Olive Oils High in Phenolic Compounds Modulate Oxidative/Antioxidative Status in Men[1]

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ABSTRACT The aim of the present study was to evaluate whether olive oils high in phenolic compounds influence the oxidative/antioxidative status in humans. Healthy men (n = 12) participated in a double-blind, randomized, crossover study in which 3 olive oils with low (LPC), moderate (MPC), and high (HPC) phenolic content were given as raw doses (25 mL/d) for 4 consecutive days preceded by 10-d washout periods. Volunteers followed a strict very low-antioxidant diet the 3 d before and during the intervention periods. Short-term consumption of olive oils decreased plasma oxidized LDL (oxLDL), 8-oxo-dG in mitochondrial DNA and urine, malondialdehyde in urine ($P < 0.05$ for linear trend), and increased HDL cholesterol and glutathione peroxidase activity ($P < 0.05$ for linear trend), in a dose-dependent manner with the phenolic content of the olive oil administered. At d 4, oxLDL after MPC and HPC, and 8-oxo-dG after HPC administration (25 mL, respectively), were reduced when the men were in the postprandial state ($P < 0.05$). Phenolic compounds in plasma increased dose dependently during this stage with the phenolic content of the olive oils at 1, 2, 4, and 6 h, respectively ($P < 0.01$). Their concentrations increased in plasma and urine samples in a dose-dependent manner after short-term consumption of the olive oils ($P < 0.01$). In conclusion, the olive oil phenolic content modulated the oxidative/antioxidative status of healthy men who consumed a very low-antioxidant diet. J. Nutr. 134: 2314–2321, 2004.

KEY WORDS: • *olive oil* • *phenolic compounds* • *oxidative stress* • *oxidized LDL* • *DNA damage*

There is growing epidemiologic evidence that the traditional Mediterranean diet has a beneficial effect on diseases associated with oxidative damage such as coronary heart disease (CHD)[3] and cancer, and also on aging (1,2). Olive oil consumption was associated with a lower coronary risk (3) and with a reduced breast-cancer risk (4). Consumption of olive oil is in agreement with the current American Heart Association guidelines (5) to replace saturated fatty acids with unsaturated fats, and with the U.S. National Cholesterol Education Program (NCEP) guidelines to liberalize total fat intake, specifically from monounsaturated fat (MUFA) (6). Oleic acid, present in MUFA-rich diets, was shown to prevent in vitro LDL oxidation (7,8). Phenolic compounds present in olive oil might also contribute to health benefits derived from the Mediterranean diet. In in vitro (9,10) and animal models (11,12), olive oil phenolic compounds protected lipids from oxidation in a dose-dependent manner. However, the available evidence from randomized, crossover, controlled clinical trials of in vivo health beneficial effects of consumption of olive oil phenolic compounds is scarce and controversial (13–16). In this study, using an experimental design, we assessed the effect of moderate, real-life doses of 3 olive oils, differing only in their phenolic content, on oxidative stress biomarkers and blood lipids, both postprandially and after short-term consumption.

SUBJECTS AND METHODS

Olive oils composition. From a natural extra virgin olive oil (produced from Tsounati olives, Island of Crete) with high phenolic content (HPC; 486 mg/kg), an olive oil with moderate phenolic content (MPC; 133 mg/kg) was obtained after 1 water-washing procedure at 30°C, and an olive oil with low phenolic content (LPC; 10 mg/kg) was obtained after 9 water-washing procedures at 30°C (**Table**

[1] Supported by Grant ALI 2000–0525-C02–01 from Comisión Interministerial de Ciencia y Tecnología (CICYT) and by a Marie Curie Individual Fellowship from the European Commission (QLK1-CT-2001–51885).

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[3] Abbreviations used: AUC, area under the curve; CHD, coronary heart disease; GR, glutathione reductase; GSH-Px, glutathione peroxidase; HDL-C, HDL cholesterol; HPC, high phenolic content; 8-iso-$PGF_{2\alpha}$, 8-epi-isoprostane prostaglandin $F_{2\alpha}$; LDL-C, LDL cholesterol; LPC, low phenolic content; MDA, malondialdehyde; MHT, 3-O-methylhydroxytyrosol; mitDNA, mitochondrial DNA; MPC, moderate phenolic content; MUFA, monounsaturated fatty acid; 8-oxo-dG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; oxLDL, oxidized LDL; TAG, triacylglyceride.

Manuscript received 23 April 2004. Initial review completed 20 May 2004. Revision accepted 14 June 2004.

1). The washing procedure was developed at the Unilever Health Institute (Vlaardingen, The Netherlands). Percentages of individual phenolic compounds present in the olive oils were ~6.5% hydroxytyrosol, 5.5% tyrosol, 40% oleuropein aglycones, 26% ligstrosids aglycones, 12% lutein, and 3% apigenine. The fatty acid composition was determined by GC, and α-tocopherol and total phenolic content were determined by HPLC as described previously (9). The peroxide index and UV light absorption (K_{270}), a measure of secondary oxidation compounds such as carbonyls (aldehydes and ketones), were determined according to the analytical methods described in CEE/2568/91 of the European Commission.

