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Antioxidant effect of virgin olive oil in patients with stable coronary heart disease: a randomized, crossover, controlled, clinical trial

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Abstract

The Mediterranean diet, in which olive oil is the main source of fat, has been associated with a reduced incidence of coronary heart disease (CHD) and low blood pressure levels. Virgin olive oil (VOO), besides containing monounsaturated fat, is rich in phenolic compounds (PC) with antioxidant properties. The aim of this study was to examine the antioxidant and anti-hypertensive effect of two similar olive oils, but with differences in their PC (refined: 14.7 mg/kg versus virgin: 161.0 mg/kg), in 40 males with stable CHD. The study was a placebo controlled, crossover, randomized trial. A raw daily dose of 50 mL of VOO and refined olive oil (ROO) were sequentially administered over two periods of 3 weeks, preceded by 2-week washout periods in which ROO was used. Lower plasma oxidized LDL ($p < 0.001$) and lipid peroxide levels ($p = 0.003$), together with higher activities of glutathione peroxidase ($p = 0.033$), were observed after VOO intervention. Systolic blood pressure decreased after intake of VOO ($p = 0.001$) in hypertensive patients. No changes were observed in diastolic blood pressure, glucose, lipids, and antibodies against oxidized LDL. Consumption of VOO, rich in PC, could provide beneficial effects in CHD patients as an additional and complementary intervention to the pharmacological treatment.

Keywords: Olive oil; Phenolic compounds; Oxidized LDL; Blood pressure; Coronary heart disease

1. Introduction

The Mediterranean diet, in which olive oil is the main source of fat, is associated with a decrease in overall and cardiovascular mortality [1]. Diets rich in monounsaturated fatty acids (MUFA) are used to manage cardiovascular disease risk, provided that they do not exceed the saturated fatty acid (SFA) recommendation and compromise weight control [2]. On the other hand, olive oil-rich diets have shown to reduce low-density lipoprotein (LDL) oxidation [3].

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Oxidation of LDL is a hallmark for atherosclerosis and coronary heart disease (CHD) development [4]. One of the earliest steps in the generation of oxidized LDL (oxLDL) is the lipid peroxidation of polyunsaturated fatty acids (PUFA). Tissue membranes that are rich in PUFA are more susceptible to oxidation by free radicals than membranes rich in MUFA [5]. However, lipid peroxidation, and its chain reaction in LDL, can be interrupted if LDL lipids are protected from free radicals by antioxidants.

Olive oil is rich in MUFA and antioxidant compounds. The concentration of antioxidants in olive oils is influenced by the olive oil extraction procedures. Virgin olive oil (VOO), obtained exclusively by physical procedures, is much more than a MUFA fat because it contains relatively high amounts of antioxidants, mainly phenolic compounds (PC). However, PC are lost when the olive oil is refined. The main PC in olive oil are oleuropein and ligstroside aglycones which by hydrolysis both give hydroxytyrosol (OHT) and tyrosol (T) [6]. Both free forms of T, OHT and their secoroid and conjugated forms, represent around 80% of the PC present in a virgin olive oil [7]. Olive oil PC have been shown to protect LDL from lipid peroxidation in in vitro experiments [8]. Animal studies suggest a protective effect of olive oil phenolics on LDL oxidation [9]. However, the information from randomized, crossover, controlled intervention trials in humans, which provides first level of scientific evidence, on the in vivo effects of olive oil rich in PC is scarce and controversial [10–13].

Compared with a saturated fat diet, the Mediterranean diet has been found to be associated with lower levels of blood pressure [14]. In the few studies concerning the relationship between olive oil consumption and blood pressure levels, olive oil consumption was effective in lowering blood pressure in hypertensive patients [15,16]. Hypertension is related to endothelial dysfunction which contributes to make the atherosclerotic plaque more unstable, thus increasing the risk of secondary events in CHD patients [17]. On the other hand, a relationship exists between oxidative stress and LDL oxidation with endothelial dysfunction [18].

Thus, the first aim of the present study was to evaluate the effect of both VOO and refined virgin olive oil (ROO) with differences in their PC concentration, on oxidative stress in stable CHD patients. The second aim was to compare the effects of olive oil on blood pressure in hypertensive and stable CHD patients. A randomized, crossover, placebo controlled trial study was designed.

