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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

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In the present study we examined whether two virgin olive oils (VOO1 and VOO2), of the same variety (*Olea europaea* var. *hojiblanca*) and with a similar composition of minor components but differing in the content of triacylglycerol molecular species, had different effects on blood pressure and plasma lipid levels in a healthy elderly population. Thirty-one participants, aged 84·9 (SD 6·4) years, were asked to participate in the study. No differences were found with regard to blood pressure after both experimental periods (VOO1 and VOO2). However, plasma total cholesterol and LDL-cholesterol were reduced only after VOO1 ($P<0·01$). The reduction of plasma cholesterol concentrations was related to the incorporation of oleic acid into plasma cholesteryl esters and phospholipids, which was higher after VOO1 ($P<0·01$). Indeed, the oleic acid concentration in cholesteryl esters and phospholipids strongly correlated with plasma total cholesterol and LDL-cholesterol levels in all experimental periods studied ($r^2>0·418$, $P<0·07$), except for phospholipids in VOO1 ($P=0·130$ for total cholesterol and $P=0·360$ for LDL-cholesterol). These results have demonstrated that blood pressure and plasma lipids can be modified by the consumption of VOO in elderly people, but that the extent of such modification depends on the composition and amount of active minor components and triacylglycerol molecular species.

Virgin olive oil: Blood pressure: Cholesterol: Fatty acids: Elderly

In recent decades, ageing has acquired great interest, not only because elderly people form an increasing percentage of the population, but also because they can now enjoy an active and productive life beyond the retirement age. In elderly people, plasma cholesterol levels decline after age 70 years, leading to a reduction of the total cholesterol:HDL-cholesterol ratio (Newschaffer *et al.* 1992; Lamon-Fava *et al.* 1994; Wilson *et al.* 1994). However, in this population, a concomitant decrease of the absolute risk of CHD is not observed (Benfante *et al.* 1992; Krumholz *et al.* 1994). Both isolated systolic hypertension and combined systolic–diastolic hypertension are considered as major risk factors for cardiovascular disease in the elderly (Moser, 1999; Forette, 1999; Forette *et al.* 2000); for the moment, results on the effect of diet on blood pressure in very old subjects (>85 years) are scarce and conflicting (Forette, 1999).

Olive oil is the major source of fat in the Mediterranean diet, and it has been firmly associated with improvements in plasma lipid and lipoprotein levels and prevention of cardiovascular disease (Mata *et al.* 1992; Perez-Jimenez *et al.* 1995). In addition, it has been suggested recently that dietary virgin olive oil (VOO) reduces blood pressure. Ferrara *et al.* (2000) reported that VOO reduces the need for medication in hypertensive subjects, and this effect was attributed to enhanced NO levels by polyphenols. In our laboratory, we demonstrated that in normotensive and in hypertensive normocholesterolaemic and hypercholesterolaemic subjects dietary VOO lowered blood pressure when compared with another oleic acid-rich oil, such as high-oleic acid sunflower oil (HOSO) (Ruiz-Gutierrez *et al.* 1996, 1997). Moreover, VOO, but not HOSO, also normalized some altered functions of the erythrocyte membrane in hypertensive subjects (Ruiz-Gutierrez *et al.* 1996).

Abbreviations: HOSO, high-oleic acid sunflower oil; VOO, virgin olive oil.

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It was proposed that some other compounds distinct from oleic acid might be responsible for such effects, since the content of this fatty acid in VOO and HOSO was almost identical. We have also observed that the differences in the composition of triacylglycerol molecular species of VOO and HOSO exerts influence on the triacylglycerol composition of atherogenic triacylglycerol-rich lipoproteins, in both fasting (Ruiz-Gutierrez *et al.* 1998, 1999) and postprandial (Abia *et al.* 1999, 2001) conditions. Despite emerging results concerning the effects of VOO on blood pressure and serum lipoprotein concentrations, there is little information regarding elderly people, in part because VOO has been frequently considered neutral for cardiovascular disease (Busnach *et al.* 1998; Prisco *et al.* 1998; Mori *et al.* 1999).

