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Effects of differing phenolic content in dietary olive oils on lipids and LDL oxidation

A randomized controlled trial

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■ **Summary** *Background* Evidence from *in vitro* studies suggests that antioxidant olive oil phenolic compounds can prevent LDL oxidation. However, *in vivo* evidence in support of this hypothesis is sparse. *Aim of the study* to establish the antioxidant effect of olive oils with differences in their phenolic compounds content in humans *Methods* A controlled, double blind, cross-over, randomized, clinical trial using three similar olive oils with increasing phenolic concentration (from 0 to 150 mg/Kg) was conducted in 30 healthy volunteers. Olive oils were administered over three periods of 3 weeks preceded by two-week washout periods. *Results* Urinary tyrosol and hydroxy-tyrosol increased ($p < 0.020$), *in vivo* plasma oxidized LDL de-

creased ($p = 0.006$), and *ex vivo* resistance of LDL to oxidation increased ($p = 0.012$) with the phenolic content of the olive oil administered. After virgin olive oil administration, an increase ($p = 0.029$) was observed in HDL cholesterol levels. *Conclusions* Sustained consumption of virgin olive oil with the high phenolic content was more effective in protecting LDL from oxidation and in rising HDL cholesterol levels than that of other type of olive oils. Dose-dependent changes in oxidative stress markers, and phenolic compounds in urine, were observed with the phenolic content of the olive oil administered. Our results support the hypothesis that virgin olive oil consumption could provide benefits in the prevention of oxidative processes.

■ **Key words** olive oil – phenolic compounds – tyrosol – oxidized LDL – Mediterranean diet – HDL cholesterol

Introduction

There is a growing evidence that the Mediterranean diet, in which olive oil is the main source of fat, has a beneficial effect on diseases associated with oxidative damage such as coronary heart diseases (CHD) and cancer, and on aging [1–3]. The Mediterranean type diet has been shown to reduce the number of recurrences in patients

with myocardial infarction [3] and is assumed to account in part for the low CHD incidence and mortality rates observed in southern European Mediterranean countries [4].

Phenolic compounds are antioxidant agents present in several foods of the Mediterranean diet among which virgin olive oil stands out. The biological benefits of olive oil consumption in preventing low-density lipoproteins (LDL) from oxidation could be linked both

to its antioxidant content, mainly phenolic compounds and vitamin E, and to its high monounsaturated fatty acid (MUFA) content. Previous dietary intervention studies have shown that compared with carbohydrate or polyunsaturated fatty acid (PUFA)-rich diets, MUFA-rich diets provided benefits on the lipid profile and reduced the LDL susceptibility to oxidation [5–8]. Olive oil phenolic compounds protect LDL from *in vitro* oxidation [9–12]. However, information about the effects of phenolic compounds, as distinct from those of other olive oil components, on human *in vivo* LDL oxidation is sparse [13–18]. Olive oils mainly used for nutritional purposes in Mediterranean countries are virgin olive oil, obtained exclusively by physical procedures, and common olive oil (olive oil, UE, 1991), a mixture of refined virgin olive oil (without phenolic content) and virgin olive oil. Olive oil phenolic compounds are lost in the refinement process. Although the content of both total and individual phenolics in virgin olive oil varies among cultivars and harvests, free forms of tyrosol and hydroxytyrosol and their secoroid derivatives have been described as representing around 30%, and other conjugated forms such as oleuropein and ligstroside aglycones representing almost half, of the total phenolic content of a virgin olive oil [9].

In this study we conducted a double-blind, cross-over, randomized, controlled clinical trial in order to investigate the effects of olive oils, with differences in their phenolic content, on *in vivo* LDL oxidation and urinary tyrosol and hydroxytyrosol levels.

Methods

The study population included 30 healthy non-smoking volunteers from a Spanish male religious center. Exclusion criteria were: intake of antioxidant supplements, aspirin or any other drug with established antioxidant properties, obesity (body mass index > 30 kg/m²), dyslipidemia, diabetes, celiac or other intestinal disease, any condition limiting mobility, life-threatening diseases, or any other disease or condition that would impair compliance. The local institutional Review Board approved the protocol. Each study participant signed an informed consent form prior to enrollment.

