Elevated Circulating LDL Phenol Levels in Men Who Consumed Virgin Rather Than Refined Olive Oil Are Associated with Less Oxidation of Plasma LDL[1,2]

Karina de la Torre-Carbot,[3,4] Jorge L. Chávez-Servín,[3,4] Olga Jaúregui,[5] Ana I. Castellote,[3,4] Rosa M. Lamuela-Raventós,[3] Tarja Nurmi,[6] Henrik E. Poulsen,[7] Antonio V. Gaddi,[8] Jari Kaikkonen,[9] Hans-Franz Zunft,[10] Holger Kiesewetter,[11] Montserrat Fitó,[12] María-Isabel Covas,[12] and M. Carmen López-Sabater[3,4]*

[3]Department of Nutrition and Food Science, Reference Center in Food Technology, Faculty of Pharmacy, University of Barcelona, 08028 Barcelona, Spain; [4]CIBER Epidemiología y Salud Pública, 08020 Barcelona, Spain; [5]Scientific and Technical Services, University of Barcelona, Josep Samitier 1-5 08028, Barcelona, Spain; [6]Research Institute of Public Health, University of Kuopio, PB 1627, 70211, Kuopio, Finland; [7]Department of Clinical Pharmacology, Rigshospitalet, University Hospital, Copenhagen 2200, Denmark; [8]GC Descovich Atherosclerosis and Metabolic Disease Research Unit, University of Bologna, 40138 Bologna, Italy; [9]Oy Jurilab, Kuopio, 70210 Finland; [10]German Institute of Human Nutrition, Potsdam-Rehbruecke 14558, Germany; [11]Charité-Universitätsmedizin, Berlin 10117, Germany; and [12]Lipids and Cardiovascular Epidemiology Unit, Institut Municipal d'Investigació Mèdica-Hospital del Mar, Parc de Recerca Biomédica de Barcelona, Barcelona 08003, Spain

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Abstract

In human LDL, the bioactivity of olive oil phenols is determined by the in vivo disposition of the biological metabolites of these compounds. Here, we examined how the ingestion of 2 similar olive oils affected the content of the metabolic forms of olive oil phenols in LDL in men. The oils differed in phenol concentrations as follows: high (629 mg/L) for virgin olive oil (VOO) and null (0 mg/L) for refined olive oil (ROO). The study population consisted of a subsample from the EUROLIVE study and a randomized controlled, crossover design was used. Intervention periods lasted 3 wk and were preceded by a 2-wk washout period. The levels of LDL hydroxytyrosol monosulfate and homovanillic acid sulfate, but not of tyrosol sulfate, increased after VOO ingestion ($P < 0.05$), whereas the concentrations of circulating oxidation markers, including oxidized LDL (oxLDL), conjugated dienes, and hydroxy fatty acids, decreased ($P < 0.05$). The levels of LDL phenols and oxidation markers were not affected by ROO consumption. The relative increase in the 3 LDL phenols was greater when men consumed VOO than when they consumed ROO ($P < 0.05$), as was the relative decrease in plasma oxLDL (P = 0.001) and hydroxy fatty acids ($P < 0.001$). Plasma oxLDL concentrations were negatively correlated with the LDL phenol levels ($r = -0.296$; $P = 0.013$). Phenols in LDL were not associated with other oxidation markers. In summary, the phenol concentration of olive oil modulates the phenolic metabolite content in LDL after sustained, daily consumption. The inverse relationship of these metabolites with the degree of LDL oxidation supports the in vivo antioxidant role of olive oil phenolics compounds. J. Nutr. 140: 501–508, 2010.

Introduction

The traditional dietary habits of the Mediterranean area have been consistently associated with a lower incidence of cardiovascular disease (1–4). Dietary phenols have been reported to have a protective effect on cardiovascular risk factors (2,5–10). Olive oil is the predominant source of fat in the Mediterranean diet. The health properties of this oil have often been attributed to its high monounsaturated fatty acid content (1). Recent evidence has shown that minor components of olive oil, particularly phenols, also contribute to the beneficial effects of this fat source (11–16).

There are no phenols in refined olive oil (ROO),[13] which is not commonly consumed as such; olive oil is generally ingested as a commercial oil composed of virgin olive oil (VOO) and ROO in different proportions. In contrast, the phenols present in VOO may range from 150 to 1000 mg/L. Hydroxytyrosol and

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* To whom correspondence should be addressed. E-mail: mclopez@ub.edu.

[13] Abbreviations used: DAD, diode array detection; MS/MS, tandem MS; oxLDL, oxidized LDL; ROO, refined olive oil; VOO, virgin olive oil.

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tyrosol in simple forms or as conjugates are the most abundant phenolic alcohols in this oil (17,18).

A potentially important factor in the absorption and metabolism of olive oil phenols is that they can be absorbed directly as simple phenols or as their conjugated forms, such as oleuropein and ligstroside, which undergo rapid hydrolysis. As a result, substantially more free hydroxytyrosol and tyrosol enter the small intestine (19). Once absorbed, olive oil phenols undergo extensive first-pass intestinal/hepatic metabolism in the body. This leads to the formation of sulfate and glucoronide conjugates to such an extent that concentrations of their free forms are almost undetectable in body fluids. Hydroxytyrosol and homovanillic acid are endogenous dopamine metabolites and homovanillic acid is a product metabolite of hydroxytyrosol (2,20,21). Hydroxytyrosol and related compounds have high metabolic activity and a range of functions that are related to cardiovascular protection, including in vitro (15,16,22–27) and in vivo (7,12,28–34) activities. These activities are exerted through: *1*) free radical scavenging properties (hydrogen donation and their capacity to improve radical stability) (5,35,36); *2*) strong metal chelation (5,15); *3*) alteration of the expression of genes related to atherosclerosis development and progression (37); *4*) stimulation of antioxidant transcription and detoxification of defense systems (12,15,24,28,30,38,39); *5*) modification of gene expression in targets that are mechanistically relevant for cardiovascular protection (29); *6*) improvement of the lipid profile (8,30,31); *7*) decrease in homocysteine-induced endothelial dysfunction and/or cell adhesion; and *8*) increase of the antithrombotic effect and decreases in inflammatory markers such as thromboxane, leukotriene, cyclooxygenase-2, tumor necrosis factor-α, interleukin-6, and C-reactive protein (38,40,41). In some studies, there is no evidence for the stimulation of nitric oxide in endothelial cells due to olive oil phenols (42). However, other studies suggest that this may occur (43). Furthermore, the phenols in VOO protect other phenols that were previously bound to LDL (7,25,44).