Subjects. Participants were 12 healthy, male, nonsmoking volunteers recruited from biology students of the University Pompeu Fabra, Barcelona, Spain. They had a mean age of 21.1 y (range 20–22 y), a mean weight of 73.8 ± 3.9 kg and a BMI of 22.9 ± 1.7 kg/m^2. Exclusion criteria included intake of antioxidant supplements, aspirin or any other drug with established antioxidant properties, obesity (BMI > 30 kg/m^2), diabetes, hyperlipidemia, intestinal diseases, physical activity > 12.6 MJ/w, or any condition that would impair compliance. All volunteers were considered healthy as assessed by medical history, a complete physical examination, and standard laboratory tests.

Experimental study design. The study was performed as a double-blind, randomized, crossover experimental trial. Two Latin squares of 3 × 3 for balancing treatment order were used to randomize olive oil administration to participants. Subjects received 1 of the 3 olive oil treatments (25 mL/d) over 4 consecutive days (intervention period) with a washout period of 10 d between treatments. The washout period was divided into 3 phases: d 1–3, habitual diet; d 4–7, controlled diet to avoid excess consumption of phenolic compounds and antioxidants; d 8–10, low phenolic compound/antioxidant diet (restricted fruits, vegetables, coffee, tea, chocolate, wine, beer, coke, and olive oil). The same low phenolic compound/antioxidant diet was followed during intervention periods in which meals were served at the IMIM Pharmacology Unit. The 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 during the intervention periods. A daily dietary record was made by all participants throughout the study. Nutrient intakes were calculated by a nutritionist and converted into nutrients using the software Medi-System 2000 (Conaycyte S.A).

TABLE 1

Characteristics of the olive oils administered

	Olive oil		
Quality variables	LPC	MPC	HPC
Peroxide value, *mEq O_2/kg*	8.56	6.96	7.4
K_{270}[1]	0.09	0.12	0.12
		%	
Fatty acid			
14:0	0.01	0.01	0.01
16:0	10.49	10.48	10.42
16:1	0.07	0.13	0.12
17:0	0.18	0.18	0.17
17:1	0.29	0.29	0.29
18:0	2.95	2.96	2.94
18:1	79.36	79.39	79.36
18:2	5.20	5.10	5.21
20:0	0.60	0.56	0.54
18:3	0.34	0.36	0.39
20:1	0.32	0.34	0.32
22:0	0.14	0.14	0.14
24:0	0.07	0.07	0.08
		mg/kg	
α-Tocopherol	240	232	234
Total polyphenols	10	133	486

[1] K_{270}, UV light absorption.

On the morning of d 1 and 4 of the intervention and after an overnight fast, 25 mL olive oil was administered as a single dose with 25 g of bread to the volunteers to obtain kinetic data for evaluating postprandial changes. On d 2 and 3, subjects received the same daily olive oil dose, but distributed among meals (breakfast: 8 mL; lunch: 8 mL; dinner: 9 mL).

Venous blood samples were collected in tubes containing 1 g/L EDTA in the morning after an overnight fast (0 h) and at 1, 2, 4, 6, 8, and 10 h after the olive oil administration on d 1 and 4 (kinetic data), and in the morning after an overnight fast on d 2, 3, and 5. Urine samples were collected on d 1 and 4 in the morning after an overnight fast (0 h) and at defined periods starting after olive oil administration (0–4, 4–8, 8–12, and 12–24 h). On d 2 and 3 of the intervention periods, 24-h urine samples were collected. Plasma was separated by centrifugation at 1000 × *g* at 4°C for 15 min. All samples were stored at −80°C until analysis. All biochemical and analytical determinations were performed in duplicate. All laboratory variables were analyzed at 0, 1, 4, and 6 h except 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG) in mitochondrial DNA, which was analyzed only at 0 and 4 h because a large amount of whole blood (10 mL) is required to perform the determination. The protocol was approved by the CEIC-IMAS Ethics Committee. Each study participant signed an informed consent form before enrollment.

Analytical measurements. Serum cholesterol, HDL cholesterol (HDL-C), triacylglycerides (TAG), and glucose were determined by enzymatic methods (Roche Diagnostic) adapted to a Cobas Mira Plus autoanalyzer (Hoffmann-La Roche). LDL cholesterol (LDL-C) was calculated by the Friedewald formula. Oxidized LDL (oxLDL) was determined in plasma by an ELISA (17). Plasma glutathione peroxidase activity (GSH-Px) and glutathione reductase activity (GR) were measured by enzymatic methods (Ransel RS 505 and Ransel GR 2368, Randox Laboratories) in a Cobas Mira Plus analyzer at 37°C (18).

The amount of 8-oxo-dG in urine and mitochondrial DNA (mitDNA) of mononuclear cells, and urinary malondialdehyde (MDA) were measured by HPLC with electrochemical and UV detection, respectively (19,20). Urine results were normalized against creatinine concentration. Plasma total 8-epi-isoprostane prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) was determined by HPLC and stable isotope dilution MS (ESI-MS-MS) (21). Concentrations of tyrosol, hydroxytyrosol, and 3-*O*-methylhydroxytyrosol (MHT), a biological metabolite of hydroxytyrosol, were determined by GC-MS in urine and plasma samples (22,23).