Characteristics of refined, common, and virgin olive oils used in the assay were previously analyzed. The olive oils selected came from the same cultivar and harvest and were tested for the present clinical trial. Fatty acid composition, α -tocopherol, and β -carotene content were similar in the three oils. Fatty acid composition was determined by gas chromatography [19], and α -tocopherol and β -carotenoid content in olive oils were determined by HPLC [20]. Total phenolic content of the olive oils was measured by the Folin-Ciocalteu method and by HPLC [11, 21]. MUFA percentage was 75%, 77%,

and 75%, in refined, common, and virgin olive oil, respectively. Saturated fatty acid percentage was 14%, 15%, and 15%, and PUFA percentage 11%, 8%, and 10%, in refined, common, and virgin olive oil, respectively. The concentrations of α -tocopherol were 153 mg/Kg, 112 mg/Kg, and 111 mg/Kg, and those of β -carotene were 0 mg/Kg, 0.65 mg/Kg, and 2.1 mg/Kg, in refined, common, and virgin olive oil, respectively. Phenolic compounds were undetectable in refined virgin olive oil. Common olive oil (a mixture of refined and virgin olive oil) contained 68 mg/Kg of phenols of which 2% was tyrosol, 9% was hydroxytyrosol, 52% were oleuropein aglycones, and 15% were ligstroside aglycones. Virgin olive oil contained 150 mg/Kg of phenols of which 3% was tyrosol, 7% was hydroxytyrosol, 42% were oleuropein aglycones, and 14% were ligstroside aglycones.

An in-person screening visit was conducted to ascertain eligibility and obtain baseline data. Of the 42 subjects screened for inclusion, 9 were ineligible. Thus, the subject pool at randomization consisted of 33 subjects. Two of these were withdrawn because of post-randomization ineligibility (hypercholesterolemia) and another requested to be withdrawn. A placebo-controlled, double-blind, cross-over, randomized, clinical supplementation trial was conducted with the three olive oils with different phenolic compound concentrations: refined, common, and virgin. A latin square for the three treatments was used in the crossover clinical trial to randomize participants into three orders of olive oil administration. Virgin, common, and refined olive oils were sequentially administered over three periods of 3 weeks preceded by two-week washout periods in which refined olive oil was used for raw and cooking purposes.

During olive oil treatment periods, participants were requested to ingest a raw daily dose of 25 mL of olive oil distributed over three meals. Other cooking fats were replaced by refined olive oil throughout the study. For compliance control, containers with the corresponding dose of raw olive oil were delivered daily to the participants. The participants were instructed to return the containers every morning when collecting the next daily dose for the amount of unconsumed olive oil to be registered.

Daily menus and individual quantities were recorded, together with the few extra food intakes between meals and those meals seldom taken outside the religious center by some participants. Participants were requested to avoid a high intake of foods listed as containing phenolic compounds. A trained physician stayed at the religious center for the management of the study. Foods were analyzed by a nutritionist and converted into nutrients using the software Medisystems, Conacyte S.A, Madrid Spain. Anthropometric variables (i. e., weight and height) were recorded. Physical activity was

assessed by the Minnesota Leisure Time Physical Activity Questionnaire which has been validated for use in Spanish men [22].

Blood samples were drawn and first spot morning urine collected before the randomization visit, and before and after each intervention period. Blood samples were taken in the morning after a fasting period of 10–12 hours. Blood was drawn without stasis after a period of 20 minutes rest in a supine position. Laboratory determinations for an individual were carried out in the same batch to avoid inter-assay imprecision. Analytical intraassay imprecision of the methods was assessed from 20 pairs of duplicate samples in the same run. Interassay imprecision was assessed from 20 day-to-day measurements of control samples. Both imprecisions were expressed as coefficient of variation (CV%).

In vivo oxidized LDL was determined in plasma by a sandwich ELISA procedure using the murine monoclonal antibody mAB-4E6 as the capture antibody, and a peroxidase conjugated antibody against oxidized apolipoprotein B bound to the solid phase (ox-LDL, Mercodia AB, Uppsala, Sweden). Intra- and interassay CVs were 2.82% and 7.29%, respectively.

