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SHORT COMMUNICATION

Anti-inflammatory effect of virgin olive oil in stable coronary disease patients: a randomized, crossover, controlled trial

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Objectives: To assess the effect of two similar olive oils, but with differences in their phenolic compounds (powerful antioxidant compounds), on inflammatory markers in stable coronary heart disease patients.
Design: Placebo-controlled, crossover, randomized trial.
Setting: Cardiology Department of Hospital del Mar and Institut Municipal d'Investigació Mèdica (Barcelona).
Subjects: Twenty-eight stable coronary heart disease patients.
Interventions: A raw daily dose of 50 ml of virgin and refined olive oil (ROO) was sequentially administered over two periods of 3-weeks, preceded by 2-week washout periods in which ROO was used.
Results: Interleukin-6 ($P<0.002$) and C-reactive protein ($P=0.024$) decreased after virgin olive oil intervention. No changes were observed in soluble intercellular and vascular adhesion molecules, glucose and lipid profile.
Conclusions: Consumption of virgin olive oil, could provide beneficial effects in stable coronary heart disease patients as an additional intervention to the pharmacological treatment.
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Keywords: olive oil; phenolic compounds; inflammation; interleukins; coronary heart disease

Introduction

The traditional Mediterranean diet in which olive oil is the main source of fat, is one candidate factor for explaining the low incidence rates of myocardial infarction in Southern-Europe countries in comparison with the Northern ones (Tunstall-Pedoe *et al*., 1999). Monounsaturated fatty acids (MUFA) rich-diets have been associated with a low risk for

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Contributors: Drs MF, MC, and MIC had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of analyses; *Study concept and design:* MF, MC, MIC, RT, JM; *Acquisition of the data:* MA, DM, MF, MC, MP-B, JM, HS; *Analysis and interpretation of data:* MF, MC, MIC, RT, MCL-S, JM; D*rafting of the manuscript*: MF, MC, MIC; *Critical revision of the manuscript for important intellectual content:* MA, MC, MIC, MF, RT, HS, JM, JM, MCL-S, JB, DM, MP-B; S*tatistical analyses:* MF,MC; *Obtained funding:* MC, MIC, JB; *Administrative, technical and material support:* MF,JM,DM, MP-B, HS, MA.
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coronary heart disease (CHD) (Kris-Etherton, 1999). Olive oil, however, is much more than a MUFA-fat because it contains high amounts of antioxidant phenolic compounds (PC) (Covas *et al*., 2006b). PC are lost when the olive oil is refined.

Transcription factors and adhesion molecules involved in the inflammatory response can be inhibited in cells culture by pc (Murase *et al*., 1999; Carluccio *et al*., 2003). Data from randomized, controlled intervention studies on the effect of olive oil and its PC consumption on systemic inflammatory markers in humans are scarce. Recently, in two randomized crossover studies, virgin olive oil, rich in polyphenols, was shown to be more effective in lowering LTB_4 and TXB_2 than refined olive oil (ROO), with a low phenolic content, both at postprandial state in healthy subjects (Bogani *et al*., 2007) and after sustained consumption in mildly dyslipidemic patients (Visioli *et al*., 2005).

We recently reported virgin olive oil (VOO) to be more effective than refined on reducing the lipid oxidative damage in healthy volunteers (Covas *et al*., 2006a). We have also reported this fact in stable CHD patients (Fito *et al*., 2005), in whom a high oxidative status was observed (Weinbrenner *et al*., 2003). Here, we report that VOO is more effective than ROO on reducing the inflammatory status in a subsample of 28 volunteers of these CHD patients.

Methods

Exclusion criteria were to be older than 80 years, the intake of antioxidant supplements, change in treatment during the study, and any condition that would impair compliance. Twenty-eight participants (68 years, s.d.: 7 years) were included. The investigation conforms with the Declaration of Helsinki.

Percentage of fatty acids were: MUFA 74 and 77%; saturated fatty acid, 16 and 15%; and polyunsaturated fatty acid (PUFA), 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.

