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Original Contribution

Postprandial LDL phenolic content and LDL oxidation are modulated by olive oil phenolic compounds in humans

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Abstract

Olive oil phenolic compounds are potent antioxidants in vitro, but evidence for antioxidant action in vivo is controversial. We examined the role of the phenolic compounds from olive oil on postprandial oxidative stress and LDL antioxidant content. Oral fat loads of 40 mL of similar olive oils, but with high (366 mg/kg), moderate (164 mg/kg), and low (2.7 mg/kg) phenolic content, were administered to 12 healthy male volunteers in a cross-over study design after a washout period in which a strict antioxidant diet was followed. Tyrosol and hydroxytyrosol, phenolic compounds of olive oil, were dose-dependently absorbed ($p < 0.001$). Total phenolic compounds in LDL increased at postprandial state in a direct relationship with the phenolic compounds content of the olive oil ingested ($p < 0.05$). Plasma concentrations of tyrosol, hydroxytyrosol, and 3-O-methyl-hydroxytyrosol directly correlated with changes in the total phenolic compounds content of the LDL after the high phenolic compounds content olive oil ingestion. A 40 mL dose of olive oil promoted a postprandial oxidative stress, the degree of LDL oxidation being lower as the phenolic content of the olive oil administered increases. In conclusion, olive oil phenolic content seems to modulate the LDL phenolic content and the postprandial oxidative stress promoted by 40 mL olive oil ingestion in humans.

Keywords: Olive oil; Tyrosol; Hydroxytyrosol; LDL phenolic content; LDL oxidation; Postprandial; Free radical

Introduction

There is increasing evidence that oxidative modification of low density lipoprotein (LDL) plays a key role in the development of atherosclerosis [1]. Elevated levels of oxidized LDL showed a positive correlation with the severity of acute coronary events [2] and have been considered a biochemical marker for coronary heart disease [3]. The process of LDL oxidation led to the modification of the protein moiety of LDL, directly, i.e. via myeloperoxidase-derived HOCl [4], or indirectly, i.e. via the peroxidation of the polyunsaturated fatty acids [5]. The modified apo B has immunogenic properties prompting the generation of autoantibodies against oxidized LDL [5]. Linoleic acid accounts for 90% of the polyunsaturated fatty acids (PUFA) present in LDL and is the major substrate for its oxidation [6]. Therefore, diets rich in PUFA may increase the risk of LDL oxidation. On the other hand, diets rich in oleic acid generate particles that appear to be more resistant to this process [7,8]. However, apart from its fatty acid profile, the formation of oxidized LDL depends upon its antioxidant content, such as vitamin E and phenolic compounds, present in LDL [8–10].

Besides containing high quantities of oleic acid, virgin olive oil, obtained exclusively by physical procedures, is rich in phenolic compounds. In animal and in in vitro studies, olive oil

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phenolic compounds have been shown to be strong antioxidants protecting lipids from oxidation [11–14]. The biological activities of olive oil phenolic compounds have prompted several studies on their potential activity in the prevention of cardiovascular diseases and cancer. However, controversial results have been obtained in the randomized, cross-over, controlled human in vivo studies performed on the olive oil phenolic compounds antioxidant capacity [15–18]. Tyrosol (T) and hydroxytyrosol (HT) are the main olive oil phenolic compounds which are present as free or conjugate forms as secoroids or aglycones [19].

Postprandial lipemia has been recognized as a risk factor for atherosclerosis development as it is associated with oxidative changes [20,21]. After a high-fat meal an oxidative stress occurs impairing endothelial function [22]. However, the consumption of fatty meals with suitable sources of antioxidants, such as red wine [23], vitamin C [24], or antioxidant drugs such as simvastatin [22], minimizes this postprandial oxidative stress. In this report, through a randomized, cross-over, controlled study, we describe the impact of three similar types of olive oils, but with differences in their phenolic content, on the postprandial oxidative stress and the antioxidant content of the low density lipoproteins (LDL).

Subjects and methods

Subjects and study design

Twelve healthy male volunteers were recruited, with a mean age of 21.2 years (range 20–22 years), and a mean body mass index of 22.9 (range 20.8–31.6 kg/m^2). Subjects were considered healthy on the basis of physical examination and routine biochemical and hematological laboratory determinations. The protocol was approved by the CEIC-IMAS Ethic Committee. The protocol was fully explained to the participants before they gave their written informed consent.