The present study was conducted with the aim of assessing the effects of dietary VOO on blood pressure and on plasma lipid and lipoprotein levels in a normocholesterolaemic and normotensive elderly population (average age 85 years). In order to evaluate the hypothetical influence of triacylglycerol molecular species composition, we compared two VOO of the same variety, with a similar composition of minor components and fatty acids, except for oleic (18 : 1*n*-9) and linoleic (18 : 2*n*-6) acids. These slight differences in the fatty acid composition were, however, reflected in the triacylglycerol molecular species composition.

Materials and methods

Experimental design

A double-blind study was conducted over two 4-week periods, during which each participant ate a diet enriched in two VOO (*Olea europaea* var. *hojiblanca*; Grupo Hojiblanca, Antequera, Spain), VOO1 and VOO2, which substituted for sunflower oil- and maize oil-based margarines consumed habitually (baseline). Subjects were assigned to the VOO diets in a random-order sequence. A 4-week washout period was included between the two periods, during which the diet was returned to the baseline composition. Before the study, the health officers had recorded the regular dietary intake of the participants for four consecutive weeks, using a 24 h recall and food-frequency questionnaires. The energy consumption and nutrient intake were calculated, approved by a dietitian and employed as a basis for the diets of the study.

Subjects

The study was performed at Residencia Heliópolis (Junta de Andalucía, Sevilla, Spain), a residential home for the elderly, where the diet of all participants was controlled. Twenty-two women and nine men, who were residents at the beginning of the study, gave written, informed consent to a protocol approved by the Institutional Committee on Investigation in Humans (Hospitales Universitarios Virgen del Rocío, Sevilla, Spain). The participants were free-living and we gave them free food but no payment. All participants had been residents of the Residencia Heliópolis for at least 5 years, and consequently all their habits were completely known. The average age of the subjects was 84·9 (SD 6·4) years and their BMI was 28·0 (SD 4·8) kg/m^2. All participants were normocholesterolaemic and normotensive and none was diabetic or suffered from glucose intolerance or hypothyroidism. Fasting glucose levels were 94·4 (SD 8·4) mmol/l. None of the subjects was receiving any antihypercholesterolaemic or antihypertensive treatment. Cigarette smokers were excluded from the study and no case of alcohol abuse was detected among participants.

Diets

The studied diets were based on ordinary food and were planned for each 4-week period and were revised every week. The only difference between the diets lay in the composition of edible fats added, in the form of oils (VOO1 and VOO2) for cooking, salad dressing and occasionally for spreading on slices of bread. The menus were the same for each experimental period, including baseline, but the daily diet was adjusted to 30 % energy as fat, 55 % as carbohydrate and 15 % as protein, from nearly 35 % energy as fat at baseline. The composition of the two oils was similar with regard to minor components and fatty acids, except for the oleic and linoleic acid content (Table 1). However, the two oils presented substantial differences in the triacylglycerol molecular species composition (Table 2). The analysis of the fatty acid composition of the oils was performed by GC as described later for fatty acid determination of

Table 1. Fatty acid (g/100 g total fatty acids) and minor components (g/kg) of the two virgin olive oils employed in the experimental diets (VOO1 and VOO2)

(Mean values and standard deviations)

	VOO1		VOO2	
	Mean	SD	Mean	SD
Fatty acids				
16 : 0	10·9	1·8	13·9	1·9
16 : 1*n*-7	1·1	0·3	1·3	0·2
18 : 0	2·4	1·3	1·8	1·0
18 : 1*n*-9t	0·3	0·2	0·3	0·2
18 : 1*n*-9	74·6	0·6	66·5**	2·0
18 : 1*n*-7	3·5	0·1	4·6	0·6
18 : 2*n*-6	4·5	0·4	9·7**	1·4
18 : 3*n*-3	0·6	0·2	0·5	0·1
18 : 3*n*-6	1·1	0·3	0·9	0·1
20 : 1*n*-9	0·2	0·1	0·1	0·0
22 : 0	0·8	0·1	0·1	0·1
SFA	13·9	2·0	15·9	0·9
MUFA	79·3	0·3	72·4**	1·7
PUFA	6·2	0·6	11·1	1·5
Unsaponifiable matter (mg/g)	1·2	0·2	1·2	0·3
Sterols (mg/100 g)				
Campesterol	3·3	0·0	3·7	0·1
Stigmasterol	1·1	0·2	0·7	0·1
Clerosterol	1·4	0·7	1·1	0·3
β-Sitosterol	85·4	2·7	86·4	1·8
Δ-5-avenasterol	7·3	1·0	7·3	1·6
Others	1·5	0·9	1·0	0·4
Polyphenols (μg/g)	202·1	18·6	233·0	15·9

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.
Mean values were significantly different from those of VOO1: ** $P<0·01$.