Oxidized LDL (oxLDL) are currently thought to be more damaging to the arterial wall than native LDL cholesterol. oxLDL are pro-oxidants that cause tissue injury and promote the development of atherosclerotic lesions. Moreover, they are a marker for the development of atherosclerosis and cardiovascular heart disease (45,46).

Dietary phenols, including those in olive oil, bind LDL (20). Data on the bioavailability and disposition of these compounds in olive oil (47) show that >90% of phenols in human biological fluids are metabolized (i.e. glucoronides or sulfates). Consequently, the bioactivity of olive oil phenols in LDL can be determined by the in vivo disposition and kinetics of the biological metabolites of these substances.

To date, several authors have addressed the impact of olive oil phenols on the total phenolic content of LDL (7,44). To our knowledge, our study is the first to use a randomized controlled trial to obtain data on the effect of specific metabolites in olive oil phenols on human LDL and their oxidation. Here, we examined the effect of these phenols on human LDL after sustained consumption of 2 olive oils with similar compositions that differed in phenol concentrations. We also evaluated the impact of the olive oils' phenol concentrations on the degree of lipid oxidative damage.

Materials and Methods

Olive oil characteristics and analysis. The oils used were specially prepared for the study. The total phenol concentration of the oils was measured by HPLC-diode array detection (DAD) (Hewlett-Packard-1050 with an automatic injector and DAD 1050 series instrument) and HPLC-tandem MS (HPLC-MS/MS) (Agilent 1100) equipped with an autosampler and coupled to an API3000 triple-quadrupole mass spectrometer (PE Sciex) (48). Picual VOO from Andalucía, Spain, with a high natural phenol concentration (629 mg/L), was selected. ROO (0.0 mg/L) was harvested from the same cultivar and the soil was tested to find an oil that had similar amounts of fatty acids and a similar micronutrient profile. The fatty acid composition of the oils was measured by conventional GC (Shimadzu GC-2010; Shimadzu) equipped with an FID and an AOC-20i autoinjector, with a fast capillary column (10 m × 0.10 mm i.d.) coated with an SGE-BPX70 cross-linked stationary phase (SGE Europe) (EEC/2568/91) (49). Concentrations of squalene and β-sitosterol were determined by capillary GC (50) and those of α-tocopherol by HPLC (Hewlett-Packard HP1050, HP-1040 mol/L DAD, and a Watters 717 Plus autosampler injector) as previously described (51). Vitamin E as α-tocopherol, at 99% purity and food grade, was purchased from Sigma-Aldrich and added to the ROO to adjust values to those found in the VOO. The homogeneity of the mix was verified by the HPLC system and the aforementioned method. Thus, the oils did not differ in fat or micronutrient composition (vitamin E, triterpenes, and phytosterols), with the exception of the phenol concentration. The oils met the specifications of the European Commission (49).

Study design and participants. The study population consisted of a subsample from the EUROLIVE study (8), which was a randomized, controlled, double-blind, crossover study performed with 3 types of olive oil that differed in phenol concentration. In this paper, we study 2 of these 3 oils. Participants were nonsmoking males from 20 to 60 y of age who were recruited from 6 centers in 5 European countries: Barcelona, Spain; Copenhagen, Denmark; Kuopio, Finland; Bologna, Italy; Potsdam, Germany; and Berlin, Germany. The intervention periods were 3 wk long and were preceded by 2-wk washout periods. Volunteers were preselected if they were nonsmokers with normal clinical records, physical examinations, and blood pressure. Subsequently, a complete blood count and biochemical laboratory analyses were undertaken to measure serum levels of glucose, cholesterol, and triglycerides using standard enzymatic automated methods (Roche Diagnostics). Candidates with values within the normal reference ranges were included in the study. Exclusion criteria were: smoking; intake of antioxidant supplements, aspirin, or any other drug with established antioxidant properties; hyperlipidemia; obesity (BMI >30 kg/m^2); diabetes; hypertension; any other disease or condition that would impair compliance, such as celiac disease or other intestinal disorders or diseases; any condition that limits mobility; life-threatening diseases; neurological disorders; and the use of special dietetic treatments. Women were excluded to avoid the possible interference of estrogens, which are considered to be potential antioxidants (52). The protocol was fully explained to the participants before they gave their written informed consent. The protocol complied with the Helsinki Declaration as revised in 1983 and was approved by the Ethics Committee or each Clinical Trial Center that was involved.

During the intervention periods, participants were requested to ingest a raw daily dose of 25 mL (22 g) of the respective oil distributed over 3 meals to replace other raw fats. This amount was chosen because it is similar to the daily consumption by populations in Southern European Mediterranean countries. Daily doses were prepared in special containers with the different types of oils and labeled with a code number. Containers with the corresponding daily doses of 25 mL of raw oil were delivered daily to the participants. They were instructed to return the containers at the end of each intervention period so that the amount of unconsumed oil could be registered. Participants were requested to avoid a high intake of foods containing antioxidants (vegetables, legumes, fruit, tea, coffee, chocolate, wine, and beer). The nutritionists personally informed the men about how to replace all types of habitually consumed raw fats with the olive oils (e.g. spreading the assigned olive oil instead of butter on bread, putting the assigned olive oil instead of margarine on boiled vegetables, and using the assigned olive oil instead of other vegetable oils or standard salad dressings on salads). Participants, with individual advice from the nutritionists, recorded their habitual diet for 3 consecutive days at baseline and at the end of the study period. They

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were also advised how to record their food consumption and follow the dietary recommendations. Food consumption was converted into the corresponding nutrient intake using validated nutrition software from each participating country.

The present study includes randomized samples of 36 participants (6 from Barcelona, Spain; 3 from Copenhagen, Denmark; 10 from Kuopio, Finland; 3 from Bologna, Italy; 7 from Potsdam, Germany; and 7 from Berlin, Germany) who were analyzed before and after high and null phenol concentration olive oil treatment.