A placebo-controlled, crossover, double-blind, randomized trial was performed by using the two olive oils with different PC concentrations (ROO:14.67, VOO:161 mg/Kg). A raw daily dose of 50 ml of VOO and ROO were sequentially administered over two periods of 3-weeks, preceded by 2-week washout periods in which ROO was used. Other cooking fats were replaced by ROO in order to maintain similar fat intake during the study. ROO was provided in enough quantity for all the family. Food intake during each intervention period was recorded by a validated food-frequency questionnaire (Schroder *et al*., 2001). Physical activity was assessed at baseline and at the end of the study by the Minnesota Questionnaire (Elosua *et al*., 1994). Interleukin-6 was measured by Enzyme-linked immunosorbent assay (ELISA) (Bender-MedSystems); sICAM-1 and sVCAM-1 were determined by ELISA (DRG Instruments, Marburg, Germany). High-sensitivity C-reactive protein (CRP) was determined by immunoturbidimety (ABX-Diagnostics). Urinary tyrosol (T), hydroxytyrosol (OHT) and *O*-methylhydroxytyrosol (MOHT), determined as biomarkers of compliance, were measured by GC-MS (Miró-Casas *et al*., 2003).

Statistical analysis

Multiple linear regression models were used to adjust post-intervention values for baseline and pre-intervention values. A general linear model for repeated measurements was used, with multiple paired comparisons corrected by Tukey's method, to assess differences for each variable in:(a) oil-intervention effects, (b) period (time) effects and (c) intervention-period interaction effects. Linearity of values across ROO and VOO was tested for the dose–response effect of PC. All analyses were carried out on a per protocol basis.

Results

No significant differences in basal characteristics were observed between the two groups of olive oil administration order at the beginning of the study (Table 1). No differences in the daily mean energy, nutrient, or antioxidant vitamin intake were observed between the two interventions (Table 2). No changes in physical activity were observed from the beginning to the end of the study. In comparison with the ROO intervention, that of VOO decreased IL6 ($P<0.002$) and CRP ($P=0.024$) and increased T, OHT and MOHT ($P\leqslant 0.001$) (oil-intervention effect) (Table 3). No changes were observed in the other assessed variables between the two olive oil intervention periods (Table 3).

Discussion

Atherosclerosis is considered to be an inflammatory disease (Ross, 1999). IL6 and CRP have been associated with atherosclerosis and have been shown to be predictors for CHD development (Tzoulaki *et al*., 2005). In the present study, consumption of VOO was more effective in decreasing IL6 and CRP than ROO in stable CHD patients.

Several *in vitro* and *in vivo* studies have examined the anti-inflammatory properties of olive oil and its pc (Perona *et al*., 2006). LDL enriched in oleic acid promote less monocyte chemotaxis, compared with linoleic acid-enriched LDL, when exposed to oxidation (Tsimikas *et al*., 1999). Esposito *et al*. (2004) found a reduction in the inflammatory markers after a 2-year follow-up of patients with metabolic syndrome consuming a Mediterranean-type diet. In a recent report, from a randomized and controlled study with 772 participants at high risk for CHD, a reduction in inflammatory

Table 1 Anthropometric variables, physical activity, blood pressure, glucose, lipids, and inflammatory markers of participants at baseline by order of administration of olive oils