Table 2. Triacylglycerol molecular species composition (mg/g) of the two virgin olive oils employed in the experimental diets (VOO1 and VOO2)

(Mean values and standard deviations)

	VOO1		VOO2	
Triacylglycerol	Mean	SD	Mean	SD
LLL	ND	ND	ND	ND
OLL	ND	ND	21	2
PLL	17	3	15**	3
OOL	70	3	152**	13
POL	34	1	69**	13
OOO	429	24	304**	30
SOL	ND	ND	17	0
POO	277	6	263	14
PPO + PLS	42	3	44	8
PPP	14	1	14	0
SOO	58	1	48	11
POS	24	4	19	4
SSO	20	4	6	1
SSP	16	1	ND	ND

ND, not detected; LLL, trilinoleoyl–glycerol; O, oleic acid; L, linoleic acid; P, palmitic acid; S, stearic acid; POO, palmitoyl–dioleoyl–glycerol; POS, palmitoyl–oleoyl–stearoyl–glycerol.

Mean values were significantly different from those of VOO1: $^{**}P<0.01$.

plasma phospholipids and cholesteryl esters. With regard to the non-fatty acid constituents, we fully determined total sterols, after extraction of the unsaponifiable matter and polyphenols as described previously (Ruiz-Gutierrez *et al.* 1997, 2000). Triacylglycerol composition was determined by reversed-phase HPLC following the method described by Perona & Ruiz-Gutierrez (1999).

Three diet samples were collected in each experimental period to be analysed for their fat content and other nutrients. One investigator was present several times in the kitchen during meal preparation without previous notification. The energy consumption was approximately 7530 kJ (1800 kcal)/d and the cholesterol intake was approximately 307 mg/d. Na and Ca intakes were identical in the baseline period and in both experimental periods.

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 Hg-gauge sphygmomanometer. At each visit three blood pressure measurements were used to determine eligibility. In addition, blood pressure was recorded at the beginning, middle and end of each experimental period. The measurement at the beginning of the first experimental period was considered as baseline and it was corroborated by a measurement at the washout period. All these measurements were performed under the same conditions.

Plasma lipid and lipoprotein analyses

Venous blood was obtained after an overnight fast, at the beginning and end of each period of the study. Blood was collected in Vacutainer® tubes (Beckton Dickinson, Meylan Cedex, France) and plasma obtained by centrifugation at 1500 rpm for 30 min at 4°C. Plasma samples were frozen below −80°C until analysed. Plasma total cholesterol and triacylglycerol concentrations and cholesterol concentrations in LDL and HDL were measured by conventional enzymatic methods (Bucolo & David, 1973; Allain *et al.* 1974).

Determination of plasma phospholipid and cholesteryl ester fatty acid composition

Total lipids were extracted following a modification of the method of Rose & Oaklander (1965), using 2,6-di-*tert*-butyl-*p*-cresol as antioxidant. Lipids were separated into fractions by TLC on silica gel 60 plates (Kieselgel 60 F254; Merck Espana, Barcelona, Spain) using an elution system of *n*-hexane–diethyl ether–acetic acid (80:20:1, by vol.) (Merck), according to the method of Ruiz-Gutierrez *et al.* (1992). The phospholipid and cholesteryl ester fractions were scrapped off the silica, eluted with chloroform–methanol (1:1, v/v) and *n*-hexane respectively, passed through a N_2 stream and stored at temperature below −20°C until analysed, always within the following few days.

Phospholipids and cholesteryl esters were transmethylated and the resulting fatty acid methyl esters analysed by GC as described by Ruiz-Gutiérrez *et al.* (1992) using a model 5890 series II GC (Hewlett-Packard Co., Avondale, PA, USA) equipped with a flame ionization detector and a capillary silica column Supelcowax 10 (Sulpelco Co., Bellefonte, CA, USA) of 60 m length and 0·25 mm internal diameter. Individual fatty acid methyl esters were identified by means of comparison of the retention times with those of standards. Fatty acid methyl esters for which no standards were available were identified by GC–MS on a Konik KNK-2000 chromatograph (Konik Co., Barcelona, Spain) interfaced directly to an AEJ MS30/790 VG MS (VG Analytical, Manchester, UK) using electron impact ionization mode. The ion source temperature was maintained at 200°C, the multiplier voltage was 4·0 kV, the emission current was 100 μA and the electron energy was 70 eV. The data were processed with a VG 11/250 data system (VG Analytical).