2. Materials and methods

2.1. Olive oil characteristics

The olive oils selected, ROO and VOO, came from the same cultivar and harvest and were prepared for the present clinical trial. Fatty acid composition, α-tocopherol, and β-carotene content were similar in the two olive oils. MUFA percentage was 74 and 77%; SFA percentage was 16 and 15%; and PUFA percentage was 11 and 9%, in ROO and VOO, respectively. The olive oil dose (50 mL) per day administered to the patients contained 0 and 0.15 mg of β-carotene; 5.99 and 8.73 mg of α-tocopherol; and 0.62 and 6.53 mg of PC (caffeic acid equivalents), in ROO and VOO, respectively. Fatty acid composition was measured by conventional gas chromatography (GC), as previously described [19]. α-Tocopherol and β-carotene content were measured by HPLC, as previously described [20]. Total phenolic content of olive oils was measured by HPLC, as previously described [21]. T, OHT, and 3-*O*-methyl-hydroxytyrosol (MOHT) in olive oil were measured by GC–mass spectrometry (GC–MS), after acidic treatment of olive oil, as previously described [6]. T, OHT, and MOHT values were 11.0 and 13.7 mg/L; 0.25 and 0.1 mg/L; and 0.1 mg/L and undetectable amounts; for ROO and VOO, respectively.

Table 1
Characteristics of participants at baseline

	n	%
Diabetes	9	22.5
Arterial hypertension	19	47.5
Smoker	4	10
Smoker in past	32	80
Ischaemic cardiopathy		
Myocardial infarction	21	52.5
Angina	19	47.5
Coronary vessels affected		
1 vessel	11	27.5
2 vessels	11	27.5
3 vessels	12	30
4 vessels	6	15
Revascularization	18	45

2.2. Subjects and recruitment

An in-person screening visit was conducted to ascertain eligibility and to obtain baseline data. The diagnosis of stable CHD was based on the history of previous myocardial infarction or unstable angina without clinical symptoms of ischemia, and without changes in treatment in the last 45 days prior to inclusion in the study. All patients had coronary arteriography and significant coronary stenosis defined as ≥50% in one or more coronary epicardic vessels. Clinical characteristics of the patients are presented in Table 1. Exclusion criteria were to be older than 80 years, intake of antioxidant supplements the last 2 months prior to their inclusion in the study, any change in treatment during all the study, and any other disease or condition that would impair compliance.

Fifty-two subjects were recruited to participate in the trial. Six subjects were ineligible and 46 were randomized. Three of them dropped out for personal reasons unconnected to the study. Forty-three subjects completed the full study protocol, but three patients were also excluded due to lack of compliance on the basis of their urinary T, OHT, and MOHT

concentrations, as they indicated a non-compliance of treatments. Finally, 40 males with stable CHD, with a mean age of 67 (S.D. 8.7), were included. Medical treatment included: aspirin in 40 patients; statines in 33; angiotensin converting enzymes inhibitors in 20; beta blockers in 26; long-acting nitrates in 11; and calcium channel antagonists in 11. The local institutional Review Board approved the protocol, and a written informed consent was obtained from all patients.

2.3. Study design

A placebo controlled, crossover, randomized trial was designed using the two olive oils with different PC concentrations: ROO (phenolic content: 14.67 mg/kg) and VOO (phenolic content: 161 mg/kg). VOO and ROO were sequentially administered over two periods of 3 weeks preceded by 2-week washout periods in which ROO was used. During intervention periods, participants were requested to ingest a raw daily dose of 50 mL of olive oil distributed over three meals. ROO was used as the source of crude fat in washout periods. Other cooking fats were replaced by ROO in order to maintain similar and unchanged fat intake during the study, ROO was provided in enough quantity for all the family. Urinary T, OHT, and MOHT were determined as biomarkers treatment compliance [6].

Laboratory determinations were carried out in fasting samples drawn by venipuncture before the first washout period (basal), and before and at the end of the VOO and ROO administration. Anthropometric variables (weight and height) were recorded, and body mass index was calculated. In patients who had a diagnosis of hypertension, blood pressure measurements were recorded by a mercury sphygmomanometer after a minimum of 10 min rest in the seated position; an average of two measurements was taken for analyses. Food intake during each intervention period was recorded on a validated food frequency questionnaire [22]. The food frequency questionnaire was administered by trained medical personnel in a face-to-face interview. Foods were converted into nutrients with the software Medisystem 2000 (Conacyte S.A. Madrid, Spain). Physical activity was assessed at baseline and at the end of the study by the Minnesota Leisure Time Physical Activity Questionnaire, which has been validated for use in Spanish men [23].