LDL isolation was performed by sequential flotation ultracentrifugation [23]. Content of α -tocopherol in isolated LDL was measured by HPLC [24]. The LDL resistance to oxidation was determined by formation of conjugated dienes after copper (5 μ M) oxidation of isolated LDL [11]. Briefly, dialysed LDL (0.05 g protein/L) was incubated with cupric sulphate (5 μ M) in phosphate buffered saline at a final volume of 1 mL. Absorbance at 234 nm was continuously monitored at 2 min intervals for 5 h at 35°C. For data presentation, the x-axis value corresponding to the intercept of the propagation phase tangent with the extrapolated line for the slow propagation reaction was calculated (lag time), oxidation rate was derived from the slope of the propagation phase tangent, and maximum dienes formation was calculated by the maximum increase of the absorbance at 234 nm. Oxidation rate and dienes formation were calculated using the molar absorbance $\epsilon_{234\text{nm}}$ for conjugated dienes (29.5000 \cdot L \cdot mol⁻¹ \cdot cm⁻¹). Intra- and inter-assay CVs were 3.29%, 5.27%, and 1.46%, and 6.69%, 7.21% and 5.09% for lag time, oxidation rate, and dienes formation respectively.

Oxidized LDL serum antibodies (OLAB) were measured by ELISA using copper-oxidized LDL as antigen, and a specific peroxidase conjugated with anti-human IgG antibodies (OLAB, Biomedica, Vienna, Austria). Intra- and interassay CVs were 4.8% and 7.9%, respectively.

Serum glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, and triacylglycerols were determined by enzymatic methods. LDL cholesterol was calculated by the Friedewald formula. Interassay CVs were 2.8%, 2.6%, 4.6%, and 2.9%, for glucose, total cho-

lesterol, HDL cholesterol, and triacylglycerols, respectively.

Tyrosol (T) and hydroxytyrosol (HT) in urine samples were determined by gas chromatography-mass spectrometry [25]. All chemicals and organic solvents used were of analytical grade. Intra-assay CVs were 2.9% and 5.7% for T and HT, respectively, and inter-assay CVs were 3.8% and 6.2% for T and HT, respectively.

Normality of variable distribution was assessed by the Kolmogorov-Smirnov test and by analysis of skewness and kurtosis. One-factor ANOVA and Kruskal-Wallis tests were used as appropriate to analyze the differences in basal characteristics among the three groups of order of olive oil administration. A general linear model for repeated measurements was used, with multiple paired comparisons corrected by Tukey's method, in order to assess differences among groups. Interaction between the type of olive oil and order of administration was assessed for each variable. Linearity of values across refined, common, and virgin olive oils was determined by these models as a test for the dose-response effect of phenolic compounds. All analyses were carried out on an intention-to-treat basis. Statistical significance was defined as $p < 0.05$ for a two-sided test. SPSS statistical software was used.

Results

Table 1 shows characteristics of participants at the beginning of the study. No differences in age, body mass index, physical activity, lipids, glycemia, and LDL oxidative status were observed among the three groups of oil administration order at the beginning of the study.

No significant difference was observed among groups of order of oil administration for average main antioxidant intake (i.e., β -carotenoid, vitamin C, α -tocopherol), energy, or any nutrient of interest at baseline (data not shown). The mean (SD) daily intake was 37.7% (4.5%) protein, 41.8% (4.9%) carbohydrate, and 20.2% (1.8%) fat (percentage of total energy). Mean values for α -tocopherol, ascorbic acid, and β -carotene were 8.3 mg (2.4 mg), 228 mg (102 mg), and 2.38 mg (0.35 mg), respectively. No differences in the daily nutrient intake were observed during each type of olive oil administration (data not shown).