Statistical analyses

Values are shown as means and standard deviations. Data were evaluated by ANOVA with Tukey's *post hoc* comparison of the mean values and Pearson test for correlations. The significance of the differences between diets was evaluated by using a two-tailed unpaired *t* test. Differences were considered significant with a 99 % CI ($P<0.01$). The analyses were done with the SPSS package (*v.* 11.0.1; SPSS Inc. Chicago, IL, USA).

Results

All participants completed the study according to schedule. The assistant personnel for the study estimated compliance with the diets to be close to 90 % from the evaluation of daily food questionnaires and the visual examination of

the food remaining on the plates after every meal. Body weight was maintained after both VOO periods (baseline 67·3 (SD 11·5) (P=0·324), after VOO1 71·1 (SD 13·7) (P=0·956), after VOO2 67·0 (SD 12·7) (P=0·348) kg).

Both VOO1 and VOO2 diets were responsible for a similar reduction in systolic pressure, accounting for 10 and 12 mmHg respectively ($P<0·01$). Baseline systolic pressure was 143 (SD 8) mmHg, whereas this value after VOO1 was 133 (SD 7) mmHg and after VOO2 131 (SD 7) mmHg. Diastolic pressure was not significantly modified after consuming both VOO, and was maintained at average levels of about 70 mmHg.

The intake of VOO1, but not VOO2, was responsible for a reduction of plasma total cholesterol (by 10 %, $P<0·01$) and LDL-cholesterol (by 12 %, $P<0·01$) levels of elderly people compared with baseline (Table 3). Conversely, an increment in the serum triacylglycerol (by 24 % after VOO1, $P<0·01$ and by 31 % after VOO2, $P<0·01$) and a decrease in HDL-cholesterol (by 8 % after VOO1, $P<0·01$ and by 7 % after VOO2, $P<0·01$) concentrations were found after both periods. This slight, though significant, decrease in HDL-cholesterol concentration prevented a reduction in the total cholesterol:HDL-cholesterol and LDL-cholesterol:HDL-cholesterol ratios.

The plasma cholesteryl ester fatty acid composition is shown in Table 4. The consumption of VOO (VOO1 and VOO2) elevated the oleic acid content in these molecules by about 30 % ($P<0·01$). Although mirystoleic (14 : 1*n*-5) and palmitoleic acids (16 : 1*n*-7) were only increased after VOO1 ($P<0·01$), the elevation in the oleic acid content resulted in a significant increase in total monounsaturated fatty acids after both dietary periods (by 28 % after VOO1 and by 20 % after VOO2, $P<0·01$). However, linoleic acid and total polyunsaturated fatty acids were lowered in the plasma cholesteryl ester fraction after VOO1 (by 11 and 10 % respectively, $P<0·01$), but not after VOO2. Correspondingly, the linoleic acid:oleic acid ratio was reduced after both experimental periods, but to a greater extent after VOO1 (about 31 % after VOO1 and about 22 % after VOO2, $P<0·01$). In addition, the consumption of VOO2 lowered the content of total saturated fatty acids by 18 % ($P<0·01$) compared with baseline and VOO1.

Table 3. Plasma lipid levels (mg/l) of elderly subjects at baseline and after consuming the two virgin olive oil diets (VOO1 and VOO2)*

(Mean values and standard deviations for thirty-one elderly subjects)

	Baseline		VOO1		VOO2	
	Mean	SD	Mean	SD	Mean	SD
chol	1862[a]	381	1666[b]	377	1884[a]	431
LDL-chol	1130[a]	355	992[b]	324	1236[a]	379
HDL-chol	576[a]	178	481[b]	149	435[b]	147
Total chol:HDL-chol	33	10	36	10	43	12
LDL-chol:HDL-chol	20	9	22	8	26	12
Triacylglycerol	776[a]	300	963[b]	431	1023[b]	579

chol, cholesterol.
[a,b]Mean values within a row with unlike superscript letters were significantly different ($P<0·01$).
* For details of diets and procedures, see Tables 1 and 2 and p. 820.