Serum cholesterol and lipids. Blood samples were taken before and at the end of each intervention. Blood was withdrawn after 10–14 h of fasting into tubes containing 1 g/L EDTA and aliquots of serum and plasma were stored at –80°C. Glucose, total cholesterol, HDL cholesterol, and triglyceride levels were measured in serum using standard enzymatic automated methods (Roche Diagnostics). LDL cholesterol was calculated by the Friedewald equation whenever triglycerides were $<$3.4 mmol/L. Quality control was performed with the External Quality Assessment UNITY (Bio-Rad).

LDL isolation. For the isolation of LDL (20), 1 mL of isotonic saline containing EDTA and NaCl was layered carefully on top of 2 mL of plasma in a centrifuge tube. The tubes were centrifuged in a Beckman-Coulter XL-70 using the Fixed-Angle Type 50.4 rotor at 199,808 $\times$ g for 18 h at 4°C. The infranatant (3 mL) was placed in a centrifuge tube containing KBr, stained with Coomassie Brilliant Blue R solution, and vortexed. Two milliliters of isotonic saline containing KBr was layered carefully on top of the infranatant up to the base of the tube vertex. The tubes were sealed before being placed in the NVT100 rotor and were then centrifuged at 697,760 $\times$ g for 5 h at 4C°. The LDL layer was aspirated and kept frozen at $-$80°C. LDL cholesterol and triglycerides were determined by standard enzymatic methods (Roche Diagnostics). The apolipoprotein B concentration was measured by immunoturbidimetry (Roche Diagnostics).

LDL phenolic metabolites. Briefly, to measure phenols (53), 100 μL of protocatechol (molecular weight = 109) solution (200 μg/L) as internal standard and 20 μL of phosphoric acid 85% (v:v) were added to acidifed LDL. An Oasis HLB (60 mg) cartridge from Waters (Mildford) was activated with methanol and formic acid (5% in water) and acidified LDL was then percolated into the cartridge. The sample was washed with water and 5% aqueous methanol. Phenols were eluted with methanol. The eluent was evaporated completely under nitrogen. The sample was dissolved in 150 μL water:acetonitrile (90:10). Samples were filtered and transposed into an amber vial. Subsequently, 20 μL of these samples was injected into the same HPLC-MS/MS as that used for measured phenols in the olive oils. The entire process was performed under conditions of darkness using brown glass. We used a 3-μm particle size C_{18} Luna column, 5 cm $\times$ 2.0 mm i.d. with a C_{18} 4-mm guard cartridge (Phenomenex). The mobile phase consisted of a binary solvent system that used water acidified with 0.1% formic acid (solvent A) and 100% acetonitrile (solvent B) kept at a flow rate of 0.6 mL/min. LDL (20 μL) samples were injected at a constant flow rate of 0.6 mL/min. All the analyses used the turbo ion-spray source in negative mode. Quantification was performed using multiple reaction monitoring. Hydroxytyrosol and tyrosol derivatives were expressed as hydroxytyrosol and the homovanillic acid metabolite was expressed as homovanillic acid. All calculations of concentration and regression for the compounds were performed using Analyst 1.4 software and all the chemicals and organic solvents were of analytical grade.

Circulating oxidation markers. oxLDL in plasma were measured by a sandwich ELISA procedure using the murine monoclonal antibody mAB-46 as the capture antibody bound to microtitration wells, and a peroxidase conjugated anti-apolipoprotein B antibody that recognizes oxLDL bound to the solid phase (oxLDL, Mercodia) (7). Plasma C18 hydroxy fatty acids were measured by GC and MS, and serum LDL cholesterol-uninduced conjugated dienes were measured by MS and adjusted for the cholesterol content of LDL (8).

Statistical methods. The normality of variable distribution was assessed by the normal probability plots, the Kolmogorov-Smirnov test, and by an analysis of skewness and kurtosis. A general linear model for repeated measurements with Tukey's correction for multiple comparisons was used to assess the effect of each type of olive oil on the phenolic content of LDL and the lipid oxidation markers (before and after olive oil ingestion). The Student's t test for paired samples analysis was used to compare the percentage changes between treatments (ROO and VOO). Spearman's test was used to study correlations between oxidation markers and phenols in LDL. Significance was defined as $P <$ 0.05 for a 2-sided test. Values in the text are means $\pm$ SEM. We used SPSS 14.0 statistical software.

Results

The HPLC-DAD and HPLC-MS/MS analyses of the olive oils administered in this study showed that whereas the ROO did not contain any phenols or phenolic compounds, the VOO had several (**Table 1**). Characteristics of the participants at baseline and at the start of each intervention are shown in **Table 2**. The phenolic metabolites that we detected and quantified in LDL were hydroxytyrosol, tyrosol, and homovanillic acid. All of these were present in sulfated forms before and after the men had consumed each kind of olive oil for 3 wk. We did not detect the free forms of hydroxytyrosol and tyrosol or their glucuronates bound to LDL.

Absolute changes during VOO and ROO consumption. Two of the 3 phenols, hydroxytyrosol monosulfate and homovanillic acid, increased in LDL after VOO ingestion only, as did the total phenol content (sum of the 3 metabolites measured) ($P < 0.05$) (**Table 3**). Phenols did not change after the men consumed ROO (**Table 4**). The cholesterol, triglyceride, and apolipoprotein B levels in LDL did not change after ROO (Table 4) or VOO (Table 3) ingestion. After VOO ingestion, plasma oxLDL, serum conjugated diene, and plasma hydroxy fatty acid concentrations decreased ($P < 0.05$) (Table 4). Concentrations of these oxidation markers did not change when the men consumed ROO (Table 3). Levels of cholesterol, triglycerides, and apolipoprotein B in LDL did not change after the men ingested either oil.