2.4. Laboratory analyses

Laboratory determinations for an individual were carried out in the same batch to avoid between-run imprecision. Analytical intra-assay imprecision of the methods was assessed from 20 pairs of duplicate samples in the same run. Inter-assay imprecision was assessed from 20 day-to-day measurements of control samples. Both precision measurements were expressed as coefficient of variation (CV%).

Serum glucose, total cholesterol, high-density lipoprotein cholesterol (HDL), and triglycerides were determined by enzymatic methods. LDL cholesterol was calculated by the Friedewald formula. Lipoprotein (a) (Lp(a)) was analysed by immunoturbidimetry. Inter-assay CVs were 2.8, 2.6, 4.6, 2.9, and 7.5% for glucose, total cholesterol, HDL, triglycerides, and Lp(a), respectively. oxLDL was determined in plasma by a sandwich ELISA procedure using the murine monoclonal antibody mAB-4E6 as capture antibody, and a peroxidase conjugated antibody against oxidized apolipoprotein B bound to the solid phase (oxLDL, Mercodia AB, Uppsala, Sweden). Intra- and inter-assay CVs were 2.8 and 7.3%, respectively. oxLDL serum antibodies (OLAB) were measured by ELISA using copper-oxLDL as antigen, and a specific peroxidase conjugated with anti-human IgG antibodies (OLAB, Biomedica, Vienna, Austria). Intra- and inter-assay CVs were 4.8 and 7.9%, respectively. Plasma lipid peroxides were assessed by the generation of malondialdehyde equivalents, and measured by the thiobarbituric acid reactive substances method. The method involves heating the sample with thiobarbituric acid under acidic conditions and reading the absorbance of the malondialdehyde–thiobarbituric acid adduct formed at 532 nm. Values were normalized by neperian logarithm. Intra-run and between-run imprecision were 4.24 and 6.87%, respectively. Glutathione peroxidase in whole blood (GSH-Px) activity was measured by a modification of the method of Plagia and Valentine (Ransel RS 505, Randox Laboratories, Crumlin, United Kingdom). Intra-run and between-run imprecision were 3.6 and 5.43%, respectively. Total antioxidant status (TAS) was measured by the generation of 2,2′-azino-di-(3-ethylbenzthiazoline sulphonate) (ATBS) radical cation (TAS, Randox Lab., Crumlin, Northern Ireland). Intra- and inter-assay CVs were 3.4 and 5.8%, respectively. GSH-Px activity and TAS were measured in a Cobas Mira Plus analyser at 37 °C (ABX Diagnostics, Madrid, Spain).

Urinary T, OHT, and MOHT were determined by GC–MS in spot first morning urine. 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, an HP5973 mass-selective detector, and an HP7683 series injector. Separation of hydroxytyrosol and tyrosol was carried out using an 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 [6]. Intra- and inter-assay CVs for T, OHT, and MOHT were 4.7 and 3.8%; 1.3 and 3.0%; and 6.0 and 6.6%, respectively. All chemicals and organic solvents used were of analytical grade.

2.5. Statistical analyses

Normality of variable distribution was assessed by the Kolmogorov–Smirnov test and by analysis of skewness and kurtosis. Student's *t*- and Mann–Whitney *U*-tests were used as appropriate to analyse the differences in basal characteristics between the two groups of order of olive oil administration. Student's *t*-test was applied to compare daily mean

diet nutrient intake during each type of olive oil intervention. Relationship among variables was assessed by means of the Spearman's correlation test. Linear regression models were used in order to adjust values at the end of the intervention periods for baseline values at the start of the study and before each intervention. A general linear model for repeated measurements was used, with multiple paired comparisons corrected by Tukey's method, in order to assess differences for each variable in: (a) intervention effects, (b) period (time) effects, and (c) intervention–period interaction effects. Interaction with medical treatments was also assessed. Intervention–period interaction effects were assessed for each variable by the sphericity or Greenhouse-Geisse test if sphericity was not assumed. Linearity of values across ROO and VOO was tested for the dose–response effect of PC. All analyses were carried out on an intention-to-treat basis. Statistical significance was defined as $p < 0.050$ for treatment effects (two-sided test). SPSS statistical software was used.