Table 2 shows T and HT urinary concentrations at baseline (after washout) and after each olive oil administration period. An increase was observed in urinary T after common ($p = 0.009$) and virgin ($p < 0.001$) olive oil administration, and in urinary HT ($p = 0.018$), after virgin olive oil administration. When the changes, as difference in percentage between measurements obtained at baseline and after each olive oil intervention period, were calculated, urinary T ($p = 0.015$) and HT ($p = 0.011$)

Table 1 Basal characteristics, serum glucose and lipids, and LDL oxidation biomarkers by sub-groups of subjects depending on the order of olive oil administration

	Group*		
	V-C-R (n = 11)	C-R-V (n = 9)	R-V-C (n = 10)
Age, y	54.8 ± 21.4	61.0 ± 19.2	56.6 ± 19.3
BMI, kg/m ²	24.2 ± 3.5	20.2 ± 8.3	23.6 ± 2.9
Physical activity, kcal/day	337 ± 231	394 ± 210	451 ± 363
Glucose, mmol/L	4.4 ± 0.7	4.2 ± 0.5	4.6 ± 0.9
Total cholesterol, mmol/L	5.4 ± 1.1	5.7 ± 1.0	5.9 ± 1.2
LDL cholesterol, mmol/L	3.5 ± 0.9	3.7 ± 1.0	3.9 ± 1.0
HDL cholesterol, mmol/L	1.4 ± 0.3	1.4 ± 0.3	1.5 ± 0.3
Triacylglycerols, mmol/L	1.1 ± 0.5	1.2 ± 0.4	1.0 ± 0.5
Oxidized LDL, U/L	41.3 (28.1)	43.7 (17.3)	31.14 (14.3)
Resistance of LDL to oxidation			
Lag time, min	108 ± 18.5	110 ± 19.5	116 ± 17.9
Oxidation rate, $\mu\text{mol dienes} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ of LDL protein	6.2 ± 0.6	6.0 ± 0.8	4.6 ± 0.4
Maximum Dienes, $\mu\text{mol dienes} \cdot \text{g}^{-1}$ of LDL protein	520 ± 40.8	516 ± 70.9	482 ± 91.9
Antibodies against oxidized LDL, U/L**	288 (172–1143)	651 (115–1802)	223 (129–611)

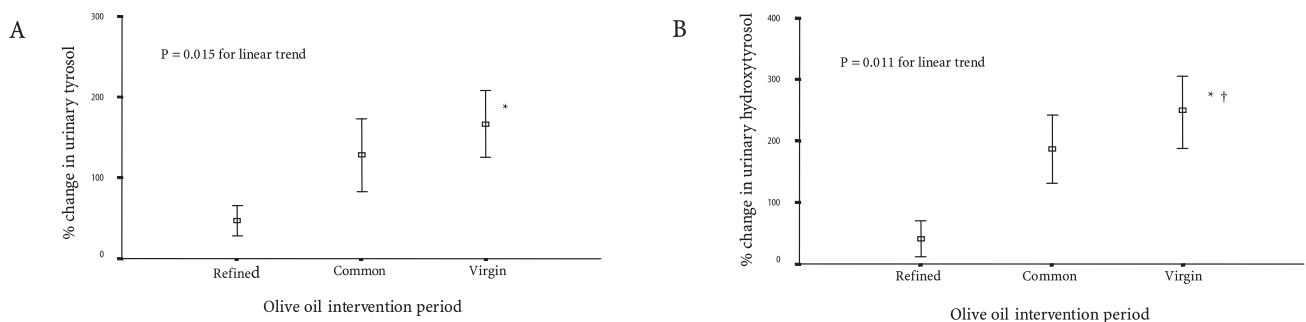
* V virgin olive oil; C common olive oil; R refined olive oil. Values are mean ± SD

** Median (interquartile range)

Table 2 Urinary tyrosol and hydroxytyrosol on each olive oil intervention period (n = 30)

Olive oil	Tyrosol ($\mu\text{mol/L}$)			Hydroxytyrosol ($\mu\text{mol/L}$)		
	Baseline	Treatment	P	Baseline	Treatment	P
Refined	28 (17, 45)	34 (18, 57)	ns	237 (141, 943)	316 (106, 896)	ns
Common	26 (17, 41)	47 (23, 78)	0.009	151 (61, 895)	360 (115, 1102)	ns
Virgin	24 (15, 31)	56 (24, 193)	< 0.001	172 (79, 547)	527 (111, 1647)	0.018

Values are expressed in median (interquartile range)

**Fig. 1** Percentage of change (from before to after olive oil intervention) with 95 % confidence interval in urinary tyrosol and hydroxytyrosol in refined, common, and virgin olive oil intervention periods (mean ± SD, n = 30). **A** Tyrosol; **B** hydroxytyrosol. * P < 0.02, versus refined olive oil intervention period, † P < 0.02 versus common olive oil intervention period

concentrations increased with the phenolic content of olive oil administered (Fig. 1).

