mg/dL)	57.6 ± 17.8 a	48.5 ± 14.7 a	58.5 ± 18.4 a	48.8 ± 15.3 a

<sup>a</sup> Mean values within a row sharing the same letter are not significantly different ( $p > 0.05$ ). BMI, body mass index; WHR, waist-to-hip ratio; HOMA2-IR, homeostatic model assessment for insulin resistance. Controls  $n = 23$ ; diabetic patients  $n = 17$ .

to a light-scattering detector (DDL31 Eurosep, Ins, Cergy-Pontoise, France). The mobile phase consisted of a ternary solvent system, starting with 0.8% of 2-propanol in hexane, raising the percentage of 2-propanol to 3.0% in 8 min and then to 10.0% after 17 min. From minute 17 to minute 22, the percentages of hexane and 2-propanol were reduced to 0%, whereas that of methanol was raised to 100%. The flow rate was 0.8 mL/min. Standard solutions of Sigma grade (99% pure) cholesterol oleate, triolein, cholesterol, dioleoyl-glycerol, monooleoyl-glycerol, and dipalmitoylphosphatidylethanolamine were used for identification and quantification. To calibrate the detector and to establish the capacity factor ( $k'$ ) of the system, triplicate runs of six concentrations of these standards (0.25 and 2.5 mg/mL in chloroform/methanol, 2:1) were injected. Equations resulting from regression curves of these standards were employed for quantification.

**Statistical Analysis.** Results are presented as means ± SD ( $n = 23$  for the group of controls and  $n = 17$  for the group of diabetic patients). The significance of the differences between dietary periods and groups was assessed by one-way ANOVA (repeated measures) with an unpaired two-tailed  $t$  test for comparison of the means. Analyses were done with Prism 3.0 (GraphPAD Software, San Diego, CA) statistical package. Differences of  $p < 0.05$  were considered to be statistically significant.

## RESULTS

### Anthropometric Parameters and Serum Biochemical Levels.

Body weight was slightly higher at the beginning of the study in the group of type 2 diabetic patients, thus showing a higher BMI than the control group, although the difference was not statistically significant (Table 3). The values for weight and BMI were nonsignificantly reduced in the group of diabetic patients after the period of consuming VOO. Fasting serum glucose values were significantly higher in diabetic patients than in controls, but although they were reduced in diabetic patients after the intervention with the VOO diet, the lowering did not reach statistical significance. In contrast to glucose, insulin levels were not significantly different between diabetics and controls, but the homeostatic model assessment for insulin resistance (HOMA2-IR) was higher in diabetics. This parameter was reduced nonsignificantly in both groups after VOO intake and glucose concentration in diabetics. Serum TG levels were also higher in the group of diabetic patients compared with controls. As occurred with glucose levels, VOO consumption did not reduce TG levels significantly in diabetic patients. Serum total, LDL-, and HDL-cholesterol levels were not significantly modified.

**Blood Pressure.** Systolic blood pressure was significantly reduced by 10 mmHg in controls and by 7 mmHg in type 2

**Table 4.** Fatty Acid Composition of Serum Cholesteryl Esters (CE) of Controls and Type 2 Diabetic Subjects at Baseline and after the Diet Rich in Virgin Olive Oil (VOO)<sup>a</sup>

	controls (mg/100 mg)		diabetic patients (mg/100 mg)	
	baseline	VOO	baseline	VOO
14:0	0.4 ± 0.3 a	0.5 ± 0.4 b	0.9 ± 0.1 c	0.4 ± 0.1 ab
14:1 (n-5)	0.5 ± 0.2 a	0.3 ± 0.1 b	0.3 ± 0.2 b	0.2 ± 0.1 b
16:0	14.0 ± 2.9 a	11.8 ± 2.1 a	9.8 ± 2.6 b	12.7 ± 4.6 a
16:1 (n-9)	2.0 ± 0.5 a	1.3 ± 0.7 b	2.2 ± 0.4 a	1.4 ± 0.7 b
16:1 (n-7)	2.1 ± 0.8 a	2.2 ± 0.6 a	4.1 ± 1.5 b	3.0 ± 1.1 c
18:0	2.1 ± 0.7 a	1.3 ± 0.6 b	2.5 ± 1.2 a	1.1 ± 0.2 b
18:1 (n-9)	15.9 ± 3.1 a	21.3 ± 2.7 b	15.3 ± 2.9 a	23.6 ± 3.3 b
18:1 (n-7)	1.8 ± 0.4 a	1.7 ± 0.3 a	1.7 ± 0.1 a	1.6 ± 0.3 a
18:2 (n-6)	49.9 ± 4.6 a	49.1 ± 3.9 a	51.8 ± 2.0 a	46.7 ± 8.7 a
18:3 (n-3)	1.5 ± 0.3 a	1.2 ± 0.4 a	0.7 ± 0.3 b	1.4 ± 0.9 a
20:2 (n-6)	1.0 ± 0.3 a	1.0 ± 0.3 a	1.5 ± 0.6 b	0.8 ± 0.2 a
20:3 (n-6)	nd	nd	1.1 ± 0.3	nd
20:4 (n-6)	8.8 ± 1.3 a	8.3 ± 1.5 a	8.1 ± 2.8 ab	7.2 ± 1.2 b
SFA	16.5 ± 4.0 a	13.4 ± 2.5 a	13.1 ± 3.8 a	14.0 ± 4.9 a
MUFA	22.3 ± 5.1 a	26.8 ± 4.5 a	23.5 ± 5.3 a	29.9 ± 5.5 b
PUFA	61.2 ± 6.6 a	59.7 ± 6.2 a	63.1 ± 6.0 a	56.1 ± 10.9 b