Statistical analysis. Results are presented as means ± SEM. The duplicate values obtained from each subject were calculated before data analysis. The normality of variables was assessed by the Kolmogorov-Smirnov test and by analysis of skewness and kurtosis. A general linear model for repeated measurements was fit, with multiple paired comparisons corrected by Tukey's post-hoc method, to assess the effect of each type of olive oil. The percentage change between concentrations at the beginning of each olive oil intervention (0 h, d 1 of intervention) and concentrations after 4 d of intervention with LPC, MPC, and HPC olive oil was calculated. Comparisons among the end values for each treatment period were made after adjustment for baseline values. Interaction between the type of olive oil and the order of administration was assessed for each variable. Linearity of values across 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 (SPSS 11.5.1) was used.

RESULTS

Baseline concentrations of blood lipids and oxidative markers (0 h, d 1 of each intervention) did not differ among the 3 treatments (**Table 2**). Overall, the plasma concentrations of tyrosol, hydroxytyrosol, and MHT were 2.3 ± 0.65, 6.75

TABLE 2

Body weight, glucose, blood lipids, and oxidative stress biomarkers in men during 5 d of HPC, MPC, and LPC olive oil treatment[1]

	LPC (10 mg/kg)		MPC (133 mg/kg)		HPC (486 mg/kg)	
	wk 0	Treatment[2]	wk 0	Treatment	wk 0	Treatment
Body weight, *kg*	73.7 ± 3.9	74.1 ± 2.87	73.8 ± 3.8	72.8 ± 2.9	73.8 ± 3.8	73.1 ± 3.4
Serum glucose, *mmol/L*	5.06 ± 0.09	5.12 ± 0.09	5.02 ± 0.06	4.96 ± 0.08	5.13 ± 0.15	5.05 ± 0.08
Serum cholesterol, *mmol/L*						
Total	3.64 ± 0.20	3.78 ± 0.22	3.58 ± 0.20	3.66 ± 0.20	3.69 ± 0.25	3.76 ± 0.21
LDL	2.02 ± 0.20	2.01 ± 0.20	1.98 ± 0.20	1.94 ± 0.19	2.08 ± 0.24	2.03 ± 0.20
HDL	1.20 ± 0.08	1.25 ± 0.09	1.16 ± 0.09	1.24 ± 0.10†	1.20 ± 0.08	1.28 ± 0.08†
Serum TAG, *mmol/L*	0.89 ± 0.05	1.10 ± 0.15	1.01 ± 0.08	1.07 ± 0.13	0.88 ± 0.06	1.07 ± 0.09
Plasma OxLDL, *U/L*	39.00 ± 6.21	39.5 ± 6.4	35.15 ± 6.32	33.21 ± 7.35	44.96 ± 7.58	29.10 ± 6.34†
Plasma 8-iso-$PGF_{2\alpha}$, *ng/L*	65.50 ± 3.72	66.83 ± 3.82	70.92 ± 4.83	66.17 ± 5.09	67.50 ± 5.0	70.58 ± 4.47
8-oxo-dG/10^6 dG mitDNA	26.12 ± 7.63	16.80 ± 6.6	29.22 ± 12.11	17.15 ± 7.44	29.30 ± 6.13	12.89 ± 4.31
Plasma GSH-Px, *U/L*	556.1 ± 26.9	578.1 ± 28.2	538.5 ± 26.7	573.6 ± 31.3†	553.9 ± 33.8	602.2 ± 32.1*
Plasma GR, *U/L*	50.6 ± 2.6	54.6 ± 2.3	51.5 ± 2.9	56.6 ± 2.5	49.6 ± 2.1	52.4 ± 2.4
8-oxo-dG in urine, *nmol/mmoL creatinine*	11.63 ± 1.39	8.05 ± 2.45	12.27 ± 2.09	8.88 ± 2.61	12.53 ± 3.90	3.97 ± 0.89
MDA in urine, *nmol/mmoL creatinine*	10.22 ± 1.34	6.60 ± 1.61	10.04 ± 1.86	7.94 ± 2.20	10.86 ± 3.31	3.62 ± 0.81

[1] Values are mean ± SEM, $n = 12$. Symbols indicate different from wk 0: * $P < 0.005$; † $P < 0.05$.
[2] wk 0 represents the mean of the intervention on d 1 at 0 h (baseline value); treatment values represent the mean of d 5 at 0 h of the respective olive oil.

± 1.49, and 1.96 ± 0.77 nmol/L, respectively, and did not differ among treatments.

Daily energy intake calculated for the LPC diet (d 8–10 of the washout period) was 8.8 ± 0.3 MJ/d, and included lipids (values given in percentage of energy) (46.9 ± 0.8%; PUFA: 5.5 ± 0.2%; MUFA: 18.6 ± 0.4%; SFA: 16.1 ± 0.5%), carbohydrates (36.1 ± 1.0%), and proteins (18.5 ± 0.5%). Baseline dietary intake for total polyphenols was 4.2 ± 1.4 mg/d; α-tocopherol, 5.6 ± 0.3 mg/d; vitamin C, 18.1 ± 1.6 mg/d; β-carotene, 0.6 ± 0.06 mg/d. Baseline data for these nutrients did not differ among the 3 olive oil treatments.

