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Olive oil phenols modulate the triacylglycerol molecular species of human very low-density lipoprotein. A randomized, crossover, controlled trial

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Abstract

Virgin olive oil phenolic compounds have been revealed to be potent antioxidants as part of the Mediterranean diet. To test the hypothesis that these phenolics can modulate the serum and very low-density lipoprotein (VLDL) triacylglycerol concentrations in humans, a double-blind, randomized, crossover trial was designed. Thirty-three participants received 25 mL/d of refined olive oil (devoid of phenolic content [PC]), common olive oil (PC = 370 mmol/kg), and virgin olive oil (PC = 825 mmol/kg) in a Latin square design. The 3 olive oils were administered over 3 periods of 3 weeks, each one preceded by 2-week washout periods. All analyses were carried out on an intention-to-treat basis. The interventions did not modify the concentrations of serum and low-density lipoprotein cholesterol and triacylglycerol; but they exerted changes in the cholesterol, triacylglycerol, and phospholipid content of VLDL. The virgin olive oil consumption led to increased oleic and palmitic acids, as well as decreased linoleic acid, in VLDL. The main outcome was the significant dose-dependent linear trend between the PC in the olive oils and the palmitic (16:0) and linoleic (18:2 n-6) acid and their corresponding triacylglycerol molecular species in VLDL.

1. Introduction

Virgin olive oil (VOO), the primary source of fat in the Mediterranean diet, is a functional food that, besides presenting a high monounsaturated fatty acid (MUFA) concentration, contains multiple minor components with relevant biological activities [1,2]. Phenolic compounds have been revealed to be potent antioxidants by counteracting lipid and DNA oxidation [3,4]. Commercially available olive oils are VOO and common olive oil (COO) [5], which is a mixture of VOO and refined olive oil (ROO). Virgin olive oil is obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions that do not lead to alteration in the oil [6]. Although the content of both total and individual phenolics in VOO varies among cultivars and harvests, free forms of tyrosol and hydroxytyrosol and their secoroid derivatives have been described as representing around 30%, with other conjugated forms such as oleuropein and ligstroside aglycones representing almost half, of the total phenolic content (PC) [7] of an olive oil. Olive oil phenolic compounds are lost in the refining process; thus, ROO is devoid of them.

Data from well-designed randomized intervention trials show that consumption of high-phenolic VOO can decrease the lipid oxidative damage [8], as well as serum total cholesterol and triacylglycerol (TG), and increase serum high-density lipoprotein (HDL) cholesterol concentrations [8,9]. Marrugat et al [10] reported, in the same population as in the present study, that sustained consumption of VOO

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with high PC was more effective in protecting low-density lipoprotein (LDL) from oxidation than that of other type of olive oils. A healthy nutrigenomic effect of olive polyphenols in the frame of the Mediterranean diet has been recently reported [11].

There is evidence supporting a potential role for fasting TG as a vascular risk factor [12,13]. Fasting TGs are strongly determined by very low-density lipoprotein (VLDL) concentrations [14]. Virgin olive oil consumption can modify the lipid composition of VLDL [15,16], mainly by modulating the incorporation of lipids into newly formed VLDL particles [17] in hepatocytes. There is, however, a controversy concerning the components responsible for such an effect. From our previous data, consumption of VOOs with a similar fatty acid composition, but differences in unsaponifiable minor components, caused differences in the molecular species composition of VLDL-TG in elderly individuals [16]. The unsaponifiable fraction of VOO, when incorporated into human lipoproteins, can also influence the uptake of TG-rich lipoproteins (TRL) via regulation of LDL receptor-related protein expression [18]. Moreover, these minor components can influence the hepatic VLDL release via modulation of the expression of enzymes involved in VLDL synthesis and secretion [19]. Dietary phenolic compounds have been shown to be able to reduce serum and VLDL-TG concentrations in streptozotocin-nicotinamide–induced diabetic rats [20] and guinea pigs [21]. However, to date, no randomized crossover study has examined the hypothesis that VOO phenolics can modulate the serum and VLDL-TG concentrations in humans. The present study was designed as a double-blind, randomized, crossover trial in which participants were assigned to receive 25 mL/d of 3 similar olive oils, but with differences in their PC (ranging from 0 to 825 mmol/kg of olive oil). Our aim was to assess whether differences in the PC of olive oil can promote differential changes in VLDL lipid and TG composition.