<sup>a</sup> Mean values within a row sharing the same letter are not significantly different ( $p > 0.05$ ). nd, not detected. Controls  $n = 23$ ; diabetic patients  $n = 17$ .

diabetic individuals after consumption of VOO (Table 3). In contrast, diastolic blood pressure was not modified in either group studied.

**Fatty Acid Composition of Serum Cholesteryl Esters (CE) and Phospholipids (PL).** The plasma CE fatty acid composition is shown in Table 4. The oleic acid content of CE in controls and diabetic patients was similar at the beginning of the study (15.9% in controls and 15.3% in diabetic patients). The consumption of VOO elevated the oleic acid content in this lipid fraction, by 34% in controls and by 54% in diabetic patients ( $p < 0.01$ ). Because this elevation was not observed for other MUFA [myristoleic [14:1 (n-5)], palmitoleic [16:1 (n-7)], and vaccenic [18:1 (n-7)] acids], the elevation in the oleic acid content resulted in a significant increase of total MUFA only in diabetic patients (by 27%,  $p < 0.01$ ). Linoleic acid [18:2 (n-6)] presented the highest concentration in CE, but VOO did not modify it in either of the groups studied. A slightly lower linoleic acid concentration in CE of diabetic patients after consumption of VOO, together with significant reductions in the concentrations of arachidonic acid



**Table 5.** Fatty Acid Composition of Serum Phospholipids (PL) of Control and Type 2 Diabetic Subjects at Baseline and after the Diet Rich in Virgin Olive Oil (VOO)<sup>a</sup>