Glucose, lipids, and LDL oxidation markers at baseline (after washout) and after each type of olive oil administration are shown in Table 3. Baseline concentrations did not differ among the 3 treatments. Comparison of the absolute values for virgin olive oil intervention with the respective control values (Table 3) showed

lower plasma oxidized LDL values (p = 0.013) and higher lag time for copper-mediated LDL oxidation (p = 0.006) and HDL cholesterol values (p = 0.029). Comparison of the absolute values for common olive oil intervention with the respective control values (Table 3) showed a slight increase in the lag time for copper-mediated LDL oxidation (p = 0.024). No significant differences were observed in other lipid parameters, or in

Table 3 Glucose and lipids in serum, and LDL oxidation biomarkers on each olive oil intervention period (n = 30)

Measure	Refined			Common			Virgin		
	Baseline	Treatment	P	Baseline	Treatment	P	Baseline	Treatment	P
Total cholesterol, mmol/L	5.4±0.9	5.6±0.9	ns	5.6±0.9	5.4±0.8	ns	5.5±0.9	5.5±0.8	ns
LDL cholesterol, mmol/L	3.4±0.8	3.6±0.9	ns	3.6±0.8	3.4±0.8	ns	3.5±0.9	3.4±0.7	ns
HDL cholesterol, mmol/L	1.58±0.34	1.62±0.34	ns	1.57±0.34	1.56±0.31	ns	1.57±0.29	1.65±0.32	0.029
Triacylglycerols, mmol/L	0.99±0.5	1.00±0.5	ns	1.06±0.6	0.94±0.4	ns	1.01±0.4	0.99±0.6	ns
Glucose, mmol/L	4.0±0.6	4.1±0.6	ns	4.1±0.6	4.2±1.0	ns	4.1±0.7	4.2±0.7	ns
Oxidized LDL, U/L	36.9±22.4	30.3±18.0	ns	38.3±24.8	33.7±21.8	ns	42.8±29.0	28.3±20.1	0.013
Resistance of LDL to oxidation									
Lag time, min	117±10.9	119±11.0	ns	117±17.8	121±16.5	0.024	114±11.2	122±15.0	0.006
Oxidation rate, $\mu\text{mol dienes} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ of LDL protein	5.30±1.6	5.41±0.7	ns	5.67±0.8	5.62±0.8	ns	5.65±0.9	5.59±0.9	ns
Maximum amount of dienes, $\mu\text{mol dienes} \cdot \text{g}^{-1}$ of LDL protein ⁵	498±56	499±57	ns	499±88	505±73	ns	520±85	495±53	ns
Antibodies against oxidized LDL, U/L	917±1227	836±1116	ns	795±1210	777±1020	ns	1100±1810	913±1524	ns

Values are mean ± SD

other resistance of LDL to oxidation variables (oxidation rate, and maximum dienes formation), or in serum OLAB.

In order to check whether changes in α -tocopherol content could account for differences observed in the lag time of conjugated dienes formation after common and virgin olive oils administration, α -tocopherol content in LDL was measured. No differences were observed among baseline and intervention periods. Percentage of changes from baseline were $1.89\% \pm 10.8\%$, $2.03\% \pm 15.2\%$, and $1.36\% \pm 17.5\%$ (mean ± SD) for refined, common, and virgin olive oil intervention periods, respectively.

When changes in LDL oxidation markers, as difference in percentage between measurements obtained at baseline (after washout) and after each olive oil intervention period, were calculated, a decrease ($p=0.006$) was observed in oxidized LDL with the phenolic content of the olive oil (Fig. 2A). The lag time for copper-mediated LDL oxidation increased ($p=0.012$) with the phenolic content of the olive oil administered (Fig. 2B).