Each subject was tested three times in a randomized cross-over, double-blind manner with regard to the administration of 40 mL of olive oil with low (LPC, 2.7 mg/kg), medium (MPC, 164 mg/kg), and high (HPC, 366 mg/kg) phenolic compounds content. The amount of phenolic compounds administered with the 40 mL olive oil dose was: 0.097 mg, 5.92 mg, and 13.2 mg, for LPC, MPC, and HPC olive oil, respectively. Two Latin squares of 3 × 3 for the three treatments were used to randomize participants into six orders of olive oil administration. Prior to each intervention volunteers followed a 10-day washout period. During the first 7 days of the washout period participants were asked to avoid excessive antioxidant intake. During the last 3 days before the day of the intervention (days 8–10 of the washout period) they followed a strict phenolic compound-low diet (Fig. 1). A nutritionist instructed them on excluding several foods, rich in phenolic compounds, from their diet (vegetables, legumes, fruit, juice, wine, coffee, tea, caffeine-containing soft drinks, beer, cacao, marmalade, and olives). LPC olive oil was given to the participants for raw and cooking purposes (including supplies for the family) during washout periods, and for cooking purposes in the intervention day. Daily dietary records were obtained from each volunteer. At 8 a.m., after an overnight fast, volunteers were provided with 40 mL of one of the three olive oils, which was administered as a single dose accompanied by a standard piece of bread. The 40 mL olive oil dose was the sole source of olive oil or antioxidants during the intervention day. Venous blood was collected in tubes containing 1g/L EDTA at baseline of each intervention period (0 h) and at several periods after olive oil administration. Plasma was obtained by centrifugation of blood at 1500 × g at 4°C for 20 min. Aliquots of the plasma samples were mixed with 3,5-di-tert-butyl-4-hydroxytoluene 100 μM to avoid auto-oxidation and stored at −80°C until analyzed. All biochemical and analytical determinations were performed in duplicate.

Nutrient intakes were calculated from the daily dietary records of each intervention period and the three previous days of the washout periods by a nutritionist using the software MediSystem 2000 (Conaycyte S.A, Madrid, Spain).

Measurement of tyrosol, hydroxytyrosol, and 3-O-methyl-hydroxytyrosol, in plasma

Tyrosol (T), hydroxytyrosol (HT), and 3-O-methyl-hydroxytyrosol (MHT) a biological metabolite of HT, were

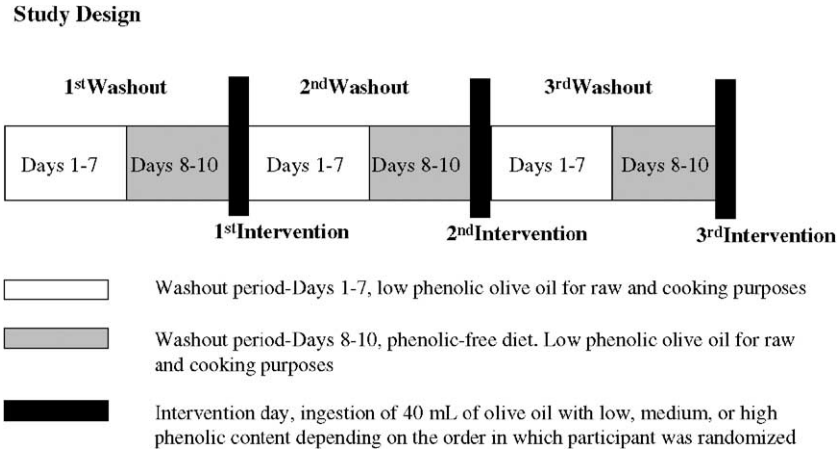

Fig. 1. Time-line showing the study design.

determined by gas chromatography-mass spectrometry (GC/MS). Analyses were carried out on a Hewlett-Packard (Palo Alto, CA) gas chromatograph coupled to a mass spectrometer detector system consisting of an HP5980 gas chromatograph, a HP5973 mass-selective detector, and a HP7683 series injector. Separation of hydroxytyrosol and tyrosol was carried out using a HP Ultra 2 (12.5 m × 0.2-mm i.d. and 0.33-μm film thickness) cross-linked 5% phenylmethyl silicone capillary column (Hewlett-Packard). Instrumental, hydrolytic and extraction conditions of samples were previously described [25]. All chemicals and organic solvents used were of analytical grade. Pharmacokinetic parameters were calculated using specific functions in a spreadsheet (PK Functions for Microsoft Excel).