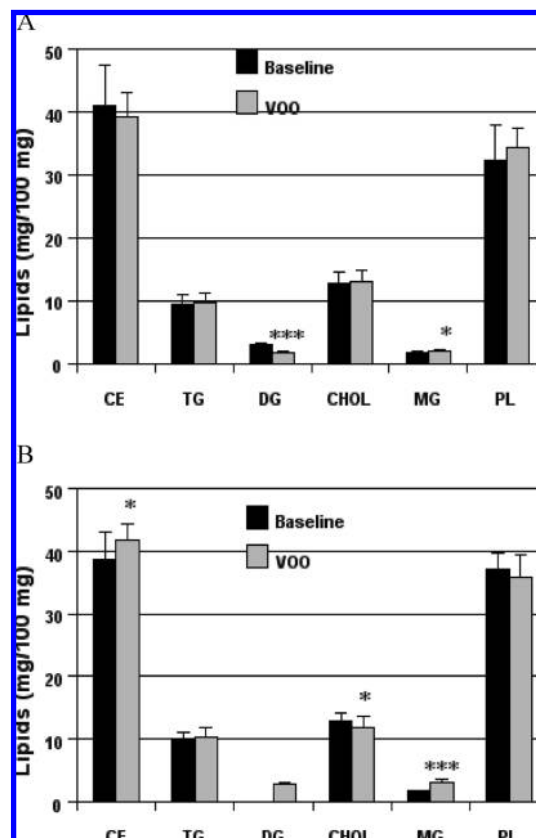
	controls (mg/100 mg)		diabetic patients (mg/100 mg)	
	baseline	VOO	baseline	VOO
14:0	0.5 ± 0.2 a	0.4 ± 0.2 a	0.6 ± 0.2 a	0.4 ± 0.2 a
14:1 (n-5)	0.6 ± 0.2 a	0.7 ± 0.2 a	0.8 ± 0.3 a	0.6 ± 0.3 a
16:0	29.0 ± 2.5 a	30.1 ± 2.7 a	27.6 ± 3.3 a	29.5 ± 0.8 a
16:1 (n-9)	0.5 ± 0.2 a	0.3 ± 0.1 b	0.6 ± 0.2 a	0.5 ± 0.2 a
16:1 (n-7)	0.7 ± 0.3 a	0.6 ± 0.2 a	0.8 ± 0.2 a	0.6 ± 0.1 a
18:0	15.2 ± 2.7 a	15.2 ± 1.4 a	12.8 ± 0.9 b	13.6 ± 1.0 b
18:1 (n-9)	14.2 ± 2.6 a	11.9 ± 1.5 a	16.7 ± 5.5 b	14.5 ± 3.0 ab
18:1 (n-7)	2.1 ± 0.6 a	2.0 ± 0.6 a	2.1 ± 1.5 a	2.9 ± 1.3 a
18:2 (n-6)	20.4 ± 2.4 a	20.6 ± 3.1 a	22.1 ± 0.8 a	19.9 ± 1.4 a
18:3 (n-3)	0.4 ± 0.1 a	0.4 ± 0.1 a	nd	0.3 ± 0.1 a
20:0	0.2 ± 0.1 a	0.1 ± 0.1 a	nd	0.3 ± 0.1 a
20:1 (n-9)	0.3 ± 0.1 a	0.2 ± 0.1 a	nd	0.3 ± 0.1 a
20:1 (n-7)	0.2 ± 0.1 a	0.1 ± 0.1 a	nd	0.1 ± 0.1 a
20:3 (n-6)	3.4 ± 0.6 a	3.9 ± 0.4 a	6.2 ± 1.8 b	3.5 ± 0.7 a
20:4 (n-6)	10.5 ± 2.3 a	11.7 ± 2.4 a	9.0 ± 0.6 b	9.5 ± 1.5 b
20:5 (n-3)	1.1 ± 0.2 a	1.0 ± 0.3 a	nd	1.5 ± 0.9 a
22:5 (n-3)	0.3 ± 0.1 a	0.4 ± 0.1 a	nd	0.6 ± 0.1 b
22:6 (n-3)	0.3 ± 0.1 a	0.4 ± 0.1 a	nd	0.5 ± 0.1 b
SFA	44.4 ± 5.6 a	45.6 ± 4.4 a	41.0 ± 4.5 b	43.8 ± 1.9 b
MUFA	19.8 ± 4.3 a	16.4 ± 3.0 b	21.8 ± 3.6 a	20.3 ± 4.8 a
PUFA	35.9 ± 6.0 a	38.1 ± 7.3 a	37.2 ± 6.3 a	35.6 ± 4.5 a

<sup>a</sup> Mean values within a row sharing the same letter are not significantly different ( $p > 0.05$ ). nd, not detected. Controls  $n = 23$ ; diabetic patients  $n = 17$ .

[20:4 (n-6)] and its precursor dihomo- $\gamma$ -linolenic acid [20:3 (n-6)], was found, which resulted in lower contents of total polyunsaturated fatty acids (PUFA) ( $p < 0.01$ ). The concentration of palmitic acid (16:0) in CE was lower in diabetic patients than in controls, but it was normalized after the consumption of VOO. In contrast, the content of stearic acid (18:0), which was higher at baseline, was reduced by administration of VOO. Consequently, the total SFA content was not modified by VOO.

Modifications in the serum PL fatty acid composition of the elderly subjects by the experimental diets were less evident (Table 5). For instance, the introduction of VOO in the diet did not increase the incorporation of oleic acid to PL. Indeed, the concentration of this fatty acid was slightly lower in serum PL after VOO in both groups studied, although the content in diabetic patients was higher at baseline (18%,  $p < 0.01$ ). As a consequence, the total MUFA content was also higher in diabetic patients ( $p < 0.01$ ). Similarly to CE, no differences were observed regarding the incorporation of linoleic acid, but the content of arachidonic acid was significantly lower in diabetic patients (15%,  $p < 0.01$ ). However, VOO consumption did not normalize the content on this fatty acid in serum PL. In contrast, dihomo- $\gamma$ -linolenic acid, which was abnormally increased in diabetic patients, returned to normal values after VOO. No significant differences were found for palmitic acid concentrations, but the content of stearic acid was lower in diabetic patients ( $p < 0.01$ ).

**LDL Oxidation and Lipid Class Composition.** The lipid class composition of LDL is presented in Figure 1. In controls, VOO did not exert any significant effect on the main lipid classes, although it reduced the diacylglycerol (DG) content ( $p < 0.001$ ) and increased that of monoacylglycerols (MG) ( $p < 0.05$ ). In contrast, the VOO diet resulted in a significant elevation of LDL-CE (2000  $p < 0.05$ ) and a reduction of free cholesterol ( $p < 0.05$ ) in diabetic patients. MG were also increased in this group after consumption of VOO ( $p < 0.001$ ).