3. Results

3.1. Baseline characteristics

Table 2 shows the basal characteristics of the participants at the beginning of the study. No significant differences in age, body mass index, physical activity, blood pressure, glycaemia values, blood lipid profile, and biomarkers of oxidative/antioxidative status were observed between the two groups of olive oil administration order at the beginning of the study. The incidence of diabetes, AMI, number of coronary vessels affected, medical treatment, and smoking habits were similar in the two orders of olive oil administration. A direct relationship between oxidized LDL basal levels and years of CHD development was observed ($R = 0.386$, $p = 0.017$). Although differences did not reach significance, years of CHD development were higher in patients randomized in order 1 of olive oil administration (6.76 (1–21), mean (range)) than those in order 2 (5.86 (1–15)). This fact could contribute to explain the higher levels of oxidized LDL in order 1 patients, at the beginning of the study, versus order 2, although differences were not significant.

3.2. Daily nutrient intake and physical activity

No differences in the daily mean energy, nutrient or antioxidant vitamin intake were observed between the two olive oil intervention periods (Table 3). No changes in physical activity practice were observed from the beginning to the end of the study (data not shown).

3.3. Laboratory analyses

Fig. 1 shows urinary T, OHT, and MOHT concentrations in all study periods. Level of urinary PC decrease after washout periods and after ROO intervention ($p < 0.05$). Urinary T, OHT, and MOHT increased as response to VOO intervention ($p < 0.039$).In comparison with ROO intervention, that of VOO decreased plasma oxLDL ($p < 0.001$) and lipid peroxide levels ($p = 0.003$), and increased GSH-Px activity ($p = 0.033$), urinary T, OHT, and MOHT ($p = 0.031$, $p < 0.001$, and $p = 0.024$, respectively) (Table 4). No changes were observed

Table 2
Anthropometric variables, physical activity, blood pressure, glucose, lipids, and oxidative markers of participants at baseline by order of administration of olive oils

	Mean (S.D.)	
	Order 1 (refined-virgin) ($n = 22$)	Order 2 (virgin-refined) ($n = 18$)
Age (years)	69 (8.42)	66 (8.92)
Body mass index (kg/m^2)	28 (3.01)	27 (3.11)
Physical activity (kcal/day)	497 (285)	505 (349)
Diastolic blood pressure (mmHg)	78 (8.25)	78.5 (12.03)
Systolic blood pressure (mmHg)	136 (10.89)	136 (12.6)
Glucose (mmol/L)	6.81 (2.44)	6.38 (1.92)
Total cholesterol (mmol/L)	5.08 (0.98)	5.32 (1.14)
HDL cholesterol (mmol/L)	1.10 (0.25)	1.12 (0.33)
LDL cholesterol (mmol/L)	3.35 (1.00)	3.57 (1.08)
Triglycerides (mmol/L)[a]	1.32 (0.92–1.61)	1.37 (0.82–1.66)
Lp(a) (g/L)[a]	0.22 (0.18–0.68)	0.39 (0.18–0.88)
Oxidized LDL (U/L)	61.1 (20.73)	53.2 (27.05)
OLAB (U/L)[a]	294 (134–529)	197 (81–344)
Lipid peroxides (μmol)	1.44 (0.62)	1.20 (0.50)
Glutathione peroxidase (U/L)	7231 (1323)	7034 (1374)
Total antioxidant status (mmol/L)	0.95 (0.15)	0.94 (0.17)
Tyrosol (μg/L urine)[a]	35.01 (23.47–94.27)	27.38 (21.21–88.51)
Hydroxytyrosol (μg/L urine)[a]	120 (77.8–208)	114 (56.49–366)
O-methyhydroxytyrosol (μg/L urine)[a]	15.86 (8.67–29.92)	14.45 (9.36–41.83)

LDL, low-density lipoprotein; HDL, high-density lipoprotein; OLAB, oxidized LDL antibodies.
[a] Median (25 and 75 percentile).