Measurement of the fatty acid profile, α-tocopherol, and total phenolic content of the LDL

The antioxidant content of the LDL was measured at several times (0–1.5 h) around the maximum peak of plasma T, HT, and MHT concentrations. α-Tocopherol in LDL was determined by HPLC [26]. Phenolic compounds in LDL were determined by HPLC-DAD [27]. The fatty acid composition of LDL was also determined following the method described by Bondía et al. [28] in which fatty acids are transformed into methyl esters and analyzed by gas chromatography (GC-FID).

Measurement of plasma and lipoproteins oxidative stress markers

Oxidized LDL (oxLDL) in plasma was measured by a sandwich ELISA procedure using the murine monoclonal antibody, mAb-4E6, as capture antibody bound to microtitration wells, and a peroxidase conjugated anti-apolipoprotein B antibody recognizing oxLDL bound to the solid phase (oxLDL, Mercodia AB, Uppsala, Sweden). Antibodies against oxidized LDL (Ab-oxLDL) were measured by ELISA using copper-oxidized LDL as antigen and a specific peroxidase conjugated with anti-human IgG antibodies (OLAB, Biomedica, Vienna, Austria) [29]. Plasma total $F_{2\alpha}$-isoprostanes (8-iso-$PGF_{2\alpha}$) were determined using HPLC-ESI-MS-MS and stable isotope dilution mass spectrometry [30]. 3-chlorotyrosine (3-CT) was measured in a mixture of Apo B containing lipoproteins. Very low density lipoproteins (VLDL) and LDL were isolated from plasma by ultracentrifugation. 3-CT and its C13 analogue (3-(13C6)-chlorotyrosine) analogue (as internal standard) were synthesized as previously described [31]. Samples containing VLDL+LDL and internal standard were submitted to a basic hydrolysis with NaOH 4M for 16 hours at 120°C. After hydrolysis, samples were adjusted to pH 3–4 with trifluoroacetic acid (TFA), and the mixture was passed over a solid/liquid phase C18 extraction column (Bond-Elut-C18, 500 mg, Varian, Palo Alto, California, USA) equilibrated with 2 mL of 0.1% TFA. The column was washed with 2 mL of the same buffer, eluted with 2mL of 20% methanol in 0.1% TFA, and the recovered amino acids were dried under vacuum. Aminoacids were derivatized with a mixture of MSTFA:NH4I:2-mercaptoethanol (1000:2:6, v/w/v) at 60°C for 1 hour and analyzed by GC/MS in the electron impact ionization mode and in the single ion monitoring acquisition mode [31]. Concentration of 3-CT was very low in plasma Apo B containing lipoproteins of participants as has been previously described for healthy individuals [4]. Thus, VLDL+LDL samples from 2 individuals were pooled on the basis of similar concentrations of Apo B in lipoproteins.

Measurement of serum glucose and lipid profile

Plasma glucose, total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), and triglyceride (TG) levels were determined using enzymatic kits (Hofmann-La Roche Diagnostic, Basel, Switzerland) adapted to a Cobas Mira Plus autoanalyzer (Hoffmann-La Roche, Basel, Switzerland). LDL cholesterol (LDL-C) was calculated by means of the Friedewald formulae.

Characteristics of the olive oils

A virgin olive oil with high phenolic content (366 mg/kg) was selected. Fatty acids and vitamin E composition were measured. Harvests of virgin olive oils from the same cultivar and soil, which were submitted to refinement, were tested in order to select one with a similar fatty acid and micronutrient profile to that of the virgin olive oil selected. An adjustment of vitamin E to similar values of that present in this virgin olive oil was performed. Phenolic compounds are lost in the refination process, due to this, the refined olive oil had a low phenolic content (2.7 mg/kg). By mixing virgin and refined olive oil, an olive oil with an intermediate phenolic content (164 mg/kg) was obtained. The characteristics of the three olive oils with high (HPC), medium (MPC), and low (LPC) phenolic content were similar to that of olive oils present in the market, and are shown in Table 1. Olive oils had similar fatty acid profile and minor components content, but, with differences in their phenolic content.