TABLE 1 Characteristics of the olive oils

Compound	ROO	VOO
Phenols	*mg/L*	
Hydroxytyrosol	—	63.5
Tyrosol	—	24.4
Vanillic acid	—	0.2
Vanillin	—	0.5
p-Coumaric acid	—	0.3
Ligstroside derivatives	—	208.0
Oleuropein derivatives	—	327.2
Luteolin	—	3.1
Apigenin	—	0.7
Methoxyluteolin	—	0.6
Total phenols	—	629
Other minor compounds		
α-Tocopherol	229	228
Squalene	2754	3671
β-Sitosterol	1438	1612
Fatty acids	*%*	
Monounsaturated fatty acids	80.4	81.8
SFA	14.5	14.0
PUFA	5.2	3.9

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TABLE 2 Anthropometrics and serum chemistry of the men at the beginning of the study and at the start of each intervention period[1]

Clinical variable	Baseline	Before ROO ingestion	Before VOO ingestion
Age, *y*	32 ± 2	—	—
Height, *m*	1.80 ± 0.01	—	—
Waist-hip ratio	0.88 ± 0.009	—	—
Weight, *kg*	76.8 ± 1.8	77.1 ± 1.8	77.4 ± 1.8
BMI, *kg/m²*	23.6 ± 0.5	23.6 ± 0.5	23.7 ± 0.5
Systolic pressure, *mm Hg*	125 ± 2	119 ± 2	122 ± 2
Diastolic pressure, *mm Hg*	78 ± 1	76 ± 1	78 ± 1
Triglycerides, *mmol/L*	1.19 ± 0.09	1.17 ± 0.12	1.22 ± 0.09
Cholesterol, *mmol/L*	4.61 ± 0.18	4.51 ± 0.17	4.62 ± 0.16
HDL cholesterol, *mmol/L*	1.19 ± 0.03	1.22 ± 0.05	1.22 ± 0.04
LDL cholesterol, *mmo/L*	2.90 ± 0.17	2.78 ± 0.16	2.89 ± 0.15
Glucose, *mmol/L*	4.7 ± 0.1	4.7 ± 0.1	5.0 ± 0.2

[1] Values are means ± SEM, n = 36.

Relative differences in changes between ROO and VOO consumption. The percentage change (relative difference) in the concentrations of homovanillic acid, hydroxytyrosol, and tyrosol in LDL was greater when the men consumed VOO than when they consumed ROO ($P < 0.05$). Percentage changes in 2 LDL oxidation markers, plasma oxLDL and hydroxy fatty acids, were greater when they consumed VOO than when they consumed ROO ($P \leq 0.001$). Relative changes (%) in cholesterol, triglycerides, and apolipoprotein B did not differ ($P > 0.05$) between the 2 treatment periods (**Table 5**).

Correlations. The plasma oxLDL concentration and the sum of phenols in LDL were negatively correlated (Spearman $r = -0.296$; $P = 0.013$) when values of all men before and after VOO ingestion were considered. The relation was stronger in Berlin ($r = -0.514$; $P = 0.043$) and in Potsdam ($r = -0.688$; $P = 0.007$), both of which are in Germany, whereas negative correlations for the other sites were not significant when they were studied individually. Plasma phenols in LDL and other oxidative markers were not associated.

Discussion

We studied the effect of olive oil phenols on phenolic forms bound to LDL and their impact on LDL oxidation after the administration of 2 olive oils with similar compositions.

We did not detect glucuronides in this study. This may be attributable to the fact that the samples were obtained under fasting conditions instead of in a postprandial state after olive oil ingestion, as occurred in a previous experiment in which glucuronides were found 60 min after olive oil ingestion (20). We hypothesized that hydroxytyrosol and tyrosol glucuronide bind to LDL, but are removed faster from LDL than sulfated compounds. This hypothesis is borne out by other studies that demonstrated that after acute VOO ingestion, the elimination half-lives of hydroxytyrosol and tyrosol (mainly as glucuronates) in urine are 2.5 and 5–8 h, respectively (47,54). Consequently, glucuronates can be detected in LDL shortly after a single olive oil dose (20). In addition, the concentrations of hydroxytyrosol, tyrosol, and homovanillic acid sulfate were greater than those recorded in the present study, which could be attributed to the same cause. To assess the in vivo physiological

TABLE 3 LDL composition and LDL oxidation markers in men before and after they consumed ROO for 3 wk[1]

Compound	Before	After	Change[2]	P[3]
LDL composition[4]				
Apo B, *g/L*	0.4 ± 0.0	0.4 ± 0.0	0.0	0.343
Cholesterol, *mg/mg Apo B*	2.0 ± 0.1	2.0 ± 0.1	0.0	0.727
Triglycerides, *mg/mg Apo B*	4.1 ± 0.3	3.8 ± 0.2	−0.2	0.177
Homovanillic acid sulfate, *ng/mg Apo B*	13.9 ± 2.0	14.4 ± 1.9	0.5	0.873
Hydroxytyrosol sulfate, *ng/mg apo B*	3.6 ± 1.0	4.1 ± 1.1	0.6	0.648
Tyrosol sulfate, *ng/mg Apo B*	4.6 ± 0.5	4.8 ± 0.9	0.2	0.753
Sum of the 3 phenols, *ng/mg Apo B*	21.3 ± 2.62	23.7 ± 2.36	2.4	0.380
Circulating LDL oxidation markers				
Plasma oxLDL, *U/L*	46 ± 3	42 ± 3	−4	0.230
Serum conjugated dienes, *μmol/L*	13.1 ± 0.6	12.0 ± 0.6	−1.1	0.091
Plasma hydroxy fatty acids, *μmol/L*	1.3 ± 0.1	1.3 ± 0.1	0.0	0.676

[1] Values are means ± SEM, n = 36.
[2] Difference is the result after ingestion minus before ingestion means.
[3] P-value is for change from before to after ROO ingestion using a general linear model for repeated measurements.
[4] Molecular weights: cholesterol, 386.7; triglycerides, 885.7; homovanillic acid sulfate, 261; hydroxytyrosol sulfate, 233 and tyrosol sulfate, 217.

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TABLE 4 LDL composition and LDL oxidation markers in men before and after they consumed VOO for 3 wk[1]

Compound	Before	After	Change[2]	*P*[3]
LDL composition[4]				
Apo B, *g/L*	0.4 ± 0.0	0.4 ± 0.0	0.0	0.779
Cholesterol, *mg/mg Apo B*	2.0 ± 0.1	2.0 ± 0.1	−0.0	0.627
Triglycerides, *mg/mg Apo B*	4.1 ± 0.3	3.8 ± 0.3	−0.3	0.224
Homovanillic acid sulfate, *ng/mg Apo B*	13.3 ± 2.0	17.0 ± 2.4	3.7	0.046
Hydroxytyrosol sulfate, *ng/mg apo B*	2.4 ± 0.4	3.6 ± 0.7	1.	0.004
Tyrosol sulfate, *ng/mg Apo B*	3.6 ± 0.1	4.3 ± 0.7	0.7	0.290
Sum of the 3 phenols, *ng/mg Apo B*	19.5 ± 2.2	24.7 ± 2.7	5.3	0.017
Circulating LDL oxidation markers				
Plasma oxLDL, *U/L*	50 ± 4	39 ± 3	−11	<0.001
Serum conjugated dienes, *μmol/L*	13.4 ± 0.8	11.9 ± 0.5·	−1.5	0.039
Plasma hydroxy fatty acids, *μmol/L*	1.6 ± 0.2	1.1 ± 0.1	−0.5	<0.001