Table 3
Daily mean (S.D.) diet nutrient intake during each type of olive oil intervention

$n = 40$	Refined (14.67 mg/kg)	Virgin (161 mg/kg)	p
Energy (MJ)	6.9 (4.1)	6.9 (3.6)	0.949
Protein (%)	20.0 (5.1)	20.5 (3.8)	0.658
Fat (%)	44.4 (11.6)	45.8 (9.1)	0.558
Carbohydrate (%)	35.5 (10.6)	32.6 (8.6)	0.187
MUFA (%)	19.3 (6.0)	20.3 (5.2)	0.432
PUFA (%)	7.2 (2.8)	6.6 (1.2)	0.192
SFA (%)	12.5 (4.9)	14.0 (5.3)	0.180
α-Tocopherol (mg)	17.3 (12.6)	15.6 (6.3)	0.442
Vitamin C (mg)	264 (160)	253 (189)	0.797
β-Carotenoid (mg)	6.8 (5.6)	8.5 (6.3)	0.217

MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

in the other assessed variables between the two olive oil intervention periods (oil intervention effect) (Table 4). The period effect observed in urinary PC is in accordance with differences observed in Fig. 1. Neither any effect of the time of olive oil consumption (p for period effect), nor interaction with the order of olive oil administration (p for intervention–period effect), were observed for the assessed variables (Table 4). No interaction with medical treatments was observed.

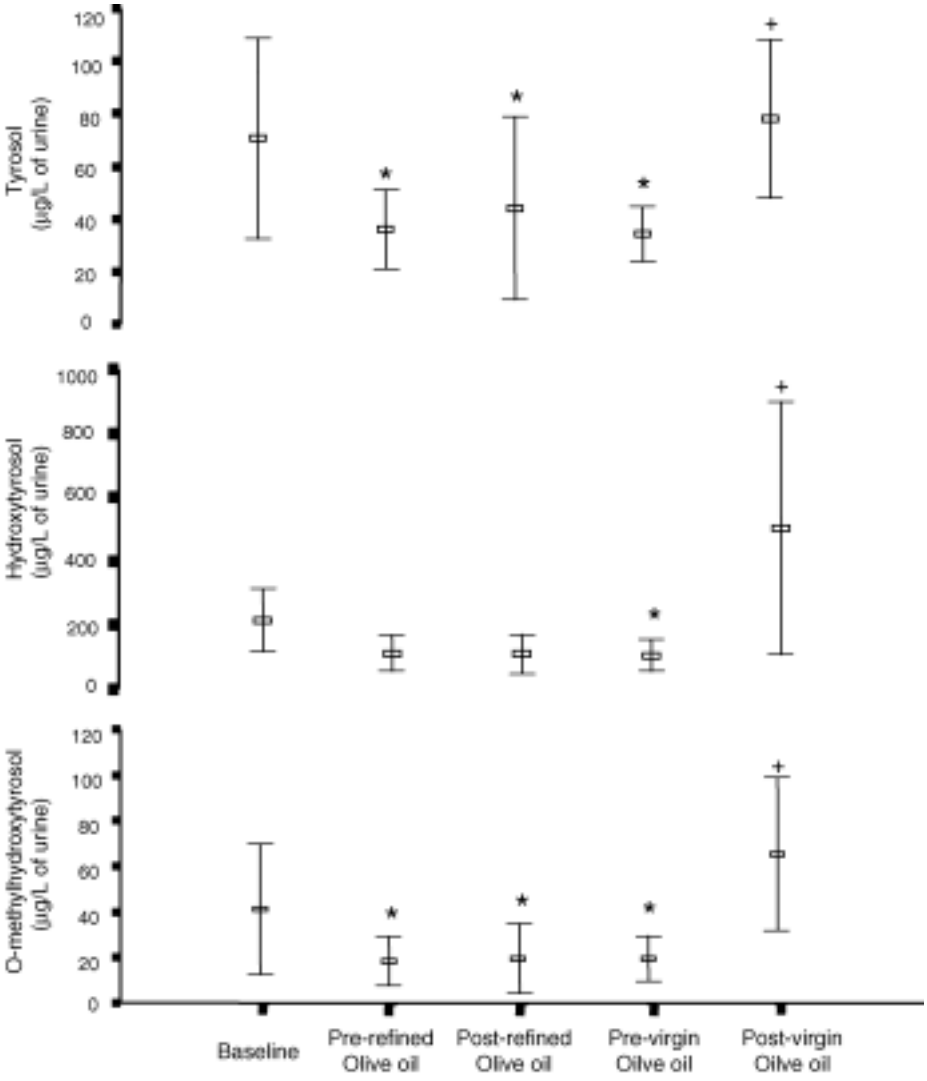

Fig. 1. Urinary tyrosol, hydroxytyrosol, and 3-*O*-methyl-hydroxytyrosol values (mean ± S.E.M.) at the start of the study (baseline), and before and after each olive oil intervention period. $^{*}p < 0.05$ vs. baseline, $^{+}p < 0.039$ vs. pre-virgin olive oil intervention.