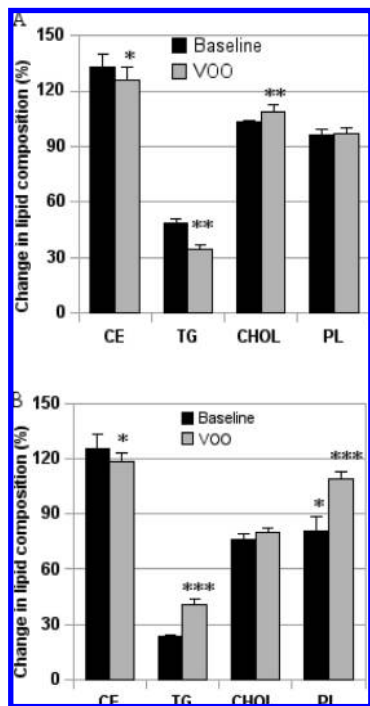


**Figure 1.** Lipid class composition of LDL (mg/100 mg of lipids) of control (A) and type 2 diabetic (B) elderly subjects at baseline and after consuming the virgin olive oil diet (VOO). \*,  $p < 0.05$ , and \*\*,  $p < 0.01$ , versus baseline values. Control  $n = 23$ ; diabetic patients  $n = 17$ . CE, cholesteryl esters; TG, triacylglycerols; DG, diacylglycerols; CHOL, cholesterol; MG, monoacylglycerols; PL, phospholipids.

Figure 2 shows changes in the lipid class composition of LDL from control (A) and type 2 diabetic (B) subjects after iron-induced oxidation in relation to baseline LDL composition. DG and MG could not be detected in LDL after oxidation, and the major loss among LDL lipid classes was observed for TG, because only an approximate 40% of the baseline TG remained. The reduction in the glyceride fraction (TG, DG, and MG) occurred concomitantly with an increase in the cholesterol species. VOO showed a protective effect against lipid peroxidation in the group of type 2 diabetic patients. Eighty percent of baseline TG disappeared from LDL after oxidation, whereas only 60% was lost after the VOO diet. Similarly, the VOO diet protected free cholesterol and PL from oxidation. In controls, the free cholesterol content was significantly higher in oxLDL ( $p < 0.01$ ) and, in diabetics, the relative content of PL  $> 100\%$  after the VOO diet, compared with 80% at baseline ( $p < 0.001$ ).

## DISCUSSION

Modification of dietary habits is central in the prevention and treatment of diabetes. Traditionally, nutrition advice for the treatment of diabetes emphasized avoiding all dietary fat, especially saturated fat (5). However, due to the scientific evidence accumulated in the past two decades on the beneficial effects of dietary MUFA, this recommendation has been relaxed, allowing consumption of more energy from fat in the form of MUFA (6). In the present study, we present the reduction in blood pressure and the protective effect against LDL oxidation in



**Figure 2.** Changes (expressed as percent in relation to baseline period) in the lipid classes of LDL of control (A) and type 2 diabetic (B) elderly subjects at baseline and after consuming the virgin olive oil diet (VOO) after iron-induced oxidation. \*,  $p < 0.05$ , \*\*,  $p < 0.01$ , and \*\*\*,  $p < 0.001$ , versus baseline values. Control  $n = 23$ ; diabetic patients  $n = 17$ . CE, cholesteryl esters; TG, triacylglycerols; CHOL, cholesterol; PL, phospholipids.

elderly subjects affected by type 2 diabetes mellitus after VOO consumption, the key dietary fat of the Mediterranean diet.

Four week VOO consumption did not modify body weight, body mass index (BMI), or waist-to-hip ratio (WHR) in either controls or diabetic elderly subjects significantly. Similarly, other serum biochemical parameters, such as glucose, insulin, HOMA2-IR, TG, total cholesterol, and LDL-cholesterol concentrations, were not significantly affected by the dietary intervention, despite being higher in the diabetic group. However, it is interesting to note that diet induced a reduction of glucose concentration in the diabetic group. The lack of significance might be related to the size of the sample. In contrast, consumption of VOO reduced systolic blood pressure significantly in both experimental groups. Therefore, the present study gives evidence for the hypotensive effect of VOO in type 2 diabetic subjects. Previous studies carried out in our laboratory have shown that long-term VOO consumption reduces systolic and even diastolic blood pressure in healthy (14) and hypertensive elderly individuals (13, 16). We have recently published a randomized controlled trial in which a Mediterranean-type diet enriched in VOO has been administered to a large group of subjects ( $n = 257$ ) with a high risk of cardiovascular disease for 3 months. One of the main outcomes of that study was the significant reduction in systolic blood pressure (by 5 mmHg) (12).