2. Methods

2.1. Participants

An individual screening visit was conducted to ascertain eligibility and to obtain baseline data. Forty-two men from a religious center in Barcelona (Spain) were screened for inclusion. Subjects with any of the following conditions were excluded: smoking, intake of any drug or supplements with established antioxidative properties in the 2 weeks before the onset of the study, obesity (body mass index [BMI] >30 kg/m^2), diabetes, and any disease or condition that would impair compliance. Nine of them were ineligible: 4 had BMI greater than 30 kg/m^2, 4 had diabetes, and 1 was taking antioxidant supplementation. Thus, 33 healthy volunteers, from 23 to 91 years old, with a regular lifestyle and dietary habits were included. The meals consumed throughout the study were prepared and consumed in the religious center. Volunteers maintained their regular physical activity and lifestyle throughout the study and gave their written consent before participation. The local Institutional Review Board (Comité Ético de Investigación Clínica del Instituto Municipal de Asistencia Sanitaria) approved the protocol according to the Helsinki Declaration of 1975 as revised in 1983.

2.2. Description of procedures

Three olive oils provided by the Olive Oil Cooperative Association of Catalonia were used. They were obtained from the same harvest; therefore, olive fruits were of the same cultivar, collection time, and soil. First, a VOO with a PC of 825 mmol caffeic acid equivalents per kilogram was selected. Afterward, we used an ROO (PC of 0 mmol/kg) and a COO (PC of 370 mmol/kg) with similar fatty acid composition, α-tocopherol and β-carotene content, to match the VOO. The characteristics of the oils are summarized in Table 1. The acidity value, the peroxide index, and the UV spectrophotometric index (K270) were determined following the analytical methods described in the European Union Commission Regulation CE/1989/200326. Fatty acids were transformed into methyl esters and analyzed by gas chromatography [22]. α-Tocopherol was measured by high-performance liquid chromatography, as previously described [23]. Phenolic compounds were measured by the Folin-Ciocalteu method [24].

2.3. Study design

A placebo-controlled, double-blind, crossover, randomized, supplementation trial was conducted. A Latin square for the 3 treatments was used in the crossover trial to randomize participants into 3 orders of olive oil administration: VOO-COO-ROO (order 1), COO-ROO-VOO (order 2), and ROO-VOO-COO (order 3). The 3 olive oils were administered over 3 periods of 3 weeks, each one preceded

Table 1
Fatty acid and TG composition of VOO, COO, and ROO

	VOO	COO	ROO
Fatty acids (%)			
16:0	14.43	16.30	14.47
16:1 n-7	0.99	1.14	1.05
18:0	1.80	2.04	2.21
18:1 n-9	72.40	68.83	69.62
18:2 n-6	7.99	9.24	10.31
Others	2.39	2.44	2.34
TGs (%)			
LOO	11.08	13.35	11.22
LOP	2.21	3.39	3.11
OOO	54.79	47.93	49.18
OOP	30.46	32.92	33.95
Others	1.46	2.40	2.54
Phenolic compounds (mmol/kg CAE)	825	370	0
α-Tocopherol (mol/kg)	65.88	48.22	47.98

CAE indicates caffeic acid equivalents.

by 2-week washout periods. Participants were requested to ingest a daily dose of 25 mL of raw olive oil, distributed over the 3 meals of the day, during intervention periods. The 25-mL dose was chosen as one less than the usual daily intake of some Mediterranean areas [25] and because higher amounts may elicit an oxidative postprandial response when taken as a single dose [26]. Refined olive oil was used as source of raw fat in washout periods. Other cooking fats were replaced by ROO to maintain energy and oleic acid intake unchanged during the entire study. Detailed description of the intervention can be found in Marrugat et al [10] and Gimeno et al [27].