Short-term effects. Comparing the percentage change obtained after olive oil treatments showed that the 4-d interventions decreased plasma oxLDL, 8-oxo-dG in mitDNA and urine, and urinary MDA, and increased HDL-C and GSH-Px dose-dependently with the phenolic content of the olive oil administered (linear trends $P < 0.05$) (**Fig. 1**). HPC olive oil intervention reduced oxLDL (−25.2%, $P = 0.059$), 8-oxo-dG in mitDNA (−49.2%, $P < 0.01$) and in urine (−51.67%, $P < 0.01$), and urinary MDA (−59.7%, $P < 0.001$), and increased GSH-Px (9.8%, $P < 0.01$) and HDL-C (7.7%, $P = 0.052$). MPC olive oil increased GR (13.0%, $P < 0.05$), GSH-Px (4.4%, $P < 0.05$), and HDL-C (7.1%, $P < 0.05$). Plasma 8-iso-$PGF_{2\alpha}$ was not affected.

Changes in the absolute values at the beginning and the end of each intervention period (Table 2) were in line with the percentage change observed in oxidative stress markers and HDL-C. Comparisons among the end values for each treatment period of the oxidative stress biomarkers showed lower concentrations of plasma oxLDL ($P < 0.05$); urinary MDA ($P < 0.001$), and 8-oxo-dG in urine ($P < 0.01$) and higher activities of GHS-Px ($P < 0.05$) and GR ($P < 0.05$) in plasma after HPC compared with the LPC intervention. Concerning the bioavailability and disposition of tyrosol and hydroxytyrosol, although plasma peak values for each phenolic after 25 mL olive oil ingested did not differ between d 1 and 4 (data not shown), sustained ingestion of MPC and HPC olive oils increased the overall plasma concentration. This was reflected in the significantly higher plasma area under the curve from 0 to 24 h (AUC_{0-24h}) values for phenolic compounds on d 4 compared with d 1 after a dose of 25 mL HPC olive oil (**Fig. 2**). Plasma levels of phenolic compounds increased significantly in a dose-dependent manner with the phenolic content of the olive oil administered as shown by the increasing linear trend of the AUC_{0-24h} of tyrosol, hydroxytyrosol, and MHT at both d 1 and 4 (Fig. 2).

Urine concentrations (24-h urine) of the phenolic compounds increased significantly in a dose-dependent manner with the phenolic content of the olive oils administered at both d 1 and 4 (**Fig. 3**).

Postprandial effects. Blood lipid and glucose concentrations did not change significantly when the men were in the postprandial state after intake of HPC, MPC, or LPC olive oils, at either d 1 or 4 of the intervention periods. At d 1 of intervention, postprandial concentrations of oxidative stress markers did not change significantly after HPC, MPC, and LPC olive oil intake. At d 4 of intervention, oxLDL decreased postprandially in a significant linear trend ($P < 0.05$) from 0 to 6 h after 25 mL of MPC (0 h: 39.0 ± 10.8 U/L; 1 h: 36.4 ± 7.2 U/L; 4 h: 25.0 ± 4.5 U/L; 6 h: 21.5 ± 4.1 U/L) and after HPC intake (0 h: 38.9 ± 8.9 U/L; 1 h: 42.3 ± 6.2 U/L, 4 h: 25.6 ± 6.0 U/L; 6 h: 29.9 ± 7.1 U/L). OxLDL and 8-oxo-dG in mitDNA were significantly lower at 4 h than at 0 h of HPC olive oil ingestion (oxLDL: $P < 0.05$; 8-oxo-dG: 9.5 ± 2.3 vs. 29.3 ± 6.1 8-oxo-dG/10^6dG, $P < 0.05$). No postprandial differences were observed in plasma 8-iso-$PGF_{2\alpha}$ or in urinary 8-oxo-dG and MDA. Plasma concentrations of tyrosol, hydroxytyrosol, and MHT rose after ingestion of a single dose of HPC and MPC olive oils, reaching a peak at 1 h after olive oil administration ($P < 0.01$ for quadratic trend) (**Fig. 4**). Plasma concentrations of the phenolic compounds at 1, 2, 4, and 6 h after HPC and MPC olive oil intake were significantly ($P < 0.05$) different from their respective baseline concentrations (Fig. 4). The phenolic compounds increased linearly with the phenolic content of the olive oils at 1, 2, 4, and 6 h ($P < 0.01$) (Fig. 4).