In the aforementioned studies, the hypotensive effect of VOO was attributed to bioactive minor compounds of VOO rather than to the fatty acid composition, and it was suggested that VOO polyphenols may play an important role. Actually, red wine polyphenols have been shown to reduce blood pressure in normal (17) and spontaneously hypertensive rats (18). The reduction in systolic blood pressure by polyphenols has been associated with reduced oxidative stress and the induction of nitric oxide release. No changes were observed in diastolic blood

pressure, similarly as observed in the present study. Fito et al. (19) published an elegant study in which they administered refined olive oil and VOO to 40 males with stable coronary heart disease for 3 weeks. The polyphenolic content of VOO was about 10 times higher in VOO than in the refined oil. These authors reported a significant reduction in blood pressure in a group of hypertensive subjects after the intake of VOO. In subjects suffering from type I diabetes, Leger et al. observed a reduced thromboxane B2 production in serum after administration of an olive oil wastewater extract for 4 consecutive days (20).

Only slight modifications were found in LDL lipid composition after consumption of VOO in both control and type 2 diabetic patients (Figure 2). However, VOO intake greatly affected LDL oxidation in this group (Figure 2). We observed a loss of glyceridic lipids (TG, DG, and MG) after LDL oxidation of particles obtained before the intervention with VOO, resulting in a proportionate increase in free and esterified cholesterol. Both DG and MG completely disappeared from LDL in all groups studied, but the loss of TG was greater in type 2 diabetic patients than in controls, suggesting that diabetes diminishes the protection of LDL against iron-induced oxidation in elderly subjects. Several studies have reported an increased susceptibility of LDL oxidation in type 2 diabetes (6), and it has been claimed that VOO protects LDL against oxidation (16, 19, 21). However, Rodriguez-Villar et al. (8) could not find a protective effect of VOO on LDL oxidation compared to a carbohydrate-rich diet in type 2 diabetic patients. In our study, TG and PL depletion were significantly reduced in oxLDL of diabetic patients after 4 weeks of consumption of VOO. The preventive effect of VOO on LDL oxidation may be attributed to the higher intake of antioxidants, such as polyphenols and tocopherols, and to the lower susceptibility of MUFA to oxidation compared with dietary oils rich in PUFA (22).

Hypercholesterolemia, diabetes mellitus, and hypertension are related to increased production of reactive oxygen species that can inactivate nitric oxide and lead to endothelial dysfunction (23). Reactive oxygen species can also react with PUFA contained in lipoproteins in the vessel wall, initiating lipid peroxidation. Antioxidants constitute a diverse group of compounds among whose effects are inhibition of LDL oxidation by both reductions in the concentration and reactivity of oxidants and improved resistance of the particle to them (24). VOO contains both  $\alpha$ -tocopherol and phenolic compounds, among which oleuropein aglycone derivatives (hydroxytyrosol and tyrosol) (Table 2) are strong antioxidants and radical scavengers. Despite SO presenting a higher tocopherol content, the synergistic effect of polyphenols and tocopherols (25) in VOO and its lower PUFA content may account for its higher protection against lipid oxidation of LDL. Nevertheless, we are aware that an important limitation of the present study is the absence of a control group to discriminate the effect of phenolics. A refined olive oil devoid of these compounds would be advisable, although it is not commercially available and suitable for human consumption.

The compositions of serum CE and PL fatty acids have been associated with the risk of developing type 2 diabetes and insulin resistance (26). In our study, we found higher concentrations of myristic, palmitoleic, eicosadienoic, and dihomo- $\gamma$ -linolenic acids in diabetic patients compared to controls in serum CE before the VOO intervention. Dihomo- $\gamma$ -linolenic was also found in higher proportion in serum PL of diabetic patients. Indeed, this fatty acid, as well as palmitoleic acid, has been found increased in serum CE of patients with non-insulin-dependent diabetes mellitus (26). Both fatty acids have shown strong association with surrogate markers for insulin resistance, such as fasting insulin

levels or the HOMA2-IR, associated with type 2 diabetes (27) and the metabolic syndrome (28). It is unlikely that the higher content in dihomo- $\gamma$ -linolenic observed in diabetic patients in our study could be related to a low intake of dietary linoleic acid, because SO, rich in linoleic acid, was the main source of fat in the baseline diet. Despite dihomo- $\gamma$ -linolenic acid being a precursor of arachidonic acid, the content of the latter was not increased in either serum CE or PL of diabetic patients compared with controls, indicating a defective  $\Delta$ -desaturase activity. A low  $\Delta$ 5-desaturase activity has been also suggested as an early sign of the risk of developing metabolic syndrome (28) and diabetes (29). Additionally, in our study long-chain n-3 PUFA, which are also formed as a consequence of the  $\Delta$ 5-desaturase activity, could not be detected in serum PL of diabetic patients. Alterations in cell membrane due to diabetes include decrease of n-3 PUFA in PL, associated with abnormalities in sodium transport system (30).