2.4. Blood sampling and laboratory analyses

Laboratory measurements were carried out on samples from fasting subjects taken at the beginning of the study (before the first washout period, baseline), and before and after the olive oil interventions. Plasma was separated by centrifugation at 1000*g* at 4°C for 15 minutes. Total cholesterol, HDL cholesterol, and TG concentrations were measured by standard enzymatic methods (ROCHE; Roche Diagnostics, Basel, Switzerland). Low-density lipoprotein cholesterol was calculated by the Friedewald formula whenever TG was inferior to 300 mg/dL. Very low-density lipoproteins were isolated from 4 mL of serum layered with 6 mL of a NaCl solution (density, 1.006 g/mL) by a single ultracentrifugation spin (227 000*g*, 18 hours, 4°C), performed using a Beckman 50.4 rotor in a BECKMAN XL-70 preparative ultracentrifuge (Beckman Instruments, Palo Alto, CA). Total lipids were extracted from VLDL following a modification of the method of Folch et al [28], using butyl-hydroxy-toluene (BHT) as antioxidant. The lipids extracted were preserved under −20°C until used. Lipid classes and TG molecular species were analyzed as described previously by Perona and Ruiz-Gutierrez [29,30] using a high-performance liquid chromatography system (2690 Separations Module; Waters, Milford, MA) coupled to a light-scattering detector (W2420, Waters). The partition number (PN) of TG was calculated as PN = CN − 2·DB (CN, carbon number; DB, number of double bonds). For fatty acid analysis, lipids were transmethylated using sodium methoxide in methanol (0.5%); and the resulting fatty acid methyl ester (FAME) was analyzed by gas chromatography using a model 5890 Series II gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a flame ionization detector and a capillary silica column Supelcowax 10 (Supelco, Bellefonte, PA) of 60-m length and 0.25-mm internal diameter as previously described [31].

2.5. Statistical analyses

Results are expressed as mean ± SD, unless otherwise stated. One-factor analysis of variance and Kruskal-Wallis tests were used, as appropriate, to analyze the differences in basal characteristics among the 3 groups of order of olive oil administration. A general linear model for repeated measurements was used, using the Pearson's coefficient, with multiple comparisons corrected by Tukey's method, to assess differences among groups. All analyses were carried out on an intention-to-treat basis. Prism 4.0 (GraphPad Software, La Jolla, CA) statistical package was used for all the analyses. Statistical significance was defined as $P < .05$ for a 2-sided test.

3. Results

3.1. Subjects and interventions

Table 2 shows the baseline characteristics of the volunteers enrolled for the study. No significant baseline differences were found among participants in each group regarding age, BMI, lipid concentrations, and LDL oxidation. Compliance with oil intakes was good as reflected in the direct dose-dependent increase of urinary tyrosol and hydroxytyrosol with the PC of the olive oil (Fig. 1). No adverse effects due to interventions were observed. The outcomes of on-treatment analysis were identical to those of intention-to-treat analysis.

3.2. Effects of the interventions on serum lipid concentrations

No changes in serum cholesterol and total TG concentrations were observed after olive oil interventions (Table 3). In contrast, the concentrations of all analyzed lipids (free cholesterol, TG, and phospholipids) were significantly increased in isolated VLDL after consumption of COO and ROO compared with VOO. The apolipoprotein B (apo B) concentration in VLDL decreased ($P < .05$) after VOO consumption compared with both the baseline and the ROO intervention. The VLDL TG/apo B ratio increased ($P < .05$) after consumption of VOO and COO compared with both the baseline and the ROO intervention.

3.3. Fatty acid composition of VLDL-TGs

Despite the fact that all olive oils provided a similar fatty acid composition, we found significant differences in the

Table 2
Serum glucose and lipids by subgroups of subjects regarding the order of olive oil administration at the time they were enrolled for the study

	Group		
	V-C-R (n = 12)	C-R-V (n = 11)	R-V-C (n = 10)
Age, y	55.1 ± 20.5	60.3 ± 18.1	56.6 ± 19.3
BMI, kg/m^2	25.5 ± 5.6	24.1 ± 4.0	23.6 ± 2.9
Waist circumference, cm	95.7 ± 12.8	92.1 ± 9.8	88.8 ± 7.9
Total cholesterol (mg/dL)	216.0 ± 43.6	218.0 ± 34,8	228.0 ± 45.5
LDL cholesterol (mg/dL)	141.0 ± 41.5	143.2 ± 37.1	150.9 ± 39.2
HDL cholesterol (mg/dL)	54.0 ± 12.3	53.3 ± 11.5	59.3 ± 11.9
TGs (mg/dL)	112.0 ± 56.3	103.1 ± 35.4	88.5 ± 44.3

Data are presented as mean ± SD.