Table 4. Plasma cholesteryl ester fatty acid composition (g/100 g total fatty acids) of elderly subjects at baseline and after consuming the two virgin olive oil diets (VOO1 and VOO2)*

(Mean values and standard deviations for thirty-one elderly subjects)

	Baseline		VOO1		VOO2	
Fatty acid	Mean	SD	Mean	SD	Mean	SD
14 : 0	0·4[a]	0·3	0·5[a]	0·2	0·2[c]	0·2
14 : 1*n*-5	0·5[a]	0·2	1·6[b]	0·1	0·3[a]	0·1
16 : 0	14·0[a]	2·9	13·3[ab]	3·1	11·8[b]	2·1
16 : 1*n*-9	2·1[a]	0·8	1·9[ab]	1·0	1·3[b]	0·7
16 : 1*n*-7	2·0[a]	0·5	3·2[b]	0·9	2·2[ac]	0·6
18 : 0	2·1[a]	0·7	2·2[a]	1·1	1·3[b]	0·6
18 : 1*n*-9	15·9[a]	3·1	20·2[b]	3·4	21·3[b]	2·7
18 : 1*n*-7	1·8	0·4	1·7	0·4	1·7	0·3
18 : 2*n*-6	49·9[a]	4·6	44·4[b]	6·8	49·1[a]	3·9
18 : 3*n*-6	1·2	0·3	1·2	0·5	1·0	0·4
18 : 3*n*-3	0·3	0·1	0·3	0·1	0·3	0·1
20 : 0	ND	ND	0·1	0·0	0·1	0·0
20 : 2*n*-6	1·0	0·3	1·0	0·2	1·0	0·3
20 : 4*n*-6	8·8	1·3	8·4	1·8	8·3	1·5
SFA	16·5[a]	4·0	16·1[a]	4·5	13·4[b]	2·9
MUFA	22·3[a]	5·1	28·6[b]	5·8	26·8[b]	4·5
PUFA	61·2[a]	6·6	55·3[b]	9·4	59·7[a]	6·2
18 : 2/18 : 1	3·2[a]	0·7	2·2[b]	0·4	2·5[c]	0·5

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; ND, not detected.
[a,b,c]Mean values within a row with unlike superscript letters were significantly different ($P<0·01$).
* For details of diets and procedures, see Tables 1 and 2 and p. 820.

Modifications in the plasma phospholipid fatty acid composition of the elderly subjects by the experimental diets were less evident (Table 5). For instance, the introduction of VOO in diet did not increase the incorporation of oleic acid to phospholipids. Indeed, the concentration of this fatty acid was significantly lower in plasma phospholipids after VOO2 compared with VOO1 and baseline diets (by 17 and 12 % respectively, $P<0·01$). As a consequence, the total monounsaturated fatty acid content was also lower after VOO2 (by 18 % after baseline and VOO1, $P<0·01$). Linoleic acid was less efficiently incorporated to phospholipids in subjects consuming VOO1 ($P<0·01$), but a significant difference in the linoleic acid:oleic acid ratio could not be detected. Arachidonic acid (20 : 4*n*-6) was slightly increased in plasma phospholipids after both VOO diets (VOO1 and VOO2) being significant only after VOO1 ($P<0·01$). Both VOO diets also induced the incorporation of polyunsaturated fatty acids (*n*-3) into phospholipids. At baseline, *n*-3 fatty acids could not be found, but after VOO1 and VOO2, α-linolenic (18 : 3*n*-3), eicosapentaenoic (20 : 5*n*-3), docosapentaenoic (22 : 5*n*-3) and docosahexaenoic (22 : 6*n*-3) acids appeared.

Plasma cholesteryl ester and phospholipid oleic and linoleic acids were plotted against plasma total and LDL-cholesterol concentrations (Table 6). Both fatty acids showed statistically significant linear correlations ($r^2>0·469$, $P<0·01$), which were inverse in the dietary periods studied (VOO1 and VOO2), except when oleic acid concentration in plasma phospholipids after VOO1 was plotted against LDL-cholesterol (P=0·120). The strongest correlation was found relating oleic acid in phospholipids with LDL-cholesterol after VOO2 (r^2 0·720, P=0·001).