Dietary VOO consumption normalized the dihomo- $\gamma$ -linolenic acid and palmitoleic acid contents in serum EC of diabetic patients and increased the content of long-chain n-3 PUFA and dihomo- $\gamma$ -linolenic acid in serum PL. However, it did not proportionally increase the concentration of arachidonic acid, which remained below the levels of the control group. Decreasing the dihomo- $\gamma$ -linolenic acid content without affecting the arachidonic acid involves the impairment of  $\Delta$ 5-desaturase activity in diabetic patients and was not restored by VOO consumption. However, it is plausible that VOO may affect the  $\Delta$ 6-desaturase activity, thus reducing the substrate availability for the synthesis of dihomo- $\gamma$ -linolenic acid (31). The desaturation of linoleic acid to  $\gamma$ -linolenic acid [18:3 (n-6)] is the rate-limiting step in the production of dihomo- $\gamma$ -linolenic acid and subsequently arachidonic acid. We have reported before increased n-3 PUFA in PL of erythrocytes of hypertensive patients after the intake of VOO, which concomitantly reduced systolic blood pressure (13), and others have reported that plasma exchange of n-3 and n-6 PUFA occurs with a reduction of blood pressure (32). These and the present results suggest that dietary VOO may increase the incorporation of n-3 PUFA into PL at the expense of arachidonic acid, which is of major importance given that n-3 PUFA competitively inhibit the utilization of arachidonic acid for the cyclooxygenase pathway and output of vasoconstrictor eicosanoids only in the control group, according to the results shown in **Table 5**. However, it seems unlikely that the reduction in systolic blood pressure could be related with the modest increase in n-3 PUFA due to VOO consumption by type 2 diabetic patients, because a proportional decrease in arachidonic acid was not observed. In addition, blood pressure was reduced in the control group despite n-3 PUFA remaining unaltered after VOO intake.

In conclusion, the present study shows that systolic blood pressure may be reduced in elderly patients affected by type 2 diabetes by long-term consumption of VOO, which concomitantly protects against LDL oxidation. We suggest that the low PUFA content, the high MUFA content, and the presence of potent antioxidants, such as polyphenols and tocopherols, may help to reduce the oxidative stress of type 2 diabetic patients, which could lead to a lower LDL susceptibility to oxidation. VOO also were shown to be effective in restoring the levels of dihomo- $\gamma$ -linolenic and palmitoleic acids in CE, which are known to be increased in diabetic patients. The reduction in dihomo- $\gamma$ -linolenic acid was not related to an increase in the  $\Delta$ 5-desaturase activity but probably to inhibition of  $\Delta$ 6-desaturase, which might be achieved by direct inhibition of the enzyme or competition with linoleic acid. This issue deserves further investigation.

## ABBREVIATIONS USED

MUFA, monounsaturated fatty acids; VOO, virgin olive oil; HOSO, high-oleic sunflower oil; SO, sunflower oil; TG, triacylglycerol; CE, cholesteryl esters; PL, phospholipids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; DG, diacylglycerol; MG, monoacylglycerol; HOMA2-IR, homeostatic model assessment for insulin resistance.

## ACKNOWLEDGMENT

This work was supported by funds from Comision Interministerial de Ciencia y Tecnologia (CICYT, AGL2008-02258/ALI), Instituto de Salud Carlos III (Thematic Network RD06/0045/002) and Consejo Superior de Investigaciones Cientificas (CSIC, 200870I208).