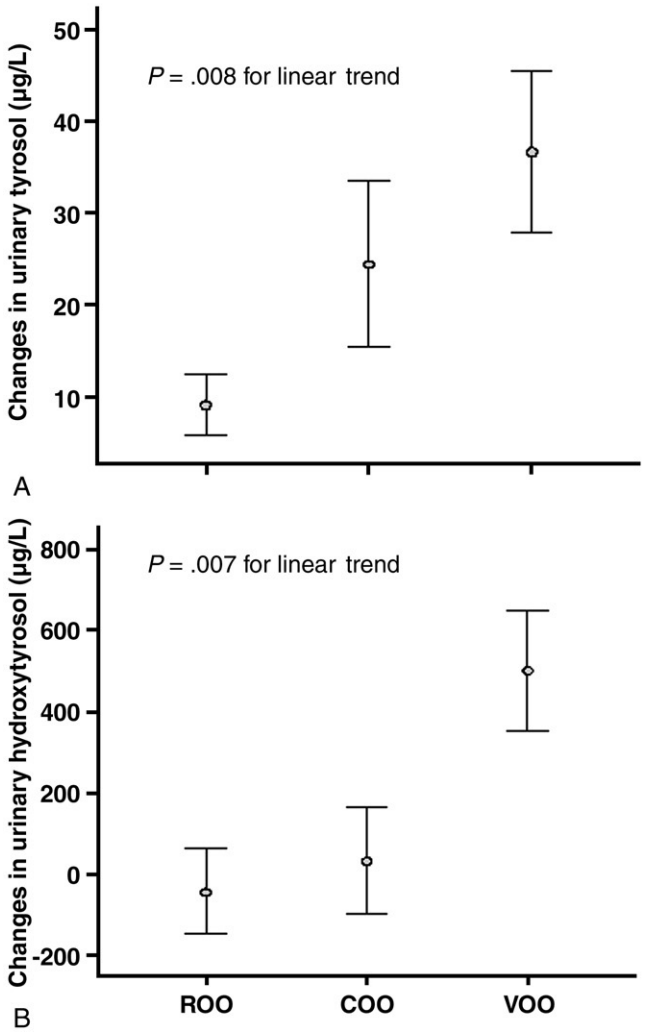

Fig. 1. Change (from before to after olive oil intervention) in urinary tyrosol and hydroxytyrosol in ROO, COO, and VOO intervention periods (mean ± SD, n = 33). A, Tyrosol. B, hydroxytyrosol.

fatty acid composition of VLDL-TG after olive oil interventions vs baseline (Table 4). Virgin olive oil consumption caused an increase in the content of palmitic (16:0) and oleic acid (18:1 n-9), but a reduction in the linoleic acid (18:2 n-6) content. As a consequence, the MUFA concentration in VLDL was significantly increased, whereas that of polyunsaturated fatty acids and total n-6 fatty acids decreased. Concentration of palmitic and oleic acids did not change after COO and ROO interventions when compared with the baseline. Consumption of COO and ROO resulted in significantly higher linoleic acid content in VLDL-TG compared with VOO consumption. The difference between the linoleic acid content after ROO and COO consumption was also significant. Linear trends between fatty acid concentrations in VLDL and the PC of the olive oils were calculated. The concentration of palmitic acid decreased linearly as the PC of the oils increased (VOO > COO > ROO), whereas that of linoleic acid increased ($P < .05$, Fig. 2A).