Statistical analyses

The normality of variables was assessed by the Kolmogorov-Smirnov test and by analyses of skewness and kurtosis. Spearmans correlation coefficients were used to assess the relationship between continuous variables. One-factor ANOVA and Kruskal-Wallis test were used to determine differences in basal characteristics and nutrient intake among the three olive oil interventions. A general linear model for repeated measurements was used with multiple paired comparisons, in order to assess the effect of each type of olive oil. The comparison of post-intervention changes in oxidative stress variables was carried out by a General Linear Mixed Model (GLMM) with the olive oil phenolic dose (high, medium, low) as a fixed factor; individual level of test subjects as random effect, and olive oil administration order

Table 1
Characteristics of the olive oils administered

	Type of olive oil		
	LPC	MPC	HPC
Quality parameters			
Free acidity (% oleic acid)	0.03	0.08	0.18
Peroxide value (mEq O_2/kg)	4.12	5.89	11.28
Fatty acids (%)			
C14:0	<0.1	<0.1	<0.1
C16:0	10.6	10.5	10.6
C16:1	0.9	0.9	0.9
C17:0	<0.1	<0.1	<0.1
C17:1	<0.1	<0.1	<0.1
C18:0	3.3	3.1	2.8
C18:1	79.1	79.8	80.6
C18:2	4.6	4.2	3.3
C20:0	0.4	0.4	0.4
C18:3	0.6	0.6	0.6
C20:1	0.3	0.3	0.3
C22:0	0.1	0.1	0.1
C24:0	<0.1	<0.1	<0.1
α-Tocopherol (ppm)	229	228	228
Phenolic compounds (mg/kg)	2.7	164	366
Squalene (mg/g)	3.0	3.2	3.4
β-sitosterol (mg/g)	1.4	1.5	1.5

LPC, MPC, and HPC, olive oils with low (2.7 mg/kg), medium (164 mg/kg), and high (366 mg/kg) phenolic content, respectively.

as covariates. Models were corrected by Tukey's method for multiple comparisons. Linearity of values across olive oil interventions was determined by these models as a test for the dose-response effect of phenolic compounds. Statistical significance was defined as $p < 0.05$ for a two-sided test. These statistical analyses were performed using the SPSS statistical software (SPSS Incorporated Co., Chicago, IL, version 11.1).

Results

Dietary intake

No significant differences were observed among groups for energy, protein, carbohydrate, and fat intake, or for the main antioxidant (i.e. β-carotenoid, vitamin C, α-tocopherol) or pro-oxidant (iron) intake during the study.

Pharmacokinetics of plasma tyrosol, hydroxytyrosol, and 3-O-methyl-hydroxytyrosol

Plasma concentration of T, HT, and MHT increased in a dose-dependent manner with the phenolic content of the olive oil administered ($p < 0.001$) (Fig. 2). The time to reach peak concentrations (Tmax) was (mean ± SD): 0.83 ± 0.58 h and 0.90 ± 0.81 h for T, 0.70 ± 0.51 h and 0.91 ± 0.84 h for HT, and 1.31 ± 1.02 h and 1.12 ± 0.74 h for MHT, for MPC and HPC olive oils, respectively. The increase in plasma phenolic compounds after LPC ingestion was negligible. The estimated elimination half-life was (mean ± SD): 3.41 ± 1.68 h and 2.89 ± 0.60 h for T, 3.01 ± 1.01h and 3.00 ± 1.46 h for HT, and 2.37 ± 1.29 and 2.96 ± 0.87 h for MHT, for MPC and HPC olive oils, respectively.

Effect on the LDL fatty acid, α-tocopherol, and total phenolic content

No changes were observed in the LDL fatty acid and vitamin E content after any type of olive oil ingestion. The phenolic content of the LDL, however, changed with the type of olive oil administered (Table 2). LDL total phenolic content decreased after 1.5 h of LPC ingestion ($p < 0.05$) whereas an increasing trend in the phenolic content of LDL was observed after HPC ingestion ($p < 0.05$). When changes in the amount of phenolic compounds in LDL from baseline among olive oils were compared, the phenolic content of LDL increased in a dose-dependent manner with the phenolic content of the olive oil administered at 1 h and 1.5 h after olive oils ingestion ($p < 0.05$) (Table 2).

Changes in the phenolic content of the LDL directly correlated with plasma T, HT, and MHT after HPC ingestion (Table 3). These relationships were significant at 30 min for HT and at 1 h and 1.5 h for T and MHT after HPC ingestion ($p < 0.05$), reaching in the remaining cases a borderline significance ($p < 0.1$). Concerning MPC, no significance was obtained in any case when the above mentioned relationships were