Table 5. Plasma phospholipid fatty acid composition (g/100 g total fatty acids) of elderly subjects at baseline and after consuming the two virgin olive oil diets (VOO1 and VOO2)*

(Mean values and standard deviations for thirty-one elderly subjects)

	Baseline		VOO1		VOO2	
Fatty acid	Mean	SD	Mean	SD	Mean	SD
14:0	0·5	0·2	0·5	0·2	0·4	0·2
14:1*n*-5	0·6	0·2	0·7	0·3	0·7	0·2
16:0	28·6	2·5	27·9	2·5	29·2	2·7
16:1*n*-9	0·5[a]	0·2	0·5[a]	0·3	0·2[b]	0·1
16:1*n*-7	0·7[a]	0·3	1·0[a]	0·4	0·6[b]	0·2
16:4*n*-7	0·6[a]	0·2	0·4[b]	0·2	0·4[b]	0·1
18:0	15·0	2·7	14·6	3·0	14·8	1·4
18:1*n*-9t	1·0	0·1	0·5	0·3	0·1	0·0
18:1*n*-9	14·0[a]	2·6	13·2[a]	2·9	11·6[b]	1·5
18:1*n*-7	2·0	0·6	2·0	0·6	2·0	0·6
18:2*n*-6	20·1[a]	2·4	18·5[b]	2·0	20·0[c]	3·1
18:3*n*-6	0·4[a]	0·1	0·3[a]	0·2	0·2[b]	0·1
18:3*n*-3	ND	ND	0·2	0·0	0·2	0·1
20:0	0·2[a]	0·1	0·2[a]	0·1	0·1[b]	0·0
20:1*n*-9	0·3[a]	0·1	0·6[b]	0·4	0·2[c]	0·0
20:1*n*-7	0·2[a]	0·0	0·1[b]	0·1	0·1[a]	0·1
20:2*n*-6	3·3[a]	0·6	3·6[a]	1·0	3·8[b]	0·4
20:4*n*-6	10·3[a]	2·3	11·9[b]	2·3	11·4[a]	2·4
22:0	ND	ND	ND	ND	0·5	0·0
20:5*n*-3	ND	ND	0·1	0·0	0·4	0·0
22:1*n*-9	0·6[a]	0·1	1·0[a]	0·8	0·1[b]	0·0
22:4*n*-6	0·5	0·1	0·5	0·2	0·5	0·2
22:5*n*-6	0·3	0·1	0·3	0·0	0·4	0·1
22:5*n*-3	ND	ND	0·8[a]	0·5	0·4[b]	0·1
22:6*n*-3	ND	ND	0·3	0·1	0·4	0·1
24:1*n*-9	ND	ND	ND	ND	0·5	0·1
SFA	44·4	5·6	43·2	5·8	45·1	4·4
MUFA	19·8[a]	4·3	19·6[a]	5·6	16·2[b]	3·0
PUFA	35·9	6·0	37·3	7·1	37·6	7·2
18:2/18:1	1·5	0·3	1·4	0·3	1·8	0·4

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; ND, not detected.

[a,b,c]Mean values within a row with unlike superscript letters were significantly different ($P<0·01$).

* For details of diets and procedures, see Tables 1 and 2 and p. 820.

Discussion

In the present study we compared the effects of two diets rich in VOO (VOO1 and VOO2), with an equivalent composition of minor components but differing in their content of oleic and linoleic acids, and thus, in the triacylglycerol molecular species composition, on plasma lipids and blood pressure of healthy elderly people. Our present results have demonstrated for the first time that dietary VOO lowers systolic blood pressure among very old people (average 85 years). Unfortunately, for the moment there are few results available on the effects of dietary oils on blood pressure in such a population group. Two double-blind randomized-controlled studies have compared the effects on blood pressure of the administration of *n*-3 (fish oil) and *n*-6 fatty acids, in the form of capsules, to elderly people, and have reported a greater reduction of blood pressure in hypertensive patients receiving fish oil (Varicel *et al.* 1999; Wensing *et al.* 1999). When the intake of capsules of fish oil was compared with olive oil as a placebo, no significant effect in the reduction of blood pressure was found (Meland *et al.* 1989; Wing *et al.* 1990; Du Plooy *et al.* 1992).