Table 3
Serum and VLDL lipids on each olive oil intervention period with VOO, COO, or ROO (in milligrams per milliliter)

	Baseline	VOO	COO	ROO
Total C	5.4 ± 0.9	5.5 ± 0.8	5.4 ± 0.8	5.6 ± 0.9
Total TG	0.99 ± 0.50	0.99 ± 0.40	0.94 ± 0.4	1.00 ± 0.50
VLDL-CE	0.52 ± 0.16^{a}	0.44 ± 0.25^{a}	0.89 ± 0.66^{b}	0.84 ± 0.85^{ab}
VLDL-TG	4.67 ± 1.58^{a}	3.82 ± 1.78^{a}	5.42 ± 1.36^{b}	5.65 ± 4.10^{b}
VLDL-C	0.50 ± 0.17^{a}	0.35 ± 0.11^{b}	0.58 ± 0.10^{a}	0.55 ± 0.21^{a}
VLDL-PL	2.84 ± 0.58^{a}	1.98 ± 0.51^{b}	3.15 ± 0.65^{a}	3.19 ± 1.51^{a}
VLDL–Apo B	0.37 ± 0.13^{a}	0.17 ± 0.84^{b}	0.23 ± 0.01^{b}	0.36 ± 0.13^{a}
TG/Apo B	11.1 ± 2.8^{a}	26.5 ± 12.7^{b}	29.2 ± 15.3^{b}	9.2 ± 1.3^{a}

Data are presented as mean ± SD. Values sharing the same latter in each row are not statistically significant ($P > .05$). n = 33. C indicates total cholesterol; CE, cholesteryl esters; PL, phospholipids.

3.4. Molecular species composition of VLDL-TGs

Differences in the molecular species composition of VLDL-TG after olive oil interventions were observed (Table 5). The main species found in VLDL were dioleoyl-palmitoyl-glycerol (OOP), linoleoyl-dipalmitoyl-glycerol (LPP), linoleoyl-oleoyl-palmitoyl-glycerol (LOP), tripalmitin (PPP), and triolein (OOO), accounting for approximately 80% of total TG. The content of LOP and LPP was significantly lower in VLDL after all olive oil interventions, with the exception of LPP after ROO. As a general rule, the concentration of all linoleic acid–containing species was lower after consumption of olive oils compared with baseline. In contrast, the content of oleic and palmitic acid–containing species in VLDL was higher than at baseline, reaching significance ($P < .05$) for OOP, oleoyl-dipalmitoyl-glycerol,

Table 4
Fatty acid composition of VLDL-TGs at baseline and after the intervention with VOO, COO, or ROO (in milligrams per 100 milligrams)

	Baseline	VOO	COO	ROO
16:0	21.38 ± 0.95^{a}	23.18 ± 2.53^{b}	22.17 ± 1.27^{ab}	21.90 ± 1.96^{a}
16:1 n-7	3.69 ± 0.31	3.71 ± 0.66	3.54 ± 0.30	3.41 ± 0.43
18:0	3.72 ± 0.63^{a}	3.67 ± 0.33^{a}	4.48 ± 0.47^{b}	3.86 ± 0.13^{a}
18:1 n-9	42.12 ± 1.08^{a}	43.62 ± 3.09^{b}	42.89 ± 1.17^{ab}	42.91 ± 1.85^{ab}
18:1 n-7	2.48 ± 0.03^{a}	2.62 ± 0.29^{b}	2.73 ± 0.12^{c}	2.57 ± 0.11^{ab}
18:2 n-6	22.14 ± 0.96^{a}	18.67 ± 2.05^{b}	19.64 ± 1.46^{c}	20.91 ± 1.18^{d}
18:3 n-3	1.09 ± 0.10^{a}	1.05 ± 0.09^{ab}	1.00 ± 0.15^{b}	1.04 ± 0.11^{ab}
20:1 n-9	0.15 ± 0.01^{a}	0.14 ± 0.02^{a}	0.14 ± 0.04^{a}	0.11 ± 0.03^{b}
20:2 n-6	0.45 ± 0.06^{a}	0.38 ± 0.03^{b}	0.37 ± 0.02^{b}	0.36 ± 0.05^{b}
20:4 n-6	1.66 ± 0.17	1.58 ± 0.01	1.66 ± 0.12	1.63 ± 0.16
20:5 n-3	0.23 ± 0.07^{a}	0.23 ± 0.05^{a}	0.26 ± 0.01^{ab}	0.27 ± 0.08^{b}
22:5 n-3	0.38 ± 0.19^{a}	0.25 ± 0.03^{b}	0.26 ± 0.02^{b}	0.29 ± 0.06^{b}
22:6 n-3	0.77 ± 0.14^{a}	0.93 ± 0.25^{b}	1.01 ± 0.30^{b}	1.05 ± 0.07^{b}
SFA	25.10 ± 0.60^{a}	26.85 ± 2.84^{b}	26.64 ± 1.01^{b}	25.76 ± 2.00^{ab}
MUFA	48.33 ± 0.75^{a}	50.07 ± 2.73^{b}	49.27 ± 1.04^{ab}	48.97 ± 1.64^{ab}
PUFA	26.56 ± 1.08^{a}	23.08 ± 2.35^{b}	23.93 ± 1.71^{b}	25.27 ± 0.83^{c}
n-3	2.31 ± 0.12^{a}	2.45 ± 0.39^{a}	2.29 ± 0.32^{a}	2.66 ± 0.32^{b}
n-6	24.25 ± 0.96^{a}	20.63 ± 2.04^{b}	21.64 ± 1.39^{c}	22.85 ± 1.04^{d}
n3/n6	0.10 ± 0.00^{a}	0.12 ± 0.01^{b}	0.11 ± 0.01^{c}	0.10 ± 0.02^{a}