Table 4
Diastolic and systolic blood pressure, glucose, lipid, and oxidative status markers at baseline and after refined and virgin olive oil administration [mean (S.D.)]

$n = 40$	Post refined olive oil (14.67 mg/kg)	Post virgin olive oil (161 mg/kg)	Mean difference between interventions (95% confidence interval)	p		
				Intervention (olive oil) effect	Period (time) effect	Intervention–period effect
Systolic blood pressure (mmHg)	135.2 (6.58)	132.6 (5.6)	−2.53 (−3.78; −1.27)	0.001	0.799	0.340
Diastolic blood pressure (mmHg)	78.4 (6.0)	79.6 (5.2)	1.16 (−0.06; 2.38)	0.061	0.688	0.729
Glucose (mmol/L)	6.46 (2.05)	6.65 (2.23)	0.212 (−0.096; 0.519)	0.171	0.467	0.354
Total cholesterol (mmol/L)	5.02 (0.99)	5.09 (0.85)	0.07 (−0.032; 0.017)	0.176	0.324	0.388
HDL cholesterol (mmol/L)	1.14 (0.32)	1.12 (0.29)	−0.021 (−0.054; 0.012)	0.207	0.385	0.612
LDL cholesterol (mmol/L)	3.30 (0.16)	3.33 (0.13)	0.033 (−0.076; 0.142)	0.542	0.281	0.234
Triglycerides (mmol/L)[a]	1.33 (0.99–1.63)	1.23 (0.88–1.71)	−0.0005 (−0.071; 0.07)	0.990	0.551	0.916
Lipoprotein (a) (g/L)[a]	0.27 (0.20–0.84)	0.34 (0.18–0.89)	0.017 (−0.008; 0.034)	0.208	0.386	0.430
Oxidized LDL (μmol/L)	58.66 (23.05)	54.01 (19.89)	−4.66 (−7.08; −2.23)	<0.001	0.941	0.705
OLAB (U/L)[a]	230 (122–495)	246 (140–487)	9.18 (−27.79; 9.42)	0.323	0.208	0.762
Lipoperoxides (μmol/L)	1.47 (1.23)	1.23 (0.72)	−0.24 (−0.40; −0.09)	0.003	0.563	0.205
Glutathione peroxidase (U/L)	7308 (711)	7668 (854)	412 (35.98; 788)	0.033	0.346	0.258
Total antioxidant status (mmol/L)	0.92 (0.12)	0.91 (0.11)	−0.01 (−0.03; 0.01)	0.301	0.715	0.172
Tyrosol (μg/L urine)[a]	23.68 (9.38–53.3)	77.5 (74.8–81.0)	32.67 (3.18–62.16)	0.031	<0.000	0.459
Hydroxytyrosol (μg/L urine)[a]	87.2 (74.1–156)	484 (439–531)	374 (310–438)	<0.001	<0.001	0.478
O-methylhydroxytyrosol (μg/L urine)[a]	10.00 (2.93–17.00)	43.18 (31.3–63.9)	33.50 (4.67–62.32)	0.024	<0.000	0.651

LDL, low-density lipoprotein; HDL, high-density lipoprotein; OLAB, oxidized LDL antibodies.
[a] Median, 25–75 percentile.

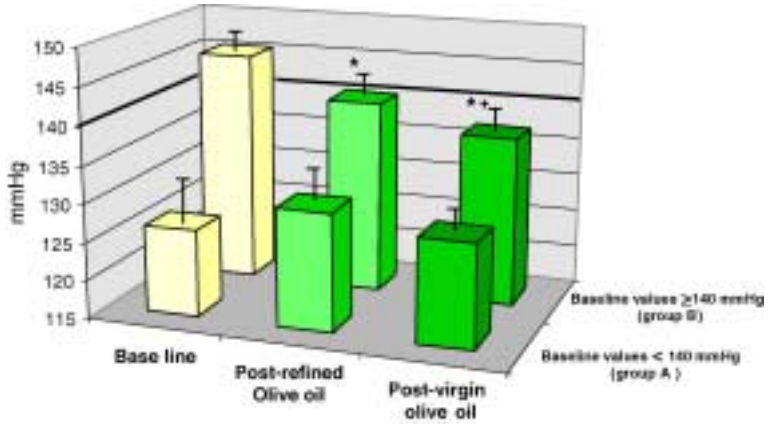

Fig. 2. Changes in systolic blood pressure (SBP) after olive oil treatments according to SBP baseline values; Group A: SBP < 140 mmHg ($n = 10$) and group B: SBP ≥ 140 mmHg ($n = 9$). $^{*}p < 0.005$ vs. baseline value, $^{+}p < 0.005$ vs. post-refined olive oil intervention.