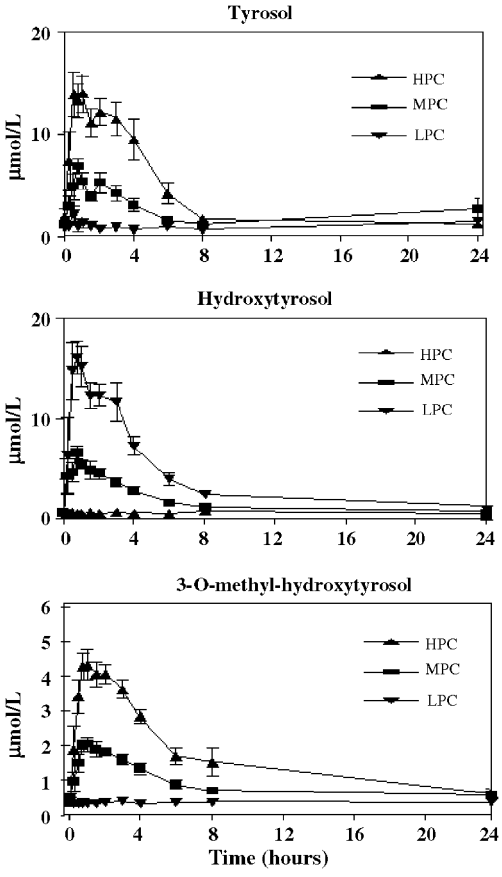

Fig. 2. Concentration versus Time curves for tyrosol, hydroxytyrosol, and 3-O-methyl-hydroxytyrosol after ingestion of 40 mL of olive oil with high (HPC), medium (MPC), and low (LPC) phenolic content.

Table 2
Percentage of changes[a] from baseline of phenolic compounds in LDL after a single dose of 40 mL of olive oil with low (LPC), medium (MPC), and high (HPC) phenolic content

Olive oil	Baseline (0 h)[b]	Changes (%) after olive oil ingestion			p[c]
		30 min	1 h	1.5 h	
LPC	0.54 (0.25)	−1.7 (15.0)	−9.3 (13.7)	−27 (5.4)[d]	0.081
MPC	0.48 (0.28)	−7.0 (17.3)	0.79 (9.3)	−9.7 (11.0)	0.442
HPC	0.43 (0.23)	14.3 (16.6)	44 (15.1)[e]	57 (14.2)[d,e]	0.046
p[f]	0.879	0.254	0.046	0.017	

[a] Data are expressed as the mean (SEM).
[b] ng/ g total fatty acids.
[c] p for linear trend across time for each olive oil evaluated, general linear model.
[d] $p < 0.05$ versus baseline, Tukey's test for multiple comparisons.
[e] $p < 0.05$ versus LPC olive oil at the evaluated time, Tukey's test for multiple comparisons.
[f] p for linear trend across olive oils for each evaluated time, general linear model.

examined, despite of a direct trend in the associations (data not shown).

Effect on the plasma and lipoprotein concentration of oxidative stress markers

After a 40 mL olive oil dose, hypertriglyceridemia together with oxidative stress occurred with all types of olive oils administered. Changes, expressed as percentage from baseline concentrations, of triglycerides, F2-isoprostanes, ox-LDL, and Ab-oxLDL are shown in Table 4 for 2, 4, and 6 h after the intake of HPC, MPC, and LPC. A significant increase in triglycerides at 2 and 4 h, and in F2-isoprostanes at 4 and 6 h, was observed after ingestion of the three olive oils ($p < 0.05$). Concerning the isoprostanes increases, the highest (21.3% and 29.9% at 4 h and 6 h postprandial, respectively) was observed after LPC ingestion, and the lowest (11.6% and 16.5% at 4 h and 6 h postprandial, respectively) after HPC ingestion. The increase in plasma oxidized LDL was only significant after LPC ingestion, Ab-ox LDL followed a different pattern depending on the type of olive oil ingested. A decreasing linear trend, a decreasing quadratic trend, and no significant changes, were observed after LPC, MPC, and HPC, respectively (Table 4). Fig. 3 shows the concentrations of 3-chlorotyrosine in Apo B containing lipoproteins after olive oils ingestion. An increase at 4 h after LPC ingestion was observed ($p < 0.05$). After 4 h of MPC ingestion concentrations of 3-chlorotirosine in VLDL+LDL increased, but significance was not reached, whereas no changes were observed after HPC ingestion. The increase (percentage of change) of 3-chlorotyrosine in Apo B containing lipoproteins at 4 h from baseline was significantly lower after HPC ingestion than after MPC ingestion ($p = 0.014$).

The data in Table 4 suggest a higher antioxidant capacity of the HPC olive oil versus the LPC one. The different effects of the olive oil phenolic dose are shown in Table 5. The decrease in ox-LDL changes from LPC to HPC observed in Table 4 was seen as a significant trend 4 h and 6 h after olive oil intake. The increase in Ab-oxLDL values from LPC to HPC was seen as a significant trend in all evaluated times. Paired comparisons showed significantly lower ox-LDL and higher Ab-oxLDL values after HPC versus LPC interventions in all evaluated times No differences were observed for F2-isoprostanes, although the decrease after HPC versus LPC at 4h reached a borderline significance at 4 h and 6 h after olive oil administration ($p = 0.062$ and $p = 0.070$, respectively).