Data are presented as mean ± SD. Values sharing the same latter in each row are not statistically significant ($P > .05$). n = 33. SFA indicates saturated fatty acids; PUFA, polyunsaturated fatty acids; n-3, n-3 fatty acids; n-6, n-6 fatty acids.

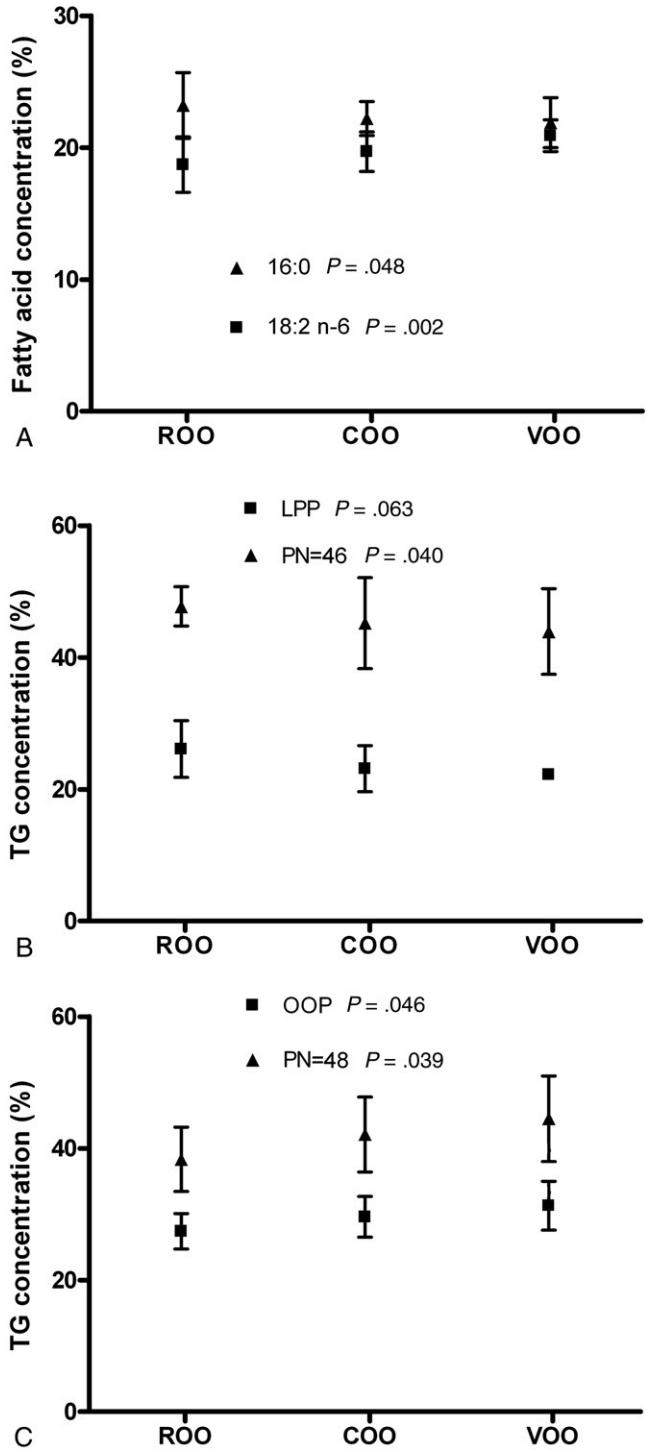

Fig. 2. Fatty acid (A) and TG (B and C) concentrations after ROO, COO, and VOO intervention periods expressed as percentage of total fatty acids or percentage of total TGs (n = 33). PN = 46, sum of all TG molecular species with PN = 46; PN = 8, sum of all TG molecular species with PN = 48.