[1] Values are means ± SEM, *n* = 36.
[2] Difference is the result from after ingestion minus before ingestion means.
[3] *P*-value is for change from before to after VOO ingestion using a general linear model for repeated measurements.
[4] Molecular weights: cholesterol, 386.7; triglycerides, 885.7; homovanillic acid sulfate, 261; hydroxytyrosol sulfate, 233 and tyrosol sulfate, 217.

importance of phenols, their availability and kinetic evolution in LDL should be determined after both acute and long-term ingestion of VOO.

The significant changes in phenols in LDL after VOO ingestion, and the markedly greater percentage changes compared with after ROO ingestion, indicate that the phenols of LDL can be affected by diet. These changes are related to the kind of olive oil administered, which is differentiated by the concentration of phenols.

The significant changes in oxidation markers due to VOO suggest that it protects against oxidation. These results are consistent with Covas et al. (8), who demonstrated that daily consumption of an olive oil with a high phenol concentration decreased oxidative damage to lipids.

The absolute decrease in oxidative damage, as assessed by oxLDL, conjugated diene, and hydroxy fatty acid concentrations, in parallel with the significant absolute increases in the levels of phenols in LDL after ingestion of VOO, indicates that the increase in phenols in LDL may be involved in reducing oxidation. Recently, Covas et al. (7) demonstrated that, in parallel to the reduction in LDL oxidation, phenols bound to human LDL increase in a dose-dependent manner with the phenol concentration of the olive oil ingested. Other studies have demonstrated the relationship between the consumption of olive oil with a high phenol concentration and the reduction of some oxidative markers in LDL in humans (12,55), animals (56,57), and in vitro experiments using olive oil phenols (16,27,58). The significant correlation between oxLDL and the sum of the phenols, in addition to better-defined correlations when groups were studied separately, strongly indicates that these compounds have an antioxidant capacity in the LDL particle.

oxLDL are pro-oxidants that causes tissue injury and may be a determining factor for cardiovascular disease development (45,46). Phenols that bind to LDL could exert their antioxidant action in vivo in the arterial intima. Some investigations have demonstrated that the phenols in olive oil modulate oxidative/antioxidant status in vivo (7,8) and that plasma antioxidant capacity improves after olive oil consumption (7,8,11–13,34,59). In addition, the phenols in VOO protect other phenols that may come from varying sources and were previously bound to LDL (7,25,44). In vitro studies have demon-

TABLE 5 Relative differences in LDL composition and LDL oxidation markers in men between the ROO and VOO consumption period[1]

Compound	Change due to ROO	Change due to VOO	*P*[2]
LDL composition	%		
Apo B	2.4 ± 1.4	0.0 ± 0.0	0.185
Cholesterol	0.5 ± 1.2	−1.0 ± 6.7	0.775
Triglycerides	−5.6 ± 12.53	−7.3 ± 8.2	0.770
Homovanillic acid sulfate	3.3 ± 4.3	27.9 ± 9.3	0.049
Hydroxytyrosol sulfate	16.8 ± 12.4	50.8 ± 12.1	0.041
Tyrosol sulfate	5.3 ± 7.9	20.0 ± 6.9	0.039
Sum of the 3 phenols	11.1 ± 19.1	27.0 ± 8.8	0.075
LDL oxidation markers in plasma and serum			
Plasma oxLDL	−9 ± 6.4	−22 ± 1.9	0.001
Serum conjugated dienes	−8.1 ± 7.1	−11.1 ± 7.2	0.799
Plasma hydroxy fatty acids	2.3 ± 3.2	−29.6 ± 4.1	<0.001

[1] Percentage change is the change, expressed as a percentage, from before to after the respective olive oil treatment, *n* = 36.
[2] *P*-value is for olive oil ingestion differences between treatments using Student's *t*-test for paired samples analysis.

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strated the antioxidant capacity of hydroxytyrosol with human LDL. Although a number of studies question the antioxidant capacity of tyrosol, the work of Di Benedetto et al. (60) suggests that, despite its weak antioxidant activity, tyrosol is effective in preserving cellular antioxidant defense. Further experiments are required on the antioxidant capacity of homovanillic acid in LDL for greater clarification. Nevertheless, some studies have demonstrated other activities of this phenol in relation to cardiovascular risk prevention, specifically the reduction of inflammatory factors and an antithrombotic effect (61,62).

Olive oil phenols undergo extensive first-pass intestinal/hepatic metabolism in the body and their biological activity is more likely to be linked to the biological metabolites of phenols than to the primary species present in the olive oil. A number of studies demonstrate that glucuronide and/or sulfate conjugates of olive oil phenols are biologically active in vitro. It appears that hydroxytyrosol has stronger antioxidant activity than tyrosol and that glucuronide conjugated compounds have a stronger activity than sulfates (63). However, further research is required in regard to these specific metabolites. Tyrosol and hydroxytyrosol are absorbed by humans in a dose-dependent manner with the phenol concentration of the olive oil administered. This occurs even with moderate doses (25 mL/d), which are similar to the traditional daily dietary intakes in Mediterranean countries. Around 98% of tyrosol and hydroxytyrosol in plasma and urine are presented in conjugated forms (21). In addition, a low concentration may be enough for them to exert their antioxidant activities (64).

Phenols that bind LDL are good candidates for the effective prevention of lipid peroxidation and atherosclerotic processes. The presence and concentration of phenols could be potential markers for the development of atherosclerosis and cardiovascular disease.

This study has strengths and limitations. One strength was the crossover design, which permitted the same participants to receive all olive oil types. This minimized interference with possible confounding variables. However, the design did not allow the modeling of potential first-order and second-order carryover effects. A limitation was the inability to assess the potential interactions between olive oil and other dietary components. This might affect the generalizability of the results, due to dietary differences among countries. As a result of the unevenly balanced number of volunteers from each center, the generalizability of the results cannot be assumed.