Discussion

Human and animal studies strongly support the hypothesis that oxidative modification of LDL plays a crucial role in the pathogenesis of atherosclerosis [26]. High levels of oxidized LDL are found in patients with different acute coronary syndromes, indicating that oxidized LDL might be a marker for atherosclerosis [27]. Recently, we have reported high levels of plasma oxidized LDL in stable CHD patients in comparison with healthy volunteers [28].

Results of animal [29, 30] and *in vitro* [9–12] studies

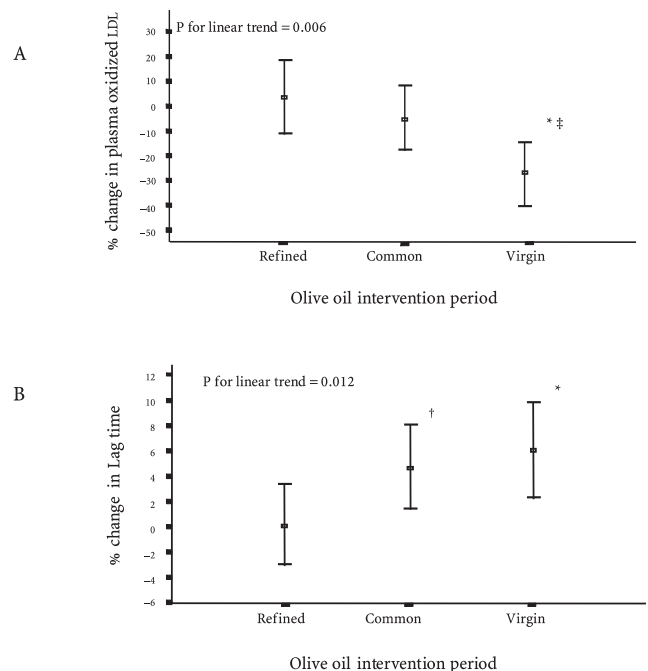


Fig. 2 Percentage of change (from before to after olive oil intervention) with 95 % confidence interval in LDL oxidation biomarkers in refined, common, and virgin olive oil intervention periods (mean ± SD, n = 30). **A** Oxidized LDL; **B** lag time of the resistance of LDL to oxidation. * $P < 0.01$, † $P < 0.05$ versus refined olive oil intervention period. ‡ $P < 0.05$ versus common olive oil intervention period

suggest a protective effect of olive oil phenolic compounds on LDL oxidation. Previous studies in humans after virgin olive oil ingestion suggest that such an effect may also apply to the *in vivo* situation [31, 32]. Concerning *in vivo* human lipid peroxidation, a decrease in the isoprostane excretion in humans after consumption

of olive oils rich in natural catecholic phenols has been reported [13]. Other studies, however, did not find differences in plasma lipid peroxides related with the phenolic content of the olive oil administered to healthy volunteers [16, 18]. Concerning measures of *ex vivo* LDL oxidation, an increase in the resistance of LDL to oxidation in both patients with peripheral vascular disease [14] and hyperlipidemic patients [17], after consumption of olive oil rich in phenolic compounds compared with olive oil with low phenolic content, has been reported. Other studies, however, did not find an effect on the resistance of LDL to oxidation related with the different phenolic content of olive oils administered [15, 16, 18].

In the present study, changes in all biomarkers were assessed through the differences between the beginning (after washout) and the end of each olive oil intervention. The same olive oil (refined) was used for cooking and raw purposes during washout periods, and no differences were obtained in the daily nutrient intake among intervention periods. The results of this study support an association between consumption of phenol-rich olive oil and a reduction of *in vivo* oxidized LDL levels. Dose-dependent changes, a decrease in plasma oxidized LDL and an increase in the resistance of LDL to oxidation, with the phenolic content of the olive oil administered were observed.

According with the design of the study, in which olive oils with similar fatty acid content were administered during the 15 weeks of the study, the lag time of conjugated dienes formation was the only parameter of LDL resistance to oxidation in which changes were expected. This parameter is considered to be mainly dependent on the antioxidant content of the LDL particle [33]. However, after oleic acid-enriched diets, oleate-rich LDL has shown to be less susceptible against oxidative modification [34]. This fact could explain that only moderate, though significant, increase in the lag time of conjugated dienes formation was observed linked to the phenolic content of the olive oil. We did not observe changes in serum antibodies against oxidized LDL related to the phenolic content of olive oil. The large interindividual variability of OLAB values found in this and other studies [35], using the same method to determine serum OLAB in healthy individuals, may account for the absence of treatment effects.