OPP, and OOO after VOO consumption, and for PPP after ROO consumption. The content of LPP and of all TGs within PN = 46 decreased linearly when plotted against the PC of the oils, whereas that of OOP and TG with PN = 48 increased (Fig. 2B, C).

Table 5
Molecular species composition of VLDL-TGs at baseline and after the intervention with VOO, COO, or ROO (in milligrams per 100 milligrams)

	Baseline	VOO	COO	ROO
LLL	0.23 ± 0.12[a]	0.21 ± 0.05[a]	0.20 ± 0.08[a]	0.27 ± 0.03[b]
LnLO	0.47 ± 0.07[a]	0.37 ± 0.04[b]	0.46 ± 0.10[a]	0.50 ± 0.03[a]
LnLP	0.36 ± 0.03[a]	0.36 ± 0.11[a]	0.29 ± 0.09[b]	0.34 ± 0.02[a]
LLO	1.88 ± 0.77[a]	1.48 ± 0.53[b]	1.36 ± 0.38[b]	1.44 ± 0.23[b]
LLP	3.82 ± 0.90[a]	2.87 ± 0.83[b]	3.41 ± 0.63[a]	3.46 ± 0.15[a]
LnOO	0.84 ± 0.06[a]	0.84 ± 0.08[a]	0.84 ± 0.09[a]	0.68 ± 0.09[b]
LnOP	1.55 ± 0.17[a]	1.34 ± 0.17[b]	1.45 ± 0.35[ab]	1.29 ± 0.29[b]
LOO	4.14 ± 1.11[a]	3.50 ± 0.74[b]	3.34 ± 0.61[b]	3.93 ± 1.11[ab]
LOP	20.96 ± 0.86[a]	18.16 ± 1.39[b]	18.80 ± 2.78[b]	18.90 ± 1.08[b]
LPP	25.66 ± 2.18[a]	22.24 ± 0.88[b]	23.09 ± 3.51[b]	25.06 ± 4.25[a]
PoOP	0.37 ± 0.04[a]	0.37 ± 0.09[a]	0.46 ± 0.04[b]	0.38 ± 0.12[a]
OOO	3.63 ± 1.20[a]	4.40 ± 1.12[b]	3.60 ± 0.79[a]	4.00 ± 1.46[ab]
OOP	24.19 ± 1.41[a]	31.32 ± 2.72[b]	29.59 ± 3.10[b]	27.38 ± 3.71[c]
OPP	1.93 ± 0.31[a]	3.00 ± 0.91[b]	2.95 ± 0.82[b]	2.39 ± 0.74[a]
PPP	5.56 ± 0.49[a]	5.61 ± 0.11[a]	5.95 ± 0.96[ab]	6.05 ± 0.61[b]
OOS	3.05 ± 0.83	2.99 ± 0.35	3.14 ± 0.59	3.37 ± 0.69
OPS	0.62 ± 0.09[a]	0.76 ± 0.06[b]	0.75 ± 0.21[b]	0.66 ± 0.02[a]
PPS	0.39 ± 0.10[a]	0.54 ± 0.17[b]	0.55 ± 0.17[b]	0.46 ± 0.20[ab]

Data are presented as mean ± SD. Values sharing the same latter in each row are not statistically significant ($P > .05$). n = 33. Ln indicates linolenic acid (18:3); L, linoleic acid (18:2); O, oleic acid (18:1); P, palmitic acid (16:0); Po, palmitoleic acid (16:1, n-7); S, stearic acid (18:0).