The measurements of dietary intake were subjective, as they relied on self-reporting. Another limitation was our failure to adequately monitor whether participants fully substituted their habitually consumed raw fats with the assigned olive oil. Finally, we consider that the short duration of the intervention periods was a limitation, because it is unknown whether additional, or different, effects in the oxidative biomarkers would have been observed over longer periods. A longer study, however, could have impaired the adherence of the participants to the protocol (8).

Here, we demonstrate that the phenolic composition in LDL can be affected by the type of olive oil ingested at a constant dose for 3 wk. The significant increase in LDL phenols after VOO ingestion, together with a reduction in oxidative markers, indicates that these compounds act as in vivo antioxidants and that they have a critical action on LDL.

In conclusion, the phenols in VOO modulate the LDL content of 3 phenolic metabolites, hydroxytyrosol, tyrosol, and homovanillic acid sulfate, after long-term ingestion of real-life doses (25 mL/d). These compounds increased significantly after the ingestion of VOO, in contrast to ROO. In parallel, the ingestion of VOO significantly reduced LDL and plasma oxidative markers. This observation suggests that the metabolic activities of phenols can be related to the capacity of these compounds to remain bound to LDL.

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M.I.C. designed research; K. de la T-C., J.L.C-S., O.J., R.M.L-R., A.I.C., and M.C.L-S. conducted the research on phenols in LDL and on the characteristics of the olive oils at Barcelona University; M.F. and M.I.C. conducted research on oxidation markers at IMIM, Barcelona; M.F., T.N., H.E.P., A.V.G., J.K., H.F.Z., and H.K. conducted patient management and collection of samples in each European center; M.F., K. de la T-C., and J.L.C-S. analyzed data and performed statistical analyses; K. de la T-C. and J.L.C-S. wrote the paper; and M.C.L-S. had primary responsibility for final content. All authors read and approved the final manuscript.

Literature Cited

1. Visioli F, Bogani P, Grande S, Galli C. Mediterranean food and health: building human evidence. J Physiol Pharmacol. 2005;56 Suppl 1:37–49.
2. Fito M, de la Torre R, Farre-Albaladejo M, Khymenetz O, Marrugat J, Covas MI. Bioavailability and antioxidant effects of olive oil phenolic compounds in humans: a review. Ann Ist Super Sanita. 2007;43:375–81.
3. Covas MI. Bioactive effects of olive oil phenolic compounds in humans: reduction of heart disease factors and oxidative damage. Inflammopharmacology. 2008;16:216–8.
4. Gonzalez-Correa JA, Navas MD, Munoz-Marin J, Trujillo M, Fernandez-Bolanos J, de la Cruz JP. Effects of hydroxytyrosol and hydroxytyrosol acetate administration to rats on platelet function compared to acetylsalicylic acid. J Agric Food Chem. 2008;56:7872–6.
5. Visioli F, Poli A, Galli C. Antioxidant and other biological activities of phenols from olives and olive oil. Med Res Rev. 2002;22:65–75.
6. Fito M. de la TR, Covas MI. Olive oil and oxidative stress. Mol Nutr Food Res. 2007;51:1215–24.
7. Covas MI, de la Torre K, Farré-Albaladejo M, Kaikkonnen J, Fitó M, López-Sabater MC, Pujadas-Bastardes MA, Oglar J, Weinbrenner T, et al. Postprandial LDL phenolic content and LDL oxidation are modulated by olive oil phenolic compounds in human. Free Radic Biol Med. 2006;40:608–16.
8. Covas MI, Nyyssonen K, Poulsen HE, Kaikkonen J, Zunft HJ, Kiesewetter H, Gaddi A, de la Torre R, Mursu J, et al. The effect of polyphenols in olive oil on heart disease risk factors: a randomized trial. Ann Intern Med. 2006;145:333–41.
9. Covas MI. Olive oil and the cardiovascular system. Pharmacol Res. 2007;55:175–86.
10. Stoclet JC, Chataigneau T, Ndiaye M, Oak MH, El BJ, Chataigneau M, Schini-Kerth VB. Vascular protection by dietary polyphenols. Eur J Pharmacol. 2004;500:299–313.
11. Ruano J, Lopez-Miranda J, Fuentes F, Moreno JA, Bellido C, Perez-Martinez P, Lozano A, Gomez P, Jimenez Y, Perez Jimenez F. Phenolic content of virgin olive oil improves ischemic reactive hyperemia in hypercholesterolemic patients. J Am Coll Cardiol. 2005;46:1864–8.
12. Fitó M, Cladellasc M, De La Torre R, Marti J, Alcantara M, Pujadas-Bastardes M, Marrugat J, Bruguera J, Lopez-Sabater MC, et al. Antioxidant effect of virgin olive oil in patients with stable coronary heart disease: a randomized, crossover, controlled, clinical trial. Atherosclerosis. 2005;181:149–58.
13. Visioli F, Caruso D, Grande S, Bosisio R, Villa M, Galli G, Sirtori C, Galli C. Virgin Olive Oil Study (VOLOS): vasoprotective potential of extra virgin olive oil in mildly dyslipidemic patients. Eur J Nutr. 2005;44:121–7.
14. Carluccio MA, Siculella L, Ancora MA, Massaro M, Scoditti E, Storelli C, Visioli F, Distante A, De CR. Olive oil and red wine antioxidant polyphenols inhibit endothelial activation: antiatherogenic properties of Mediterranean diet phytochemicals. Arterioscler Thromb Vasc Biol. 2003;23:622–9.