	Mean (s.d.)	
	Order 1 (refined-virgin) (n = *13*)	*Order 2 (virgin-refined)* (n = *15*)
Age (years)	68 (6.45)	68 (7.64)
Body mass index (kg/m^2)	28 (3.08)	27 (2.89)
Physical activity (kcal/day)	568 (426)	627 (277)
Diastolic blood pressure (mm Hg)	79.3 (8.17)	79.0 (9.58)
Systolic blood pressure (mm Hg)	134 (9.83)	135 (13.24)
Glucose (mmol/l)	6.67 (2.60)	6.41 (1.87)
Total cholesterol (mmol/l)	4.94 (0.82)	5.27 (1.26)
HDL cholesterol (mmol/l)	1.03 (0.19)	1.20 (0.31)
LDL cholesterol (mmol/l)	3.29 (0.81)	3.52 (1.19)
Triglycerides (mmol/l)[a]	1.23 (0.92–1.54)	1.08 (0.80–1.45)
C-Reactive protein (mg/dl)[a]	0.16 (0.07–0.65)	0.27 (0.19–0.93)
Interleukin-6 (pg/ml)[a]	1.38 (0.71–1.99)	1.15 (0.96–1.83)
sICAM-1 (ng/ml)[a]	385 (335–543)	362 (293–390)
sVCAM-1 (ng/ml)[a]	740 (330–939)	663 (168–792)
Tyrosol (μg/l urine)[a]	38.54 (21.37–82.76)	27.07 (20.73–75.25)
Hydroxytyrosol (μg/l urine)[a]	114 (32.36–205)	98 (50.61–329)
O-methyhydroxytyrosol (μg/l urine)[a]	15.23 (8.26–26.82)	14.28 (7.42–41.73)

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; sICAM-1, soluble intercellular adhesion molecule 1; sVCAM-1, soluble vascular adhesion molecule 1.
Data are presented as mean (s.d.) otherwise indicated.
[a]Median (25th–75th percentile).

Table 2 Daily mean (s.d.) energy and nutrient intake during each type of olive oil intervention

	Refined		*Virgin*		P
	Mean (s.d.)	*Change (%)*[a]	*Mean (s.d.)*	*Change (%)*[a]	
Energy (MJ)	11.50 (5.93)	15.4	12.52 (7.13)	22.3	0.586
Protein (% of energy)	20.54 (4.39)	14.9	20.92 (3.53)	16.5	0.227
Fat (% of energy)	45.24 (10.59)	4.3	46.97 (10.41)	7.8	0.470
Carbohydrate (% of energy)	33.64 (9.01)	−14.7	31.95 (9.49)	−20.7	0.938
MUFA (% of energy)	19.30 (4.95)	−4.7	20.69 (5.47)	2.3	0.625
PUFA (% of energy)	7.78 (3.32)	22.9	6.41 (0.96)	6.4	0.326
SFA (% of energy)	12.83 (4.57)	13.8	15.25 (5.61)	27.4	0.808
α-tocopherol (mg)$^+$	12.40 (9.86–21.93)	−7.5	14.10 (10.75–18.35)	5.2	0.307
Vitamin C (mg)$^+$	220 (185–271)	4.8	209 (123–325)	−0.48	0.711

Abbreviations: MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.
[a]Mean change (%) from baseline value; $^+$Median, 25th–75th percentile.

markers was observed after 3 months of a Mediterranean diet consumption, versus a low fat diet (Estruch *et al*., 2006).

In cells culture models olive oil PC were able to inhibite VCAM-1 expression (Carluccio *et al*., 2003) and other antioxidants, such as gallate (Murase *et al*., 1999) or vitamin E (Wu *et al*., 1999), reduced the expression of VCAM-1 and ICAM-1. However, soluble adhesion molecule expression is not always responsive to changes in oxidative status. In this way, mildly ox-LDL induced endothelial cells to produce potent monocyte activators (Berliner *et al*., 1990), but failed in inducing VCAM-1 or ICAM-1 expression (Kim *et al*., 1994). We did not observe *in vivo* changes in the cell adhesion molecules concentrations associated to VOO consumption in comparison with ROO. Changes in expression of ICAM by mononuclear cells, but not in serum concentrations, associated to a MUFA consumption have been reported after a 2-month intervention (Yaqoob *et al*., 1998). Perhaps an intervention longer than 3 weeks could be needed to observe changes in serum cell adhesion molecules associated to VOO consumption. Recently, an ibuprofen-like activity *in vitro* has been described for oleocanthal, a ligstroside aglycone present in olive oil (Beauchamp *et al*., 2005). Although given its complex structure it is doubtful whether the whole molecule could be absorbed *in vivo*. In diabetic patients, a 46%-decrease in thromboxane-B_2 production was observed after a 4-day consumption of olive mill waste (OHT: 12.5 mg/day)