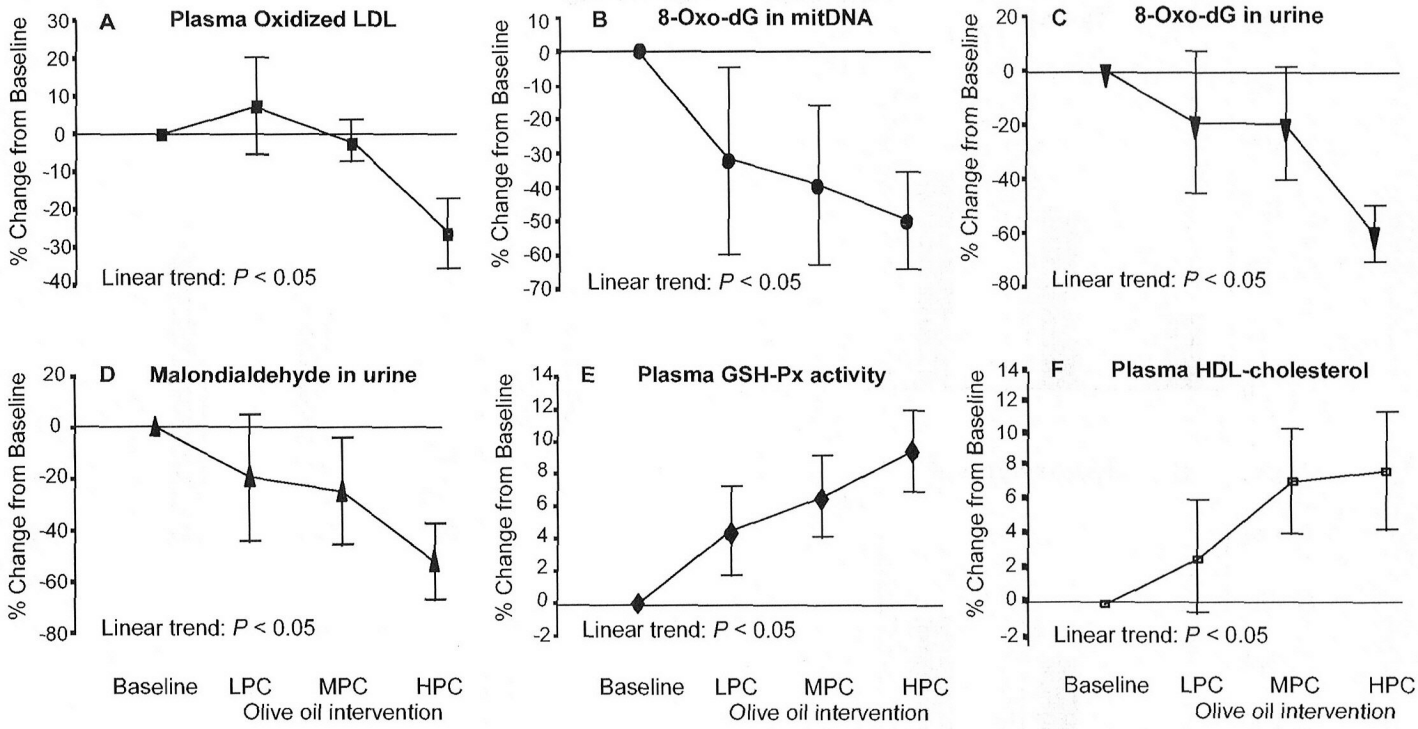

FIGURE 1 *The percentage change in plasma oxidized LDL (A), 8-oxo-dG in mitDNA (B), and urine (C), malondialdehyde in urine (D), plasma GSH-Px activity (E), and serum HDL-C (F) in men after 4 d of sustained consumption of olive oil with low (LPC), moderate (MPC), and high (HPC) phenolic content. Values are means ± SEM, n = 12.*

DISCUSSION

The aim of the study was to evaluate whether olive oil phenolic compounds in steady-state concentrations influence the oxidative/antioxidative status in humans. Short-term consumption of olive oil decreased plasma oxLDL, 8-oxo-dG in mitDNA and urine, and MDA in urine, and increased HDL-C and GSH-Px, in a dose-dependent manner with the phenolic content of the olive oil administered. At d 4, oxLDL after MPC and HPC, and 8-oxo-dG in mitDNA after HPC, were reduced postprandially. Phenolic compounds in plasma and urine increased in a dose-dependent manner both postprandially after a 25 mL dose of MPC and HPC, and after short-term consumption of the olive oils.

From our results, olive oil phenolic compounds accumulate in plasma and urine after short-term olive oil consumption, and the amount of phenolic compounds ingested with the olive oil seems to modulate the oxidative/antioxidative status in the human body. The mechanisms by which phenolic compounds present in olive oil can protect lipids and DNA are related to the abilities of the phenolics to counteract both metal-and radical-dependent oxidation (9) and to act as chelating agents, thus depressing the superoxide-driven reactions and breaking the chain-like propagation of the lipid peroxides (10). In ex vivo studies, olive oil phenolic compounds showed greater antioxidant capacity against LDL oxidation than α-tocopherol (9,10). Incubation of plasma with olive oil phenolic extracts increased both the total phenolic content of the LDL and the resistance of LDL to oxidation in a dose-dependent manner with the amount of phenolic compounds incubated with plasma (24). In addition, tyrosol was shown to be able to bind LDL in ex vivo experiments (24). Although it is not known whether olive oil phenolic compounds can bind human LDL lipoproteins in vivo, ingestion of virgin olive oil increased the vitamin E and phenolic content of the LDL lipoproteins (25). In vivo oxLDL in plasma was shown to be a risk marker for CHD (17,26). Our observation of decreased concentrations of oxLDL after short-term intake of HPC olive oil agrees with the results of some previous studies analyzing changes in in vivo oxLDL (14) and in the resistance of LDL to ex vivo oxidation, in relation to virgin olive oil intake (11–13,27). Aviram and Eias (28) showed that dietary olive oil reduced LDL uptake by macrophages, and decreased susceptibility of the lipoprotein to undergo lipid peroxidation, after 1 wk of olive oil diet. Other studies performed in humans did not find an effect on LDL resistance to oxidation or lipid peroxides in relation to the phenolic content of the olive oil (15,16,29,30).