4. Discussion

The observations of the present study reveal that VLDL-TG may be modulated by dietary olive oil phenolics and that this effect occurs in a dose-dependent fashion. Previous studies have provided evidence that VOO consumption can reduce serum TG concentrations by modifying the lipid composition of VLDL [15,16]. There is, however, some discrepancy concerning the components responsible for the effect. From our previous data, minor components contained in dietary olive oils may be reliable candidates for the observed changes in VLDL composition [17].

Olive oil interventions did not significantly modify the concentrations of serum total cholesterol, LDL cholesterol, and TG. These results are in line with previous studies in which different doses of phenolics from olive oil were administered [32]. We observed significant changes in the cholesterol, TG, and phospholipid content of VLDL. The concentrations of these lipids in VLDL increased after the intake of COO and ROO compared with VOO and the baseline. Few studies have addressed the influence of VOO on VLDL lipid composition. To the best of our knowledge, this is the first study assessing the influence of olive oil phenolic compounds on the TG composition of human VLDL.

Triglyceride and fatty acid compositions of VLDL are decisive for the metabolic fate of these particles [16]. We have previously reported that minor olive oil components, other than phenolic compounds, such as the unsaponifiable fraction of VOO can influence both the uptake and secretion of TRL via modulation of the expression of receptors and molecules involved in VLDL formation such as apo B [19].

In the present study, VOO consumption caused a significant reduction in the apo B content of VLDL compared with both the baseline and ROO consumption.

Differences in the fatty acid composition of a dietary oil can lead to significant differences in molecular species composition in VLDL. In previous studies, we found that (1) consumption of VOO led to insignificant fatty acid differences but higher OOO, OOL, and OOP contents in VLDL from healthy subjects compared with high-oleic sunflower oil [33] and (2) the content in LLO and OOO was higher in VLDL formed after administration of the VOO with the highest oleic acid concentration [16]. Now, we report that even differences in the PC can have important effects on the TG composition of VLDL. In the present study, we examined the influence of the PC content of the olive oil on VLDL-TG. As expected, consumption of all olive oils led to increased oleic and palmitic acids, as well as decreased linoleic acid in VLDL. However, the concentration of palmitic acid was significantly higher, and that of linoleic acid was lower, in VLDL after VOO vs ROO consumption. As a consequence, the concentration of linoleic acid–containing TG (mainly LOP and LPP) was lower in VLDL after ROO than after VOO, whereas the concentration of palmitic acid–containing TG was higher (mainly OOP). A progressive increase in the palmitic acid content and a decrease in the linoleic acid content were observed in a direct relationship with olive oils PC. In agreement with this, significant direct relationships with the olive oil PC were also found for OOP, LPP, and grouped TG within PN = 46 (LOO, LOP, and LPP) and PN = 48 (OOO, OOP, OPP, and PPP). The PN is used in chromatography as a determinant of the elution order of TG and, thus, of their polarity [29,34]. The lowest the PN of a TG, the highest its polarity. Because this parameter is inherent of TG molecules, the PN can also be used as an indicator of their polarity in lipoproteins, which can, in turn, determine their propensity to be hydrolyzed by lipoprotein lipase (LPL).

The dose-dependent modifications of fatty acid and TG molecular species in VLDL with the PC of an olive oil implies a role of olive oil phenolic compounds in the metabolism of TG in the liver. Molecules with a phenolic structure, such as probucol or epicatechin, have been shown to have an inhibitory effect on LPL [35,36]. Because LPL presents a fatty acid specificity [37,38], changes in the enzyme activity or expression might lead to alterations in the VLDL-TG composition. Phenolic compounds have also been shown (1) to reduce the expression of genes involved in TG synthesis and secretion in the form of VLDL, such as apo B48 and microsomal triglyceride transfer protein (MTP) [39], and (2) to modulate the expression or activity of receptors involved in the uptake of these lipoproteins by the liver [40,41]. Although VOO phenolics have been found in LDL [42], it is presently unknown whether they are present in TRL, which is unlikely because of their hydrophilic nature.

In summary, the results reported in the present study provide first-level evidence that olive oil phenolic compounds can modulate the composition of human VLDLs by modifying their TG composition. A synergistic effect between phenolics, other minor olive oil components, and the MUFA content of the olive oil cannot be discarded. The mechanisms by which olive oil phenolics modulates the TG molecular species in human VLDLs remain to be elucidated.

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