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15. Moreno JA, Lopez-Miranda J, Gomez P, Benkhalti F, el Boustani ES, Perez-Jimenez F. Effect of phenolic compounds of virgin olive oil on LDL oxidation resistance. Med Clin (Barc). 2003;120:128–31.
16. Turner R, Etienne N, Alonso MG, de Pascual-Teresa S, Minihane AM, Weinberg PD, Rimbach G. Antioxidant and anti-atherogenic activities of olive oil phenolics. Int J Vitam Nutr Res. 2005;75:61–70.
17. Servili M, Selvaggini R, Esposto S, Taticchi A, Montedoro G, Morozzi G. Health and sensory properties of virgin olive oil hydrophilic phenols: agronomic and technological aspects of production that affect their occurrence in the oil. J Chromatogr A. 2004;1054:113–27.
18. Angerosa F, Dalessandro N, Konstantinou P, Digiacinto L. Gc-Ris evaluation of phenolic-compounds in virgin olive oil. J Agric Food Chem. 1995;43:1802–07.
19. Corona G, Tzounis X, Dessi MA, Deiana M, Debnam ES, Visioli F, Spencer JPE. The fate of olive oil polyphenols in the gastrointestinal tract: implications of gastric and colonic microflora-dependent biotransformation. Free Radic Res. 2006;40:647–58.
20. De La Torre-Carbot K, Chavez-Servin JL, Jauregui O, Castellote AI, Lamuela-Raventos RM, Fito M, Covas MI, Munoz-Aguayo D, Lopez-Sabater MC. Presence of virgin olive oil phenolic metabolites in human low density lipoprotein fraction: determination by high-performance liquid chromatography-electrospray ionization tandem mass spectrometry. Anal Chim Acta. 2007;583:402–10.
21. de la Torre R. Bioavailability of olive oil phenolic compounds in humans. Inflammopharmacology. 2008;16:245–7.
22. Leenen R, Roodenburg AJC, Vissers MN, Schuurbiers JAE, van Putte KPAM, Wiseman SA, van de Put FHMM. Supplementation of plasma with olive oil phenols and extracts: influence on LDL oxidation. J Agric Food Chem. 2002;50:1290–7.
23. Manna C, Napoli D, Cacciapuoti G, Porcelli M, Zappia V. Olive oil phenolic compounds inhibit homocysteine-induced endothelial cell adhesion regardless of their different antioxidant activity. J Agric Food Chem. 2009;57:3478–82.
24. Zhang X, Cao J, Jiang L, Zhong L. Suppressive effects of hydroxytyrosol on oxidative stress and nuclear factor-kappaB activation in THP-1 cells. Biol Pharm Bull. 2009;32:578–82.
25. Covas MI, Fito M, Lamuela-Raventos RM, Sebastia N, De la Torre-Boronat C, Marrugat J. Virgin olive oil phenolic compounds: binding to human low density lipoprotein (LDL) and effect on LDL oxidation. Int J Clin Pharmacol Res. 2000;20:49–54.
26. Masella R, Vari R, D'Archivio M, Di Benedetto R, Matarrese P, Malorni W, Scazzocchio B, Giovannini C. Extra virgin olive oil biophenols inhibit cell-mediated oxidation of LDL by increasing the mRNA transcription of glutathione-related enzymes. J Nutr. 2004;134:785–91.
27. Franconi F, Coinu R, Carta S, Urgeghe PP, Ieri F, Mulinacci N, Romani A. Antioxidant effect of two virgin olive oils depends on the concentration and composition of minor polar compounds. J Agric Food Chem. 2006;54:3121–5.
28. Weinbrenner T, Fito M, De La Torre R, Saez GT, Rijken P, Tormos C, Coolen S, Albaladejo MF, Abanades S, et al. Olive oils high in phenolic compounds modulate oxidative/antioxidative status in men. J Nutr. 2004;134:2314–21.
29. Konstantinidou V, Khymenets O, Covas MI, de la Torre R, Munoz-Aguayo D, Anglada R, Farre M, Fito M. Time course of changes in the expression of insulin sensitivity-related genes after an acute load of virgin olive oil. OMICS. 2009;13:431−8.
30. Hamden K, Allouche N, Damak M, Elfeki A. Hypoglycemic and antioxidant effects of phenolic extracts and purified hydroxytyrosol from olive mill waste in vitro and in rats. Chem Biol Interact. 2009;180:421–32.
31. Ruano J, Lopez-Miranda J, de la Torre R, gado-Lista J, Fernandez J, Caballero J, Covas MI, Jimenez Y, Perez-Martinez P, et al. Intake of phenol-rich virgin olive oil improves the postprandial prothrombotic profile in hypercholesterolemic patients. Am J Clin Nutr. 2007;86:341–6.
32. Masella R, Giovannini C, Vari R, Di Benedetto R, Coni E, Volpe R, Fraone N, Bucci A. Effects of dietary virgin olive oil phenols on low density lipoprotein oxidation in hyperlipidemic patients. Lipids. 2001;36:1195–202.
33. Fito M, Gimeno E, Covas MI, Miro E, Lopez-Sabater MD, Farre M, De La Torre R, Marrugat J. Postprandial and short-term effects of dietary virgin olive oil on: oxidant/antioxidant status. Lipids. 2002;37:245–51.
34. Marrugat J, Covas MI, Fito M, Schroder H, Miro-Casas E, Gimeno E, Lopez-Sabater MC, De La Torre R, Farre M. Effects of differing phenolic content in dietary olive oils on lipids and LDL oxidation: a randomized controlled trial. Eur J Nutr. 2004;43:140–7.
35. Rietjens SJ, Bast A, Haenen GR. New insights into controversies on the antioxidant potential of the olive oil antioxidant hydroxytyrosol. J Agric Food Chem. 2007;55:7609–14.
36. Stupans I, Kirlich A, Tuck KL, Hayball PJ. Comparison of radical scavenging effect, inhibition of microsomal oxygen free radical generation, and serum lipoprotein oxidation of several natural antioxidants. J Agric Food Chem. 2002;50:2464–9.
37. Khymenets O, Fito M, Covas MI, Farre M, Pujadas MA, Munoz D, Konstantinidou V, de la Torre R. Mononuclear cell transcriptome response after sustained virgin olive oil consumption in humans: an exploratory nutrigenomics study. OMICS. 2009;13:7–19.
38. Zhang X, Cao J, Zhong L. Hydroxytyrosol inhibits pro-inflammatory cytokines, iNOS, and COX-2 expression in human monocytic cells. Naunyn Schmiedebergs Arch Pharmacol. 2009;379:581–6.
39. Masella R, Di Benedetto R, Vari R, Filesi C, Giovannini C. Novel mechanisms of natural antioxidant compounds in biological systems: involvement of glutathione and glutathione-related enzymes. J Nutr Biochem. 2005;16:577–86.
40. Fito M, Cladellas M, de la Torre R, Marti J, Munoz D, Schroder H, Alcantara M, Pujadas-Bastardes M, Marrugat J, et al. Anti-inflammatory effect of virgin olive oil in stable coronary disease patients: a randomized, crossover, controlled trial. Eur J Clin Nutr. 2008;62:570–4.
41. Bogani P, Galli C, Villa M, Visioli F. Postprandial anti-inflammatory and antioxidant effects of extra virgin olive oil. Atherosclerosis. 2007;190:181–6.
42. Schmitt CA, Handler N, Heiss EH, Erker T, Dirsch VM. No evidence for modulation of endothelial nitric oxide synthase by the olive oil polyphenol hydroxytyrosol in human endothelial cells. Atherosclerosis. 2007;195:e58–64.
43. Dudley JI, Lekli I, Mukherjee S, Das M, Bertelli AA, Das DK. Does white wine qualify for French paradox? Comparison of the cardioprotective effects of red and white wines and their constituents: resveratrol, tyrosol, and hydroxytyrosol. J Agric Food Chem. 2008;56:9362–73.
44. Gimeno E, Fito M, Lamuela-Raventos RM, Castellote AI, Covas M, Farre M, Torre-Boronat MC, Lopez-Sabater MC. Effect of ingestion of virgin olive oil on human low-density lipoprotein composition. Eur J Clin Nutr. 2002;56:114–20.
45. Witztum JL. The oxidation hypothesis of atherosclerosis. Lancet. 1994;344:793–5.
46. Meisinger C, Baumert J, Khuseyinova N, Loewel H, Koenig W. Plasma oxidized low-density lipoprotein, a strong predictor for acute coronary heart disease events in apparently healthy, middle-aged men from the general population. Circulation. 2005;112:651–7.
47. Miró-Casas E, Albaladejo MF, Covas MI, Rodriguez JO, Colomer EM, Raventos RML, De La Torre R. Capillary gas chromatography-mass spectrometry quantitative determination of hydroxytyrosol and tyrosol in human urine after olive oil intake. Anal Biochem. 2001;294:63–72.
48. De La Torre-Carbot K, Jauregui O, Gimeno E, Castellote AI, Lamuela-Raventos RM, Lopez-Sabater MC. Characterization and quantification of phenolic compounds in olive oils by solid-phase extraction, HPLC-DAD, and HPLC-MS/MS. J Agric Food Chem. 2005;53:4331–40.
49. Commission Regulation. Commission Regulation (EEC) no. 2568/91 of July 11 1991 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. European Union: Office for Official Journal of the European Union; 1991.
50. Rodriguez-Palmero M. de lP-O, Castellote-Bargallo AI, Lopez Sabater MC, Rivero-Urgell M, de La Torre-Boronat MC. Determination of sterol content in different food samples by capillary gas chromatography. J Chromatogr A. 1994;672:267–72.
51. Gimeno E, Castellote AI, Lamuela-Raventos RM, de la Torre MC, Lopez-Sabater MC. Rapid determination of vitamin E in vegetable oils by reversed-phase high-performance liquid chromatography. J Chromatogr A. 2000;881:251–4.
52. Badeau M, Adlercreutz H, Kaihovaara P, Tikkanen MJ. Estrogen A-ring structure and antioxidative effect on lipoproteins. J Steroid Biochem Mol Biol. 2005;96:271–8.
53. de la Torre-Carbot K, Jauregui O, Castellote AI, Lamuela-Raventos RM, Covas MI, Casals I, Lopez-Sabater MC. Rapid high-performance liquid chromatography-electrospray ionization tandem mass spectrometry method for qualitative and quantitative analysis of virgin olive oil