To our knowledge, until now, no in vivo studies have investigated the effect of olive oil consumption on 8-oxo-dG concentrations. 8-oxo-dG, one of the oxidized bases formed by free-radical attack to DNA, is linked pathogenically to a variety of diseases such as cancer and also to aging (31). DNA oxidative damage occurs at a high rate in in vivo systems, and multiple repair mechanisms counteract the damage (32). Data on how antioxidants in diet can affect the steady-state levels of DNA oxidative damage in humans are at present not clear, and results of antioxidant intervention studies are mixed (33). Intervention studies with polyphenol-rich foods, such as green tea and red wine, showed a beneficial effect with regard to 8-oxo-dG concentrations in DNA from white blood cells (34,35). Rehman et al. (36) demonstrated in healthy volunteers a decrease of oxidative DNA base damage in white cell DNA within 24 h after intake of a single serving of tomatoes.

Changes in HDL-C were in line with those previously reported with virgin olive oil (14,37) and oleic acid–rich diets (38). From the design of the present study, the increasing

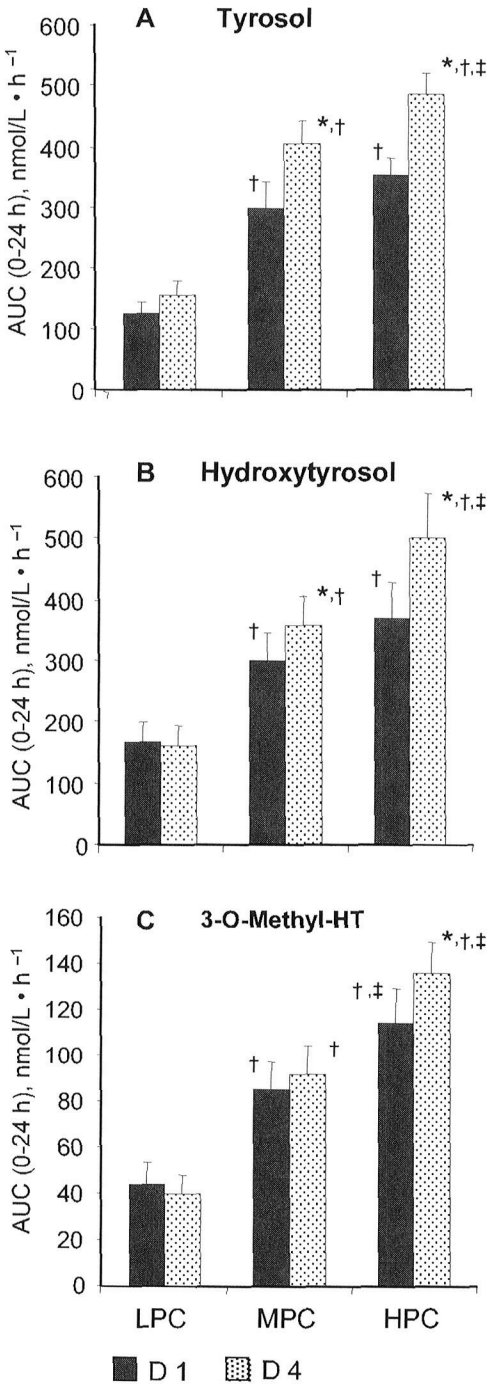

FIGURE 2 Plasma area under curve from 0 to 24 h (AUC 0–24h) on d 1 and 4 of tyrosol (*A*), hydroxytyrosol (*B*), and MHT (*C*) in men after intake of a single dose of 25 mL olive oil at d 1 and 4, respectively, with low (LPC), moderate (MPC), and high (HPC) phenolic content. Values are means ± SEM, $n = 12$. AUC values of tyrosol, hydroxytyrosol, and MHT increased at both d 1 and 4 in a significant linear trend ($P < 0.01$). *Different from respective values at d 1, $P < 0.01$; †different from LPC olive oil, $P < 0.01$; ‡different from MPC olive oil, $P < 0.05$.

linear trend of HDL-C occurred in a dose-dependent manner with the phenolic content of the olive oils. This observation can be supported by animal studies that demonstrated beneficial effects on HDL concentrations after consumption of olive oil phenolic compounds (39), polyphenol-rich plant extracts (40), and flavonoid supplements (41). Kurowska et al. (42)