Ruiz-Gutierrez *et al.* (1996, 1997) demonstrated that VOO reduces blood pressures of healthy and hypertensive subjects compared with HOSO, and suggested that the lack of effect found after the intake of HOSO was due to differences in non-fatty acid constituents, such as triacylglycerol molecular species or minor components. In the present study we have found no significant differences in the effect of VOO1 and VOO2 in regard to blood pressure. The analysis of the oils employed in the diets revealed differences in the triacylglycerol molecular species composition between them but a virtually identical composition of the minor component fraction. Therefore, our present results support the idea that minor components might be related to the reduction of blood pressure, at least in elderly people.

Table 6. Correlations between cholesteryl ester (CE) and phospholipid (PL) oleic (O) and linoleic (L) acids concentrations with variations in plasma total (T) and LDL-cholesterol (chol) concentrations from baseline, after consuming the two virgin olive oil diets (VOO1 and VOO2)*

	Variables	r^2	*P*	Equation	SE†	SE‡	SE§	*n*
CE								
VOO1	O–Tchol	0·523	0·015	$y=-5·743x+101·100$	18·9	1·5	28·6	26
	L–Tchol	0·485	0·039	$y=2·199x-102·812$	18·5	2·6	18·3	26
	O–LDL-chol	0·511	0·018	$y=-6·461x+119·863$	21·8	1·7	32·9	26
	L–LDL-chol	0·574	0·010	$y=2·919x-137·739$	20·6	0·7	31·4	26
VOO2	O–Tchol	0·544	0·040	$y=-3·919x+89·352$	18·7	1·1	23·8	22
	L–Tchol	0·485	0·027	$y=1·722x-78·379$	14·2	0·5	22·6	22
	O–LDL-chol	0·609	0·006	$y=-4·969x+113·804$	19·2	1·1	24·3	22
	L–LDL-chol	0·666	0·002	$y=4·447x-212·689$	26·0	0·9	41·4	22
PL								
VOO1	O–Tchol	0·598	0·032	$y=-7·088x+86·451$	15·3	1·8	27·8	26
	L–Tchol	0·469	0·010	$y=6·241x-109·708$	18·1	2·0	38·0	26
	O–LDL-chol	0·225	0·120	$y=-5·286x+60·554$	26·1	3·1	47·2	26
	L–LDL-chol	0·548	0·038	$y=7·585x-150·673$	18·8	2·1	39·4	26
VOO2	O–Tchol	0·696	0·000	$y=-6·680x+84·794$	11·1	1·2	15·3	22
	L–Tchol	0·688	0·002	$y=5·847x-124·128$	12·7	1·2	27·1	22
	O–LDL-chol	0·720	0·001	$y=-9·150x+120·385$	13·1	1·4	17·7	22
	L–LDL-chol	0·492	0·016	$y=9·350x-190·823$	25·7	2·4	52·9	22

* For details of diets and procedures, see Tables 1 and 2 and p. 820.

† Standard error of the correlation.

‡ Standard error of the slope.

§ Standard error of the independent term.

Interestingly, our present results have revealed that total cholesterol and LDL-cholesterol concentrations were reduced after the period of consuming VOO1, but not after VOO2, compared with baseline diet, which was rich in sunflower oil- and maize oil-based margarine (Table 3). Numerous studies have suggested that dietary intake of olive and sunflower oils has similar effects on plasma lipid concentrations (Gustafsson *et al.* 1992; Mata *et al.* 1992; Trautwein *et al.* 1999). However, there is still need for consensus on this effect. Some recent studies designed to compare the effects of olive oil with other *n*-6 fatty acid-rich oils on human plasma lipid concentrations have shown increasing cholesterol and LDL-cholesterol levels after consuming olive oil (Howell *et al.* 1998; Pedersen *et al.* 2000). In contrast, others have reported cholesterol reductions by olive oil (Sirtori *et al.* 1992; Madigan *et al.* 2000). These discrepancies may be due to differences in experimental conditions, including the employment of different varieties of olive oil. Since the dietary VOO employed in the present study were of the same variety (*hojiblanca*) and had a similar content of minor components, the difference observed in the reduction of total cholesterol and LDL-cholesterol levels in elderly people cannot be attributed to this fraction. The adjustment of dietary fat content might result in the concomitant reduction in HDL-cholesterol levels observed, according to Morgan *et al.* (1993).