## LITERATURE CITED

- (1) American Diabetes Association. Nutrition recommendations and interventions for diabetes. A position statement of the American Diabetes Association. *Diabetes Care* **2008**, *31*, S61–S78.
- (2) Mancía, G. The association of hypertension and diabetes: prevalence, cardiovascular risk and protection by blood pressure reduction. *Acta Diabetol.* **2005**, *42*, S17–S25.
- (3) Bakris, G. L.; Williams, M.; Dworkin, L.; Elliott, W. J.; Epstein, M.; Toto, R.; Tuttle, K.; Douglas, J.; Hsueh, W.; Sowers, J. Preserving renal function in adults with hypertension and diabetes: a consensus approach. *Am. J. Kidney Dis.* **2000**, *36*, 646–661.
- (4) Stamler, J.; Vaccaro, O.; Neaton, J. D.; Wentworth, D. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care* **1993**, *16*, 434–444.
- (5) American Diabetes Association. Role of cardiovascular risk factors in prevention and treatment of macrovascular disease in diabetes. *Diabetes Care* **1989**, *12*, 573–579.
- (6) Ros, E. Dietary cis-monounsaturated fatty acids and metabolic control in type 2 diabetes. *Am. J. Clin. Nutr.* **2003**, *78*, 617S–625S.
- (7) Thomsen, C.; Storm, H.; Holst, J. J.; Hermansen, K. Differential effects of saturated and monounsaturated fats on postprandial lipemia and glucagon-like peptide 1 responses in patients with type-2 diabetes. *Am. J. Clin. Nutr.* **2003**, *77*, 605–611.
- (8) Rodríguez-Villar, C.; Pérez-Heras, A.; Mercadé, I.; Casals, E.; Ros, E. Comparison of a high-carbohydrate and a high-monounsaturated fat, olive oil-rich diet on the susceptibility of LDL to oxidative modification in subjects with type-2 diabetes mellitus. *Diabetic Med.* **2004**, *21*, 142–149.
- (9) Alonso, A.; Ruiz-Gutierrez, V.; Martínez-González, M. A. Mono-unsaturated fatty acids, olive oil and blood pressure: epidemiological, clinical and experimental evidence. *Public Health Nutr.* **2006**, *9*, 251–257.
- (10) Rasmussen, O. W.; Thomsen, C.; Hansen, K. W.; Vesterlund, M.; Winther, E.; Hermansen, K. Effects on blood pressure, glucose, and lipid levels of a high-monounsaturated fat diet compared with a high-carbohydrate diet in NIDDM subjects. *Diabetes Care* **1993**, *16*, 1565–1571.
- (11) Rasmussen, O. W.; Thomsen, C.; Hansen, K. W.; Vesterlund, M.; Hermansen, K. Comparison of the effects on the diurnal blood pressure, glucose, and lipid levels of a diet rich in monounsaturated fatty acids with a diet rich in polyunsaturated fatty acids in type 2 diabetic subjects. *Diabetes Med.* **1995**, *12*, 600–606.
- (12) Estruch, R.; Martínez-González, M. A.; Corella, D.; Salas-Salvadó, J.; Ruiz-Gutiérrez, V.; Covas, M. I.; Fiol, M.; Gómez-Gracia, E.; López-Sabater, M. C.; Vinyoles, E.; Arós, F.; Conde, M.; Lahoz, C.; Lapetra, J.; Sáez, G.; Ros, E. PREDIMED Study Investigators. Effects of a Mediterranean-style diet on cardiovascular risk factors. *Ann. Intern. Med.* **2006**, *145*, 1–11.
- (13) Ruiz-Gutiérrez, V.; Muriana, F. J.; Guerrero, A.; Cert, A. M.; Villar, J. Plasma lipids, erythrocyte membrane lipids and blood pressure of hypertensive women after ingestion of dietary oleic acid from two different sources. *J. Hypertens.* **1996**, *14*, 1483–1490.