Discussion

In this study, three similar types of olive oils, but with difference in their phenolic content, were used. Participants were submitted to a strict low-antioxidant diet 3 days before and during the intervention periods. The use of LPC olive oil during wash-out periods for raw and cooking purposes, and for cooking purposes the day of the study, avoided differences in the main fat ingestion, and permitted an homogenization of the LDL fatty acid composition. The type of fat ingested is a key factor concerning LDL oxidation. Oleate rich-LDL is less susceptible to oxidation than linoleate-rich LDL (8). With our design we avoided the interference of oleic acid when assessing the in vivo antioxidant capacity of phenolic compounds from olive oil. Phenolic compounds from olive oils were also the only differential source of antioxidants during the intervention day. Phenolic compounds were absorbed and had a pharmacokinetic profile which was dose-dependent on the phenolic content of the olive oil administered. These results agree with those obtained by Visioli et al [32] in which the absorption of T and HT from olive oils enriched with high levels of free forms of these phenolic compounds were also absorbed in a dose-dependent manner.

Dietary phenolic compounds can bind human LDL lipoprotein [33]. Phenolic compounds which can bind LDL are likely to exert their peroxyl scavenging activity in the arterial intima, where oxidation of LDL mainly occurs in microdomains sequestered from the richness of antioxidants present in plasma [1]. In ex vivo studies, we observed that plasma incubation with virgin olive oil extracts led to an increase of the phenolic compounds previously bound to LDL [34]. Here, we report an in vivo increase at postprandial time in the total phenolic content of LDL in a dose-dependent manner with the phenolic content of the olive oil administered. The decrease in the LDL phenolic content after LPC ingestion could be attributed to an oxidation of the LDL phenolics by the postprandial oxidative stress. Our data suggest that in the case of MPC and HPC ingestion, the

Table 3
Spearman's correlation coefficients (p for significance) between percentage of changes of phenolic compounds in LDL and plasma tyrosol, hydroxytyrosol, and 3-O-methyl-hydroxytyrosol after 40 mL of olive oil with high phenolic content

Time	Tyrosol	Hydroxytyrosol	3-O-methyl-hydroxytyrosol
30 min	0.510 (0.090)	0.780 (0.009)	0.524 (0.080)
1 h	0.608 (0.036)	0.517 (0.085)	0.636 (0.026)
1.5 h	0.699 (0.011)	0.507 (0.089)	0.629 (0.028)

Table 4
Percentage of changes[a] of triglycerides, F_2-isoprostanes, oxidized LDL, and antibodies against oxidized LDL (Ab-ox LDL), after ingestion of 40 mL of olive oil with low (LPC), medium (MPC), and high (HPC) phenolic content

Olive oil	Baseline (0 h)	Changes (%) after olive oil ingestion (time in hours)			p (trend)[b]
		2 h	4 h	6 h	
HPC					
Triglycerides (mmol/L)	1.25 (0.46)	39 (14)[c]	43 (13)[c]	12 (10)	0.012 (quadratic)
F_2-isoprostanes (pg/ml)	24 (5.5)	4.9 (3.5)	11.6 (3.3)[c]	16.5 (4.8)[c]	0.005 (linear)
Oxidized LDL (U/L)	51 (21)	−9.5 (9.2)	5.2 (8.6)	−15.2 (8.7)	n.s.
Ab-ox LDL (U/L)	251 (113)	20.8 (13.6)	22.5 (13.1)	22.1 (18.9)	n.s
MPC					
Triglycerides (mmol/L)	1.16 (0.33)	36 (12)[c]	33 (15)[c]	9.2 (10)	0.039 (quadratic)
F_2-isoprostanes (pg/ml)	26 (7.1)	11.9 (6.0)	14.2 (5.6)[c]	23.8 (5.4)[d]	0.002 (linear
Oxidized LDL (U/L)	45 (20)	5.6 (9.2)	10.0 (8.5)	8.9 (9.2)	n.s.
Ab-oxLDL (U/L)	304 (196)	−17.8 (9.1)	−14.25 (7.7)	−0.20 (13.5)	0.0 42 (quadratic)
LPC					
Triglycerides (mmol/L)	1.24 (0.50)	32 (11)[c]	50 (21)[c]	27 (20)	0.038 (quadratic)
F_2-isoprostanes (pg/ml)	26 (6.3)	5.6 (5.0)	21.3 (6.8)[d]	29.9 (5.1)[d]	0.001 (linear)
Oxidized LDL (U/L)	46 (20)	23.2 (8.2)[c]	24.7 (8.6)[c]	20.9 (8.9)	n.s.
Ab-ox LDL (U/L)	262 (130)	−19.2 (11.2)	−28.7 (12.6)[c]	−27.1 (8.2)[c]	0.011 (linear)