Table 3 Glucose, lipid, inflammatory, and compliance markers after olive oils administration

Variable	*Post -refined olive oil*	*Post -virgin olive oil*	*Mean difference between interventions (95% confidence interval)*	P *for intervention (olive oil) effect*	P *for period (time) effect*	P *for intervention-period effect*
Glucose (mmol/l)	6.27 (2.04)	6.44 (2.08)	0.149 (−0.248; 0.546)	0.448	0.681	0.182
Total cholesterol (mmol/l)	4.95 (0.10)	5.02 (0.88)	0.083 (−0.006; 0.174)	0.067	0.239	0.750
HDL cholesterol (mmol/l)	1.15 (0.30)	1.15 (0.263)	0.005 (−0.042; 0.032)	0.240	0.060	0.926
LDL cholesterol (mmol/l)	3.24 (0.98)	3.32 (0.88)	0.085 (−0.009; 0.18)	0.139	0.316	0.668
Triglycerides (mmol/l)[a]	1.28 (0.89–1.47)	1.19 (0.83–1.68)	0.003 (−0.069; 0.075)	0.932	0.444	0.654
C-reactive protein (mg/dl)[a]	0.329 (0.158–0.510)	0.279 (0.175–0.488)	−0.063 (−0.119; −0.007)	0.024	0.548	0.223
Interleukin-6 (pg/ml)	1.65 (0.92)	1.49 (0.41)	−0.166 (−0.261; −0.071)	0.002	0.751	0.983
sICAM-1 (ng/ml)[a]	423 (345–512)	402 (365–455)	−3.840 (−33.98; 26.30)	0.796	0.303	0.277
sVCAM-1 (ng/ml)[a]	781 (441–1038)	711 (540–956)	−21.01 (−89.24; 47.21)	0.533	0.663	0.776
Tyrosol (μg/l urine)[a]	13.38 (7.48–41.05)	77.23 (74.58–81.05)	49.40 (31.57; 67.23)	$<$0.001	0.648	0.908
Hydroxytyrosol (μg/l urine)[a]	83.24 (71.85–149.13)	484 (439–525)	398 (330; 467)	$<$0.001	0.366	0.748
O-methylhydroxytyrosol (μg/l urine)[a]	6.41 (0.74–12.49)	39.36 (28.72–61.04)	45.56 (20.56; 70.56)	0.001	0.342	0.255

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; sICAM-1, soluble intercellular adhesion molecule 1; sVCAM-1, soluble vascular adhesion molecule 1.
Data are presented as mean (s.d.) otherwise indicated.
[a]Median (25th–75th percentile).

(Leger *et al*., 2005). From our knowledge this is the first data concerning the effect of PC from olive oil on IL-6 and CRP in a randomized study in humans.

Our trial had several strengths. The crossover design minimized the interference with other possible confounders. Compliance was good, as is reflected in the changes of T and OHT in urine. One limitation of the study, however, was the inability to determine whether an interaction between olive oil and other components from diet could account for the changes in the inflammatory markers observed. Also it can be argued that 50 ml (44 g) is a high daily amount for raw olive oil. Daily amounts from 30g to 50 g/day have been reported as usual in the Mediterranean diet (Helsing, 1995). A third limitation could be the short duration of the intervention periods. Whether additional effects in cell adhesion molecules would have been observed over longer periods is unknown. Although a longer duration of the study, could have impaired the adherence of the participants.

In conclusion, consumption of VOO during 3 weeks led to a decrease of IL6 and CRP higher than that observed after ROO consumption, in patients with stable CHD. Further studies are needed to establish the protective role of VOO on the inflammatory status in humans.

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