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phenolic metabolites in human low-density lipoproteins. J Chromatogr A. 2006;1116:69–75.
54. Miró-Casas E, Covas MI, Fito M, Farre-Albadalejo M, Marrugat J, De La Torre R. Tyrosol and hydroxytyrosol are absorbed from moderate and sustained doses of virgin olive oil in humans. Eur J Clin Nutr. 2003;57:186–90.
55. Gimeno E, De La Torre-Carbot K, Lamuela-Raventos RM, Castellote AI, Fito M, De La Torre R, Covas MI, Lopez-Sabater MC. Changes in the phenolic content of low density lipoprotein after olive oil consumption in men. A randomized crossover controlled trial. Br J Nutr. 2007;98:1243–50.
56. Deiana M, Rosa A, Corona G, Atzeri A, Incani A, Visioli F, Paola MM, Assunta DM. Protective effect of olive oil minor polar components against oxidative damage in rats treated with ferric-nitrilotriacetate. Food Chem Toxicol. 2007;45:2434–40.
57. Ochoa JJ, Quiles JL, Ramirez-Tortosa MC, Mataix J, Huertas JR. Dietary oils high in oleic acid but with different unsaponifiable fraction contents have different effects in fatty acid composition and peroxidation in rabbit LDL. Nutrition. 2002;18:60–5.
58. Benkhalti F, Legssyer A, Gomez P, Paz E, Lopez-Miranda J, Perez-Jimenez F, el Boustani ES. Effects of virgin olive oil phenolic compounds on LDL oxidation and vasorelaxation activity. Therapie. 2003;58:133–7.
59. Weinbrenner T, Fito M, Farre AM, Saez GT, Rijken P, Tormos C, Coolen S, De La Torre R, Covas MI. Bioavailability of phenolic compounds from olive oil and oxidative/antioxidant status at postprandial state in healthy humans. Drugs Exp Clin Res. 2004;30:207–12.
60. Di Benedetto R, Vari R, Scazzocchio B, Filesi C, Santangelo C, Giovannini C, Matarrese P, D'Archivio M, Masella R. Tyrosol, the major extra virgin olive oil compound, restored intracellular antioxidant defences in spite of its weak antioxidative effectiveness. Nutr Metab Cardiovasc Dis. 2007;17:535−45.
61. Miles EA, Zoubouli P, Calder PC. Differential anti-inflammatory effects of phenolic compounds from extra virgin olive oil identified in human whole blood cultures. Nutrition. 2005;21:389–94.
62. Dell'Agli M, Fagnani R, Mitro N, Scurati S, Masciadri M, Mussoni L, Galli GV, Bosisio E, Crestani M, et al. Minor components of olive oil modulate proatherogenic adhesion molecules involved in endothelial activation. J Agric Food Chem. 2006;54:3259–64.
63. Tuck KL, Hayball PJ. Major phenolic compounds in olive oil: metabolism and health effects. J Nutr Biochem. 2002;13:636–44.
64. Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev. 1998;56:317–33.

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