n.s., not significant.
[a] Data are expressed as the mean (SEM).
[b] p for trend from general linear model.
[c] $p < 0.05$ from baseline, general linear model with Tukey's correction for multiple comparisons.
[d] $p < 0.01$ from baseline, general linear model with Tukey's correction for multiple comparisons.

phenolic content of the olive oil protected the LDL phenolic content from degradation. The direct relationship observed among plasma T and HT with the changes in the phenolic content of LDL after HPC ingestion also supports the idea that the postprandial increase in the total phenolic content in LDL observed could be attributed to the olive oil phenolic compounds ingested with the olive oil. The fact that phenolic compounds from olive oil can protect the phenolic content of LDL reinforces their role as antioxidants in vivo.

In previous studies, the ingestion of a 25 mL dose of virgin olive oil did not promote postprandial oxidative stress [35], whereas a 50 mL dose did [36]. The balance of pro-oxidant and antioxidant reactions is well regulated in the organism. Thus, an intervention with an antioxidant-rich compound, without any additional impact of oxidative stress, may exert only a marginal effect, if any. The data presented in this report support the action of reactive oxygen species oxidizing TG-rich lipoproteins after 40 mL olive oil ingestion. These TG-rich lipoproteins are sequentially converted to LDL. Thus, a residence time of 2 to 4 hours (required for the conversion of TG-rich lipoproteins to LDL) may be adequate to propagate the oxidative process once the oxidation-initiating element has been transferred to the lipoprotein [21]. In the present study study the degree of postprandial oxidative stress, however, was lower depending

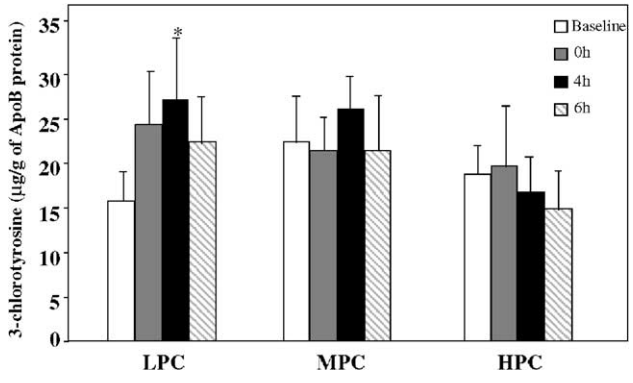

Fig. 3. Concentration of 3-chlorotyrosine (µmoL/g of ApoB protein) in pooled samples of VLDL+LDL after ingestion of 40 mL of olive oil with high (HPC), medium (MPC), and low (LPC) phenolic content ($n = 6$). *$p < 0.05$ from LPC baseline.

Table 5
p values obtained by ANOVA[a] and paired comparisons

Time	F2-isoprostanes	Oxidized LDL	Ab-oxLDL
2 hours			
p for trend	0.414	0.056	**0.016**
HPC versus LPC	0.227	**0.017**	**0.011**
HPC versus MPC	0.875	0.258	**0.014**
MPC versus LPC	0.290	0.190	0.782
4 hours			
p for trend	0.137	**0.043**	**0.003**
HPC versus LPC	0.062	**0.013**	**0.001**
HPC versus MPC	0.702	0.169	**0.010**
MPC versus LPC	0.128	0.229	0.413
6 hours			
p for trend	0.188	**0.028**	**0.031**
HPC versus LPC	0.070	**0.009**	**0.009**
HPC versus MPC	0.344	0.074	0.173
MPC versus LPC	0.356	0.362	0.190

Ab-oxLDL, antibodies against oxidized LDL.
[a] Data from Table 4 were analyzed for statistical significance for the intake of the sequence: high-phenolic (HPC), medium-phenolic (MPC), and low-phenolic (LPC) olive oil by a general lineal mixed model (GLMM) with Tukey's correction for multiple comparisons. Bold values indicate significance.