Urinary T and HT rose in a dose-dependent manner with the phenolic content of the olive oils administered. This fact points out the potential utility of T and HT urinary determinations as biomarkers of the ingestion of olive oil phenolic compounds. Although, due to its lack of ortho-diphenolic structure, the *in vitro* antioxidant activity of T is weak, in contrast to that of HT [9, 36], T has been shown to be effective in inhibiting the oxidation of cholesterol in LDL and preventing the modification of the apoproteic moiety [37]. T has also been ef-

fective in inhibiting leukocyte 5-lipoxygenase [36]. HT is considered to be a powerful antioxidant in *in vitro* studies [9, 10, 37–39], and has been shown to prevent passive smoking-induced oxidative stress in rats [30]. Besides their own antioxidant activity, olive oil phenolic compounds could protect the activity of other biological antioxidants. HT has been shown to preserve the LDL vitamin E content [10]. After virgin olive oil consumption an increase in the vitamin E and total phenolic content of LDL has been observed [31]. In the present study we did not observe changes in the vitamin E content of LDL among olive oil intervention periods. Lee et al. [40] recently reported that consumption of tomato products with virgin olive oil, but not with sunflower oil, improves the antioxidant activity of the plasma. The rise in T and HT levels after virgin olive oil administration observed in this study confirms our previous results about the absorption of these phenolic compounds from sustained and moderate doses (25 mL/day) of virgin olive oil in its natural form [41].

In this study, an increase in HDL cholesterol after virgin olive oil administration was observed. This result is consistent with the observations made in animal models by Mangas-Cruz [42], which showed that HDL cholesterol levels increased with olive oil enriched with its phenolic compounds, and decreased with olive oil impoverished in phenolic compounds. In human studies, an HDL cholesterol-raising effect, after the administration of polyphenol-rich plant extracts or juices, has also been reported [43, 44]. The mean change in HDL cholesterol values after virgin olive oil ingestion was 0.08 mmol/L. HDL cholesterol is known to be a significant and independent predictor of CHD. Results of a meta-analysis of four large prospective studies [45] indicated that for every 0.026 mmol/L increase in circulating HDL cholesterol levels the incidence of coronary events decreases by 2% in men and 3% in women. Further investigation is needed to establish the underlying mechanisms accounting for the effect of virgin olive oil consumption on HDL cholesterol levels.

One advantage of this study was the kind of participants involved. In the religious center the same type of meals was served at scheduled intervals, and participants performed the same individual level of physical activity during the study. Changes in diet and physical activity may both be confounder variables of the effect of a dietary intervention on oxidative stress biomarkers [46]. Participants' compliance was excellent, despite the extended duration of the study (15 weeks). Such compliance was reflected in the increase in urine T and HT concentrations after common and virgin olive oil intervention periods. One potential limitation of the study was, although the trial was blinded, some participants might have identified the refined olive oil by its organoleptic characteristics.

Market prices of olive oil vary according to its phe-

nolic content. Olive oils of the highest quality (in terms of their organoleptic characteristics, i. e. virgin oil), and with the highest phenolic content, are more expensive than common (UE 1991) or refined (pomace) olive oils. It is, therefore, important to distinguish the effects of each type of olive oil compound in order to determine which yields the best cost-benefit balance for the consumer in terms of health and disease prevention.

After virgin olive oil consumption a decrease in *in vivo* LDL oxidation was observed. Also observed were increases in HDL cholesterol, in the resistance of LDL to *ex vivo* oxidation, and in T and HT in urine. Dose-dependent changes, a decrease in plasma oxidized LDL and an increase in the resistance of LDL to oxidation, and in urinary T and HT, with the phenolic content of the olive oil administered were observed. Our results support the hypothesis that consumption of virgin olive oil could provide more benefits on risk factors for car-

diovascular diseases than that of other types of olive oils.

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Appendix

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