3.4. Blood pressure assessment

In the group of hypertensive patients ($n = 19$) systolic blood pressure (SBP) values decreased after VOO intervention ($p = 0.001$) versus ROO consumption period (Table 4). No significant changes were observed in diastolic blood pressure levels (Table 4). Neither period (time) effect, nor intervention–period interaction, were observed (Table 4). No interaction with medical treatments was observed.

In order to evaluate the efficacy of the olive oil interventions on SBP according to baseline values, hypertensive patients were divided into two groups at the start of the study: group A, patients with SBP < 140 mmHg, and group B, patients with SBP ≥ 140 mmHg. In group B, a decrease of SBP after both ROO and VOO intervention periods was observed ($p < 0.005$) (Fig. 2). The SBP decrease was greater after VOO administration than after ROO administration ($p < 0.005$) (Fig. 2). A decrease of SBP levels after VOO intervention (linear trend, $p < 0.001$) was observed in all cases of group B (Fig. 3).

4. Discussion

In the present study, we compared the effects of two similar olive oils, but with differences in their phenolic content, on oxidative/antioxidative biomarkers and blood pressure levels in stable CHD patients. The design of the present study allowed an independent assessment of the effects of the minor components from olive oil ingestion. From our results, consumption of VOO, rich in PC, decreased oxidative stress and increased the antioxidant endogenous defence more than refined olive oil with low phenolic content. Furthermore, a decrease of SBP was observed after VOO ingestion in stable CHD patients with a diagnosis of hypertension.

Animal and human studies strongly support the hypothesis that oxidative modification of LDL plays a crucial role in the pathogenesis of atherosclerosis [24]. Therefore, mechanisms preventing LDL oxidation appear to be antiatherogenic. OxLDL is directly involved in atherosclerotic plaque formation and CHD development [4] and it has been related with the atherosclerotic plaque instability [25]. The high plasma oxLDL concentrations observed in CHD patients are in direct relationship with the severity of acute coronary syndromes [25,26]. Thus, oxLDL has been proposed as a marker for CHD risk [25].

Previous studies in humans have shown the ability of MUFA-rich diets to prevent lipid peroxidation [27]. The in vivo role of the olive oil PC, however, remains to be elucidated. Wiseman et al. [28], comparing olive oils with the same fatty acid and Vitamin E content, but with differences in their phenolic content (VOO, RRO, and sunflower oils), showed that LDL resistance to oxidation was higher after VOO intervention in rabbits. No effects of high phenolic- versus low phenolic-olive oil consumption on oxidative stress biomarkers have been reported in some studies with healthy volunteers [11–13]. The results obtained in the present study agree with those obtained in our previous one [29] in which, after administration to healthy volunteers of three types of

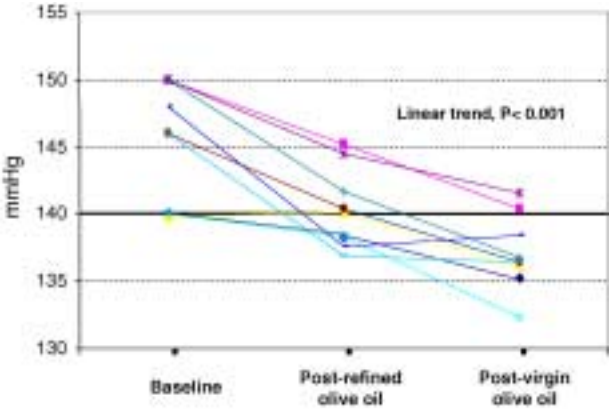

Fig. 3. Individual changes in systolic blood pressure (SBP) after olive oil interventions according to SBP baseline values, in patients with SBP ≥ 140 mmHg ($n = 9$) at the start of the study (group B).

olive oil with only differences in their phenolic content, a dose-dependent decrease in in vivo oxLDL was observed with the phenolic content of the olive oil administered. The controlled nature of the trial and the homogenization of fat intake during both studies may have contributed in detecting differences in the effects of the olive oils tested. On the other hand, in comparison with healthy volunteers, patients with stable CHD have high levels of oxidative stress [30] which would be more susceptible to be lowered by an antioxidant intake than normal values. In this sense, Ramírez-Tortosa et al. [10] have reported a higher increase of the resistance of LDL to oxidation after VOO consumption than after a refined one in patients with peripheral vascular disease, in which high levels of lipid peroxidation have also been reported [31].