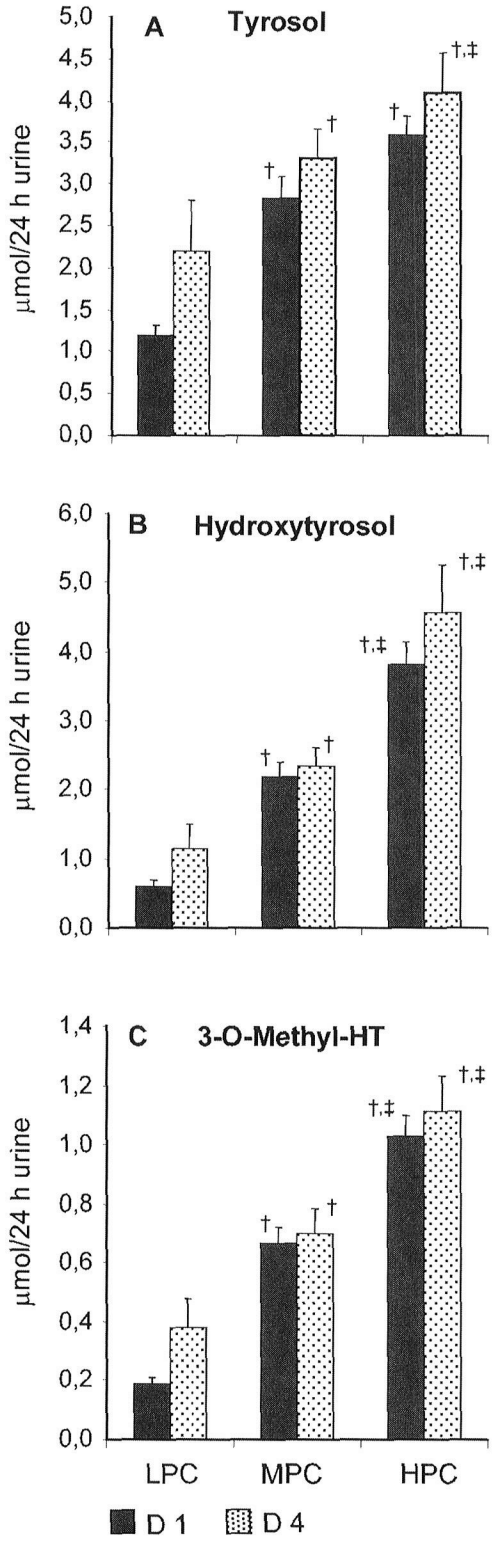

FIGURE 3 Absolute urinary concentrations of tyrosol (*A*), hydroxytyrosol (*B*), and MHT (*C*) in men at d 1 and 4, respectively, after 25 mL olive oil intake with low (LPC), moderate (MPC), and high (HPC) phenolic content. Values are means ± SEM, $n = 12$. Concentrations of tyrosol, hydroxytyrosol, and MHT increased both at d 1 and 4 in a significant linear trend ($P < 0.01$). †Difference from LPC olive oil, $P < 0.05$; ‡different from MPC olive oil, $P < 0.05$.

