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Postprandial anti-inflammatory and antioxidant effects of extra virgin olive oil

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Abstract

High postprandial serum lipid concentrations are associated with increased oxidative stress which, in turn, increases the risk of atherosclerosis. Epidemiological studies correlate lower incidence of cardiovascular disease with adherence to the Mediterranean diet.

The aim of this study was to evaluate changes in inflammatory (TXB_2 and LTB_4) and oxidative stress markers (urinary hydrogen peroxide levels and serum antioxidant capacity), in addition to classic lipid parameters, after a fat-rich meal administered to 12 normolipemic, healthy subjects. Following a Latin square design, subjects were divided into three groups, each one receiving a different kind of oil (extra virgin olive oil; EVOO, olive oil; OO or corn oil; CO, together with 150 g of potatoes), with 2-week washout periods between treatments. Blood samples were drawn at baseline and after 1, 2, and 6 h after the meal. A significant decrease in inflammatory markers, namely TXB_2 and LTB_4, after 2 and 6 h after EVOO (but not OO or CO) consumption and a concomitant increase of serum antioxidant capacity were recorded. These data reinforce the notion that the Mediterranean diet reduces the incidence of coronary heart disease partially due to the protective role of its phenolic components, including those of extra virgin olive oil.

Keywords: Postprandial lipemia; Olive oil; Polyphenols; Inflammation; Antioxidants

1. Introduction

Extensive scientific evidence shows that the Mediterranean diet prevents the onset and progression of coronary heart disease (CHD), metabolic disorders, and several types of cancer [1]. These effects might in part be due to specific dietary factors such as consumption of fresh fruit, vegetables, legumes, cereals containing large amounts of fibre, antioxidants, minerals, vegetable proteins, vitamins, and concomitant low consumption of red meat [1]. However, a clear understanding of the possible mechanisms underlying the cardioprotective effects has not yet been reached.

Olive oil is the main source of fat in the Mediterranean regions. The alleged beneficial effects of extra virgin olive oil have been linked to both its monosaturated fatty acids (MUFA, namely oleic acid) and its antioxidant components, e.g., hydroxytyrosol and oleuropein, most of which phenolic in nature. Much data show that olive oil phenolics exert in vitro and in vivo antioxidant and potentially cardioprotective activities (reviewed in [2]).

In the past few years, attention has been paid to postprandial lipemia as an independent cardiovascular risk factor. High-fat meals produce several effects on postprandial plasma concentrations of triacylglycerols and their remnants, but these effects are also influenced by the type of fat consumed [3]. Moreover, oxidative stress associated with postprandial lipemia might contribute to increase cardiovascular risk, e.g., by inducing endothelial dysfunction [4], and is preventable by antioxidant supplementation [5]. As an example, the unsaponifiable fraction of extra virgin olive oil reduces

Abbreviations: CVD, cardiovascular disease; EVOO, extra virgin olive oil; OO, olive oil; CO, corn oil; AA, arachidonic acid

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proinflammatory and vasoconstrictor eicosanoid (PGE_2 and TXB_2) synthesis by endothelial cells exposed to tryglicerides isolated post prandially [6].

In turn, both the type of dietary fat and the antioxidant content of the meal might influence postprandial lipemia and its effects on cardiovascular risk. Hence, we investigated whether a bolus ingestion of phenol-rich extra virgin olive oil affects the postprandial lipid profile, as well as selected surrogate markers of cardiovascular risk, of healthy volunteers. Moreover, we compared the effects of extra virgin olive oil with those of olive oil, i.e. an oil with identical fatty acid composition but negligible content of phenolic antioxidants, and those of corn oil, i.e. a seed oil.

2. Materials and methods

2.1. Oils

The oils at study were carefully selected and kindly provided by Carapelli s.p.a. (Tavarnelle Val di Pesa, Italy). Their characteristics are shown in Table 1.

Extra virgin olive oil (EVOO) and olive oil (OO) had nearly identical fatty acid composition but very different phenolic content, as evaluated by HPLC [7]: whereas the former contained 607 ppm of total phenols (300 ppm of hydroxytyrosol derivatives), the latter contained only 16 ppm (2 ppm of hydroxytyrosol derivatives).

All reagents were from Sigma (Milan, Italy) and were of the highest purity available.

2.2. Subjects

Twelve healthy, normolipidemic men aged 25 ± 3 with normal body weight (BMI $22 \pm 1.8\,kg/m^2$) were included in this study. The protocol was fully explained to the participants and all subjects gave their written informed consent.

The protocol was approved by the University of Milan Ethic's committee.