The mechanisms by which olive oil rich in PC can exert its protective antioxidant effect can be explained by the activity of PC or by the combined protective effect of both the PC and the MUFA content of the olive oil. Olive oil PC have been shown to counteract both metal- and radical-dependent LDL oxidation, and to act as chain-breaking antioxidants for lipid peroxidation [32]. Besides their own antioxidant activity, olive oil PC could protect the activity of other biological antioxidants such as Vitamin E [33] and PC [34] bound to LDL.

Low levels of GSH-Px have been shown to be a risk marker for CHD development [35]. In this study, together with a decrease in plasma oxLDL and lipid peroxides, an increase in GSH-Px activity was observed after VOO intervention. Our results support an association between consumption of phenol-rich olive oil and an enhancement of the endogen antioxidant system. In rabbits with experimental atherosclerosis, with low levels of hepatic antioxidant enzymes, a VOO rich-diet enhanced the hepatic GSH-Px activity [36]. An increase in glutathione-related enzyme activities in humans after 1 week of 20 mL of VOO consumption has been previously described [37]. The mechanisms by which olive oil rich in phenolics could increase the GSH-Px activity can be avoiding its consumption by reducing the free radical pool in the body. On the other hand, a direct effect on in vivo GSH-Px gene expression cannot be discarded. An increase in gene expression of GSH-Px after incubation of murine macrophage-like cells with olive oil PC has been recently described [38].

Another finding in this study is the SBP reduction observed after VOO intervention in stable CHD hypertensive patients. In patients who, despite being under anti-hypertensive treatment, had SBP equal to or greater than 140 mmHg at the beginning of the study, the degree of SBP reduction was higher than in patients with SBP lower than 140 mmHg (5.81 and 1.74% of SBP reduction, respectively). An olive oil-rich diet was shown to be able to attenuate the vascular reactivity response of the aorta ring, in spontaneously hypertensive rats [39]. Ferrara et al. [15] reported a reduced need for antihypertensive treatment in hypertensive patients after 6 months of a VOO-rich diet in comparison with a rich polyunsaturated (sunflower oil) diet. Ruiz-Gutiérrez et al. [16] compared the effect of two similar MUFA-rich diets (olive oil and high-oleic sunflower oil) in hypertensive women. These authors [16] reported that only the olive oil-rich diet induced a significant reduction of blood pressure, suggesting a role for the minor olive oil components on blood pressure levels. A major cause for endothelial dysfunction in essential hypertension is a decreased availability of nitric oxide. Oxidative stress, through superoxide anion production, decreases nitric oxide availability [18]. On the other hand, an inhibition of the nitric oxide synthase expression by oxLDL has also been reported [40]. The reduced oxidative stress and LDL oxidation after VOO intervention observed in this study in stable CHD patients could also account for the SBP reduction in the hypertensive ones. PC from red wine have been shown to be able to enhance the expression of nitric oxide synthase, with subsequent nitric oxide release in endothelial cultured cells [41]. However, data of a direct enhancement of nitric oxide synthase expression by olive oil PC has, at present, not been reported.

The olive oil intervention in the present study was designed with a daily dose (50 mL) which is the current raw olive oil intake in some Spanish regions. Participants' compliance was excellent, as reflected in the increase in urinary T, OHT, and MOHT after VOO intervention. The decrease in urinary PC observed after washouts (in which ROO was used as source of fat) and the ROO intervention, points out that this population habitually consumed olive oil.

5. Summary

Consumption of VOO during 3 weeks led to a decrease of in vivo oxLDL and lipid peroxide plasma levels, together with an increase in GSH-Px activity, higher than those observed after refined olive oil consumption. Furthermore, a decrease in the SBP was observed after VOO intervention in hypertensive stable CHD patients, especially those who were SBP $\geq$ 140 mmHg at the beginning of the study. From our knowledge, the present study is the first report on the possible protective effect of olive oil rich in PC on oxidative stress and blood pressure levels in stable CHD patients. Our results support the hypothesis that VOO consumption could provide beneficial effects on cardiovascular risk factors, as an additional and complementary intervention to the pharmacological treatment and life-style recommendations.

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