- (14) Perona, J. S.; Canizares, J.; Montero, E.; Sánchez-Domínguez, J. M.; Ruiz-Gutierrez, V. Plasma lipid modifications in elderly people after administration of two virgin olive oils of the same variety (*Olea europaea* var. hojiblanca) with different triacylglycerol composition. *Br. J. Nutr.* **2003**, *89*, 819–826.
- (15) Folch, J.; Lees, M.; Sloane-Stanley, G. H. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497–509.
- (16) Perona, J. S.; Canizares, J.; Montero, E.; Sánchez-Domínguez, J. M.; Catalá, A.; Ruiz-Gutiérrez, V. Virgin olive oil reduces blood pressure in hypertensive elderly subjects. *Clin. Nutr.* **2004**, *23*, 1113–1121.
- (17) Diebolt, M.; Bucher, B.; Andriantsitohaina, R. Wine polyphenols decrease blood pressure, improve NO vasodilatation and induce gene expression. *Hypertension* **2001**, *38*, 159–165.
- (18) Negishi, H.; Xu, J. W.; Ikeda, K.; Njelekela, M.; Nara, Y.; Yamori, Y. Black and green tea polyphenols attenuate blood pressure increases in stroke-prone spontaneously hypertensive rats. *J. Nutr.* **2004**, *134*, 38–42.
- (19) Fito, M.; Cladellas, M.; de la Torre, R.; Martí, J.; Alcántara, M.; Pujadas-Bastardes, M.; Marrugat, J.; Bruguera, J.; López-Sabater, M. C.; Vila, J.; Covas, M. I. The members of the SOLOS Investigators. Antioxidant effect of virgin olive oil in patients with stable coronary heart disease: a randomized, crossover, controlled, clinical trial. *Atherosclerosis* **2005**, *181*, 149–158.
- (20) Léger, C. L.; Carbonneau, M. A.; Michel, F.; Mas, E.; Monnier, L.; Cristol, J. P.; Descomps, B. A thromboxane effect of a hydroxytyrosol-rich olive oil wastewater extract in patients with uncomplicated type I diabetes. *Eur. J. Clin. Nutr.* **2005**, *59*, 727–30.
- (21) Ramirez-Tortosa, M. C.; Urbano, G.; Lopez-Jurado, M.; Nestares, T.; Gomez, M. C.; Mir, A.; Ros, E.; Mataix, J.; Gil, A. Extra-virgin olive oil increases the resistance of LDL to oxidation more than refined olive oil in free-living men with peripheral vascular disease. *J. Nutr.* **1999**, *129*, 2177–2183.
- (22) Covas, M. I. Olive oil and the cardiovascular system. *Pharmacol. Res.* **2007**, *55*, 175–86.
- (23) Perona, J. S.; Cabello-Moruno, R.; Ruiz-Gutierrez, V. The role of virgin olive oil components in the modulation of endothelial function. *J. Nutr. Biochem.* **2006**, *17*, 429–445.
- (24) Quinn, M. T.; Parthasarathy, S.; Fong, L. G.; Steinberg, D. Oxidatively modified low density lipoproteins: a potential role in recruitment and retention of monocyte/macrophages during atherogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 2995–2998.
- (25) Dai, F.; Chen, W. F.; Zhou, B. Antioxidant synergism of green tea polyphenols with  $\alpha$ -tocopherol and L-ascorbic acid in SDS micelles. *Biochimie* **2008**, *90*, 1499–1505.
- (26) Vessby, B.; Aro, A.; Skarfors, E. The risk to develop NIDDM is related to the fatty acid composition of the serum cholesterol esters. *Diabetes* **1994**, *43*, 1353–1357.
- (27) Lovejoy, J. C.; Champagne, C. M.; Smith, S. R.; DeLany, J. P.; Bray, G. A.; Lefevre, M.; Denkins, Y. M.; Rood, J. C. Relationship of dietary fat and serum cholesterol ester and phospholipid fatty acids to markers of insulin resistance in men and women with a range of glucose tolerance. *Metabolism* **2001**, *50*, 86–92.
- (28) Warensjo, E.; Riserus, U.; Vessby, B. Fatty acid composition of serum lipids predicts the development of the metabolic syndrome in men. *Diabetologia*. **2005**, *48*, 1999–2005.
- (29) Brenner, R. R. Hormonal modulation of delta6 and delta5 desaturases: case of diabetes. *Prostaglandins, Leukotrienes, Essent. Fatty Acids*. **2003**, *68*, 151–162.
- (30) Ruiz-Gutierrez, V.; Stiefel, P.; Villar, J. Cell membrane fatty acid composition in type I (insulin-dependent) diabetic patients: relationship with sodium transport abnormalities and metabolic control. *Diabetologia* **1993**, *36*, 850–856.
- (31) Muriana, F. J.; Ruiz-Gutierrez, V.; Vazquez, C. M. Influence of dietary cholesterol on polyunsaturated fatty acid composition, fluidity and membrane-bound enzymes in liver microsomes of rats fed olive and fish oil. *Biochimie* **1992**, *74*, 551–556.
- (32) Yosefy, C.; Viskoper, J. R.; Varon, D.; Ilan, Z.; Pilpel, D.; Lugassy, G.; Schneider, R.; Savyon, N.; Adan, Y.; Raz, A. Repeated fasting and refeeding with 20:5, n-3 eicosapentaenoic acid (EPA): a novel approach for rapid fatty acid exchange and its effect on blood pressure, plasma lipids and hemostasis. *J. Hum. Hypertens.* **1996**, *10*, S135–S139.

---

Received for review July 6, 2009. Revised manuscript received October 21, 2009. Accepted October 27, 2009. This work was supported by funds from Comision Interministerial de Ciencia y Tecnologia (CICYT, AGL2008-02258/ALI) and Consejo Superior de Investigaciones Cientificas (CSIC, 2008701208).