Table 1
Characteristics of the oils

Fatty acid (%)	Oil		
	CO	EVOO	OO
14:0	0.03	0.01	0.01
16:0	10.7	10.0	10.8
16:1	0.1	0.4	0.7
17:0	0.1	0.1	0.1
17:1	0.0	0.1	0.1
18:0	1.9	2.3	3.0
18:1	27.8	78.5	75.0
18:2	57.1	7.2	8.6
18:3	0.7	0.6	0.5
20:0	0.4	0.4	0.5
20:1	0.2	0.3	0.3
22:0	0.2	0.3	0.1
24:0	0.2	0.1	0.1
Total polyphenols (ppm)	n.d.	607	16
Hydroxytyrosol (ppm)	n.d.	300	2

EVOO: extra virgin olive oil; OO: olive oil; CO: corn oil; n.d.: non-detected.

2.3. Study design

The study followed a Latin square design, i.e. each participant was given all three oils with 2-week washout periods between treatments. For 5 days before the study, all participants followed a run-in diet that excluded several food items rich in phenolic compounds (vegetables, fruits, juices, wine, coffee, tea, cacao, and olive oil) and refrained from alcohol consumption and strenuous exercise.

For each experiment, volunteers were divided into three groups of four men each. After an overnight fast of at least 12 h (T_0), venous blood was drawn into evacuated tubes, some of which contained disodium ethylenediaminetetraacetic acid (EDTA) as the anticoagulant; other tubes did not contain anticoagulants to allow for the preparation of serum. Then, subjects were given a morning meal, which consisted of 150 g of mashed potatoes dressed with 50 ml of either one of the three different kinds of oil. At 1, 2, and 6 h after the meal, further blood samples were drawn as described above. Blood aliquots were allowed to clot at 37 °C for 1 h. Both serum and plasma were separated by centrifugation at $2100 \times g$ for 20 min at 4 °C, aliquoted, and stored at −80 °C.

Urine samples were collected at T_0 and 2 h after the meal.

2.4. Glucose and lipid assays

Total (TC) and high density lipoprotein-cholesterol (HDL-C) were quantified by enzymatic colorimetric methods (ABX Diagnostics, Montpellier, France), which included the precipitation of apolipoprotein B-containing lipoproteins with phosphotungstic acid. Triacylglycerol (TG) concentrations were also determined colorimetrically (ABX Diagnostics, Montpellier, France).

Low density lipoprotein-cholesterol (LDL-C) was calculated by means of the Friedewald formula.

Finger-prick blood samples for glycemia evaluation were withdrawn using mini-lancets (Accu-Check Compact, Roche) shortly before and 1, 2, and 6 h after test meals.

Plasma total fatty acids (FA) were analyzed by gas–liquid chromatography (GLC). Briefly, fatty acids was methylated with 1 ml of 3N MeOH/HCl (Supelco), maintained at 90 °C for 1 h, and subsequently extracted twice with 2 ml of water, 2 ml of a KCl saturated solution, and 2 ml of *n*-hexane. The resulting fatty acid methyl esters were analyzed by GLC (85.10; Dani Instruments s.p.a., Cologno Monzese, Italy) equipped with a capillary column (Omegawax 320 Supelco), FID and PTV injector. The absolute concentrations of the individual fatty acids were calculated by reference to an internal standard, i.e. 0.1 μg/μl of total volume of nonadecanoic acid, added during methylation [8].

2.5. Total antioxidant status

The total antioxidant capacity of serum was measured by an established method based on the reduction of Cu^{++} to Cu^{+}, as described, using uric acid as the reference compound [9].

The urinary concentrations of hydrogen peroxide were determined by a colorimetric method [10]. Freshly voided urine was centrifuged for 8 min at 2500 × *g* at 4 °C, and the measurement was performed immediately. Briefly, a urine sample (40 μl) was mixed with FOX reagent (360 μl), vortexed, and then incubated at room temperature for 20 min. Absorbance at 590 nm was read against and compared with a series of standards prepared with H_2O_2 stock 30% solution [10]. Each value was normalized by the concentration of creatinine (Metra Creatinine Assay Kit, Quidel, San Diego, USA) and is expressed as μM H_2O_2 per mole creatinine.

2.6. Inflammatory and pro-thrombotic parameters

In addition to the blood aliquots allowed to clot as described above, other aliquots were immediately added with the calcium-ionophore A23187 (50 μM; Calbiochem., Darmstadt, Germany) and incubated for 30 min at 37 °C to stimulate eicosanoid, namely leukotriene, production. At the end of incubation period, all samples were centrifuged to separate serum.

The productions of leukotriene B_4 (LTB_4) and of thromboxane B_2 (TXB_2) were evaluated by immunoassay (Cayman Chemical, Ann Arbor, MI).

2.7. Statistical analysis

All the parameters (TC, TG, LDL-C, HDL-C, glycemia, plasma FA, TXB_2, LTB_4, total antioxidant status and urinary concentrations of hydrogen peroxide) were studied one at a time. In order to evaluate the effects of the oils on each parameter, two different analyses were performed (softwares: Stata version 8.0 and SAS version 8.2). (1) An analysis of covariance was used to assess differences between baseline values and those recorded after 2 h from meal consumption. (2) Random effects regression was used to assess whether the parameter followed different time trends depending on the oil; in such a model, the parameters were entered as the dependent variable whereas time, indicators for the kind of oil, and subjects (as random effect) were entered as covariates. A *P*-value less than 0.05 was considered as statistically significant.