Several authors have reported results showing that not all monounsaturated oils have the same effect on plasma cholesterol (Perez-Jimenez *et al.* 1995; Ruiz-Gutierrez *et al.* 1996; Truswell & Choudhury, 1998). Studies comparing the effects of VOO and HOSO revealed that the former improved plasma lipid metabolism and erythrocyte membrane homeostasis (Ruiz-Gutierrez *et al.* 1996, 1997; Abia *et al.* 1999, 2001). These oils have a similar fatty acid composition, but differ in their triacylglycerol molecular species content. For this reason, those studies suggested that triacylglycerols were not merely fatty acid transporters and might have a relevant role in such effects. The oils employed in the present study were selected to present differences in this lipid class: whereas VOO1 contained a greater amount of triolein, VOO2 was significantly enriched in species containing linoleic acid (Table 2). We have previously demonstrated that linoleoyl-species of triacylglycerols from HOSO were incorporated to a greater extent into chylomicrons and VLDL compared with VOO (Ruiz-Gutierrez *et al.* 1999; Abia *et al.* 1999, 2001).

The present results show that in addition to triacylglycerols, dietary linoleic acid is also relatively conserved in plasma as phospholipids and cholesteryl esters. The plasma cholesteryl ester fatty acid composition of the elderly subjects confirmed the addition of VOO to the diet. Cholesteryl oleate was increased in plasma by about 30 % compared with baseline after both periods of consuming VOO. However, a concomitant reduction in the content of cholesteryl linoleate was observed only after the VOO1 period. Indeed, the concentration of linoleic acid was also lower in plasma phospholipids after VOO1. Interestingly, this was the only period in which total cholesterol and LDL-cholesterol was reduced from baseline. Thus, we investigated for a link between oleic and linoleic acids in cholesteryl esters and phospholipids with plasma total cholesterol and LDL-cholesterol. Both fatty acids showed a statistically significant linear correlation with these plasma variables, being inverse in the experimental periods studied (VOO1 and VOO2). Thus, the greater concentration of oleic acid in cholesteryl esters and phospholipids, the lower the levels of plasma total cholesterol and LDL-cholesterol. In plasma, the transfer of cholesteryl esters and phospholipids from HDL towards apolipoprotein B-containing lipoproteins (LDL and VLDL) are mediated by the cholesteryl ester transfer protein and phospholipid transfer protein respectively, which can be regulated by dietary fatty acids (Lagrost *et al.* 1999). In particular, oleic acid reduces plasma cholesteryl ester transfer protein activity compared with dietary linoleic (Kurushima *et al.* 1995), palmitic (Lagrost *et al.* 1999) and elaidic (Abbey & Nestel, 1994) acids. In human subjects, cholesteryl ester transfer protein activity and concentration have been proposed as determinants of plasma LDL-cholesterol levels since these variables correlate positively (Kinoshita *et al.* 1996; Moulin, 1996). Accordingly, the decreased levels of LDL-cholesterol found in plasma of elderly people in the present study after VOO1 might be related to the higher concentration of plasma oleic acid in phospholipids and cholesteryl esters and/or to the diminishing concentrations of linoleic acid in these molecules.

In conclusion, we observed that both dietary oils studied (VOO1 and VOO2) exhibited the same effect on blood pressure and as an equivalent composition of minor components was found in them, we suggest that this fraction might be responsible for the reduction of blood pressure documented here and in other studies. However, we found that, compared with VOO2, VOO1 reduced plasma total cholesterol and LDL-cholesterol in elderly people and that this reduction was related to the incorporation of oleic acid into plasma phospholipids and cholesteryl esters. This incorporation was related to differences in the triacylglycerol molecular species compositions of the oils, since VOO1 was enriched in triolein, whereas VOO2 was enriched in triacylglycerols containing linoleic acid. Therefore, the present results stress the importance of the dietary oil choice, since not all VOO have the same effects on risk factors related to cardiovascular disease. We suggest that the VOO variety and its content of minor components and triacylglycerol molecular species must be considered in that choice.

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