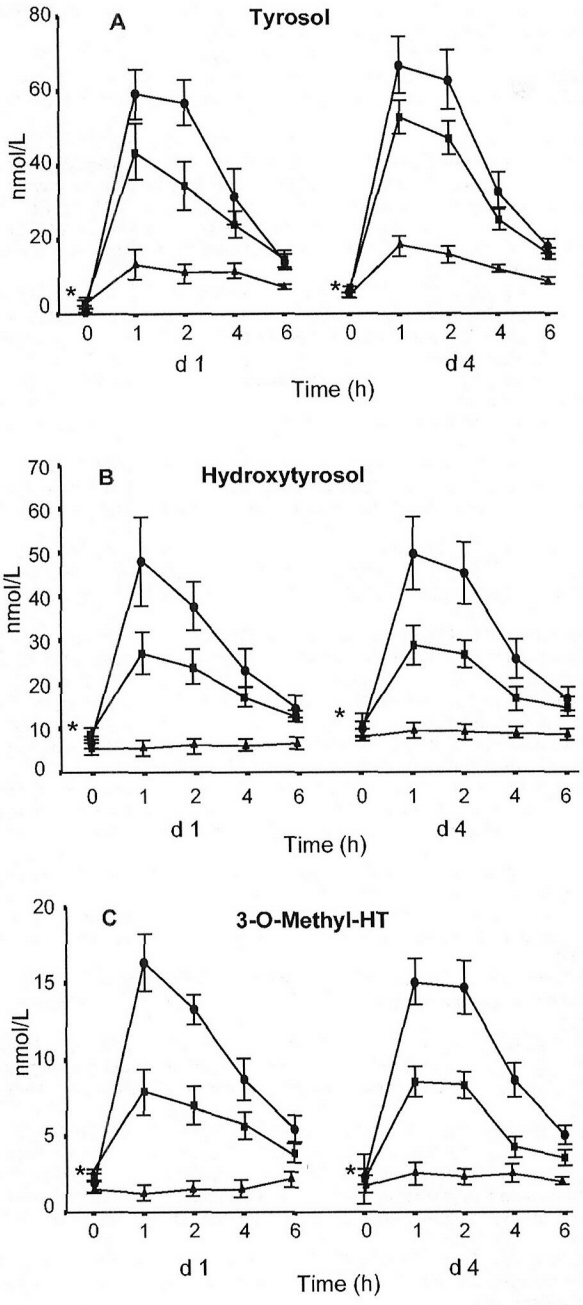

FIGURE 4 Postprandial concentrations of tyrosol (*A*), hydroxytyrosol (*B*), and MHT (*C*) at baseline (0 h), 1, 2, 4, and 6 h in men after intake of a single dose (25 mL) of olive oil with low (LPC, closed triangle), moderate (MPC, closed square), and high (HPC, closed circle) phenolic content at d 1 and 4 of treatment. Values are means ± SEM, $n = 12$. *Different from 1, 2, 4, and 6 h for MPC and HPC olive oils, $P < 0.05$.

reported that flavonoid-rich orange juice intake (750 mL/d) increased HDL-C concentrations. Changes in the present study were observed after 4 d of olive oil intake. Thus, the mechanisms involved act on a short-term effect basis and could be related to increased lipoprotein lipase activity (43) which, in addition to other factors, is regulated by insulin. Citrus flavonoids demonstrated insulin-like effects in vivo by interacting with cholesterol metabolism enzymes (44,45). However, the specific mechanisms by which olive oil beneficially alters HDL-C are beyond the scope of this study. Further studies are warranted to investigate these interactions. In addition to HDL-C, as an antioxidant defense mechanism, we observed an increase in the antioxidant enzyme GSH-Px. An increase in glutathione-related enzyme activity was reported in humans after virgin olive oil consumption (18) and nutritional supplementation with parsley (46), and in animals after the consumption of flavonoid supplements (41) and a diet rich in olive oil (47). At present, the mechanisms explaining the enhancement of scavenger enzymes after short-term antioxidant consumption are not clear. In human prostate cancer cell line studies, genistein, a soy isoflavone, upregulated GSH-Px gene expression accompanied by an elevation of the enzyme activity (48). A sparing effect of the administered antioxidants on the glutathione-related enzyme activities could also account for the increase.

From our results, a 25-mL olive oil dose did not promote plasma postprandial oxidative stress. After an oral fat load of 5 g/MJ, postprandial TAG concentrations were reported to be greater after ingestion of virgin olive oil than after sunflower or rapeseed oil (49). A MUFA-rich diet, however, is related to a faster clearance of postprandial TAG and apolipoprotein B48 from plasma than other fat-rich diets after a test meal (50). In one of our previous studies (18), after consumption of 50 mL of virgin olive oil, postprandial hypertriglyceridemia together with oxidative stress was observed in plasma. This stress was reflected by an increase in lipid peroxides and a decrease in glutathione-related enzymes. In the present study, 25 mL of olive oil ingestion did not promote hypertriglyceridemia or hyperglycemia, factors that promote postprandial oxidative stress (51). The increased overall plasma content of tyrosol and hydroxytyrosol at d 4 compared with d 1 after a dose of 25 mL HPC olive oil was observed mainly in the postprandial state, and could reflect an increased "pool" of the phenolic compounds from olive oil in the body because these 2 phenols can be used as markers of compliance (14). This increase in the antioxidant "pool" could be related to the observed postprandial decrease in plasma oxLDL and 8-oxo-dG in mitDNA. Further studies are required to investigate this relation.

It can be argued that one of the limitations of the study is the short-term period of sustained olive oil consumption. The short-term design, however, permitted volunteers to be restricted to a strict and controlled very low-antioxidant diet, thus avoiding the interference of other antioxidants as well as other possible confounder variables, such as changes in lifestyle factors, which often mask the results of nutritional intervention studies.

One advantage of the study was the fact that the 3 olive oils came from the same matrix (HPC), which implied that only changes in the polar components, the phenolic compounds, were present after the washing procedure. Therefore, we avoided the interference of other olive oil components, mainly MUFA, with the oxidative/antioxidative status. In addition, the administration of LPC olive oil during the washout periods and the sustained use of LPC olive oil for cooking purposes throughout the study avoided differences in the main fat ingestion of the volunteers. The 25 mL/d olive oil dose administered to the participants was a real-life dose of olive oil, close to that consumed daily in the traditional Mediterranean diet pattern (52).

In summary, after sustained olive oil consumption, an increase in tyrosol and hydroxytyrosol occurred in plasma and urine in a dose-dependent manner with the phenolic content of the olive oil administered. The olive oil phenolic content modulated the oxidative/antioxidative status of healthy volunteers consuming a very low-antioxidant diet.

ACKNOWLEDGMENTS

The authors thank Unilever Health Institute, Vlaardingen, for supplying the olive oils used in the study, and C. López-Sabater and Eva Gimeno for kindly determining the fatty acid profile of the olive oils used in the study.

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