on the phenolic content of the olive oil. Oxidized LDL only increased with significance after LPC olive oil. Postprandial changes in in vivo oxidized LDL decreased in a dose-dependent manner with the phenolic content of the olive oil administered. Ab-oxLDL changes followed a different pattern depending on the olive oil ingested. No changes in Ab-oxLDL occurred after HPC ingestion, in concordance with the lack of significant changes in ox-LDL. The transient reduction in Ab-oxLDL after LPC and MPC olive oils could be explained by the excess generation of oxidized LDL at postprandial state. Although discrepancies exist in the interpretation of the clinical significance of Ab-oxLDL levels [37], these levels are lower in acute oxidative stress situations, such as immediately after smoking a cigarette [38] or acute myocardial infarction [39]. It has been hypothesized that the physiological role of Ab-oxLDL is to remove oxidized LDL by means of soluble antigen-antibody complexes, these may interfere with Ab-oxLDL determination [40]. In accordance with this hypothesis, an inverse correlation has been observed between Ab-oxLDL and oxLDL in healthy individuals [41] using the same antibodies and methods for analyses as in the present study. In one of our previous studies we observed low levels of Ab-oxLDL together with high levels of oxidized LDL in stable coronary heart disease patients [29]. Transient reduction in the level of circulating autoantibodies against oxidized LDL linked with postprandial lipemia in atherosclerotic patients, after the ingestion of a test meal that provided 26 g of polyunsaturated fatty acids (PUFA), had been previously reported [42]. Although the protective or pathogenic role of the ox-LDL-Ab-oxLDL antigen-antibody complexes remains to be elucidated [37], immunization of laboratory animals with oxLDL increased the complexes inhibiting the progression of atherosclerosis [43]. An inverse relationship between Ab-oxLDL and carotid artery intima-media thickness, an indicator of subclinical atherosclerosis [44], in a healthy population has also been reported using the same antibodies and method as in the present study [45].

The ingestion of olive oil with high phenolic compounds also avoided the postprandial rise in 3-chlorotyrosine concentrations in ApoB containing lipoproteins observed after low- and medium-phenolic content olive oil ingestion. 3-chlorotyrosine is reported to be a specific marker for direct LDL protein oxidation by the myeloperoxidase-H_2-O_2-Cl^- system [4]. The tyrosyl radical generated by myeloperoxidase is also a physiological catalyst for the initiation of lipid peroxidation in lipoproteins [46]. Olive oil phenolic compounds have been shown to counteract both metal- and radical-dependent LDL oxidation and to act as chain-breaking antioxidants for lipid peroxidation [11–13]. Differences in the postprandial degree of oxidative stress were reflected in markers directly associated with LDL oxidation; the dose-dependent increase in the LDL phenolic content observed after MPC and HPC ingestion could be an explanation for this fact. The effect of a high phenolic content in an olive oil dose versus a medium or low one on the LDL oxidation is in line with some long-term studies in which high phenolic content olive oil was more effective than low phenolic content olive oil in both decreasing circulating oxidized LDL levels and increasing the resistance of LDL oxidation (16–18). Despite the high sensitivity of F2-isoprostanes [47], derived from arachidonic acid and with a more broad spectra of sources in blood (i.e. cell membranes), perhaps a higher dose of phenolic compounds or a larger number of individuals in the study would be required to achieve significance in the differences observed among olive oil interventions. Recently, a decrease in postprandial F_2-isoprostanes after a high-flavonol cocoa drink providing 187 mg of flavonols, combined with physical exercise, has been described [48]. Visioli et al. [49] showed that the administration of 50 mL olive oil with high concentrations of phenolic compounds (≥975 mg/L) resulted in a dose-dependent reduction in the 24 h urinary excretion of F_2-isoprostanes in humans.

Concluding remarks

From this work, as well as from other recent reports, we may conclude that the phenolic compounds content of an olive oil can modulate the oxidative/antioxidative balance in plasma and LDL, in an oxidative stress situation. Further clinical studies are warranted with individuals such as diabetics, hypertensive, endurance sportsmen, and smokers, who are prone to oxidative stress.

From the comparison of high- and low-phenolic content olive oil it follows that the content of phenolic compounds in an olive oil is an important determinant for its nutritional value. The phenolic content of the olive oils depends on several factors, such as the crop, variety, ripeness, conservation of the olives, technological processes used for oil extraction, olive oil transport, and harvesting systems. If the beneficial effects of high phenolic content olive oil are substantiated by further human studies, then measures for phenolic compound enhancement in crops, and its conservation in manufacturing processes, may be required to enhance the nutritional properties of olive oil.

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