3. Experimental results

TC, TG, LDL-C, and HDL-C concentrations did not change significantly 1 and 2 h after oil consumption

Table 2
Time course of the plasma lipid profile of 12 healthy normolipidemic subjects administered 150 g of potatoes and 50 ml of different types of oil

	Time after oil ingestion (h)			
	0	1	2	6
TC (mg/dl)				
EVOO	183.99 ± 48.40	202.83 ± 57.24	178.34 ± 29.80	179.90 ± 36.53
OO	208.17 ± 34.59	204.87 ± 30.00	201.36 ± 32.09	210.39 ± 19.73
CO	166.16 ± 37.57	171.53 ± 36.29	172.61 ± 29.54	193.42 ± 52.48**
LDL-C (mg/dl)				
EVOO	116.97 ± 55.04	136.49 ± 64.28	105.05 ± 40.27	115.81 ± 46.04
OO	140.15 ± 44.27	136.86 ± 37.84	134.24 ± 29.50	142.73 ± 24.51
CO	102.12 ± 39.62	96.15 ± 43.39	95.14 ± 34.39	117.69 ± 59.49
HDL-C (mg/dl)				
EVOO	55.85 ± 10.53	53.73 ± 11.26	57.47 ± 12.10	56.28 ± 18.11
OO	56.47 ± 10.67	52.64 ± 11.18	53.98 ± 14.67	53.05 ± 12.41
CO	52.26 ± 7.82	57.72 ± 14.89	58.44 ± 12.19	62.16 ± 13.93*
TG (mg/dl)				
EVOO	55.87 ± 30.93	63.62 ± 29.50	73.63 ± 32.41	71.71 ± 30.56
OO	65.19 ± 35.25	76.86 ± 32.02	85.20 ± 39.70	73.06 ± 36.70
CO	61.25 ± 20.09	88.29 ± 32.15	95.13 ± 25.40	84.04 ± 22.18
Glycemia (mg/dl)				
EVOO	95.58 ± 5.70	110.75 ± 16.77	90.00 ± 5.08	86.42 ± 3.96
OO	94.83 ± 6.94	108.50 ± 13.67	87.00 ± 7.03	88.33 ± 6.45
CO	96.83 ± 6.26	100.33 ± 10.93	91.00 ± 11.66	88.58 ± 6.78

Blood samples were collected at baseline (0 h),1, 2, and 6 h after the meal. TG: triacylglycerols; HDL-C: high density lipoprotein-cholesterol; LDL-C: low density lipoprotein-cholesterol; EVOO: extra virgin olive oil; OO: olive oil; CO: corn oil. Data are expressed as means ± S.D., *n* = 12 for each group. Differences between EVOO and other oils were assessed through analysis of random effects regression for the entire period.

* $P < 0.05$.

** $P < 0.01$, as compared with EVOO.

Table 3
Baseline and postprandial changes in serum TXB_2 concentrations (ng/ml)

	Time after oil ingestion (ng/ml)			
	Baseline	1 h	2 h	6 h
EVOO	514.60 ± 239.01	447.35 ± 363.54	347.13 ± 252.00	401.90 ± 317.64
OO	540.93 ± 249.91	901.62 ± 973.07	776.55 ± 428.69**	992.07 ± 594.52*
CO	749.79 ± 541.53	817.80 ± 477.79	855.98 ± 589.00*	1035.85 ± 664.78

Values are means ± S.D., $n = 12$ for each group. Differences between EVOO and other oils were assessed through analysis of covariance at time 2 and random effects regression for the entire period.
* $P < 0.05$.
** $P < 0.01$ as compared with EVOO.

Table 4
Baseline and postprandial changes in plasma LTB_4 concentrations (ng/ml)

	Time after oil ingestion (ng/ml)			
	Baseline	1 h	2 h	6 h
EVOO	277.20 ± 120.51	229.38 ± 119.28	189.50 ± 106.89	232.86 ± 218.44
OO	311.48 ± 150.83	390.42 ± 165.32	524.12 ± 307.79**	460.61 ± 244.57**
CO	436.47 ± 263.65	458.43 ± 338.13	501.19 ± 300.56*	474.46 ± 471.06

Values are means ± S.D., $n = 12$ for each group. Differences between EVOO and other oils were assessed through analysis of covariance at time 2 and random effects regression for the entire period.
* $P < 0.05$.
** $P < 0.01$ as compared with EVOO.

(Table 2). The random effects regression analysis from T_0 to 6 h revealed a significant decrease of TC and HDL-C plasma concentrations after ingestion of EVOO, as compared with CO (TC: from 183.99 ± 48.40 mg/dl at T_0 to 179.90 ± 36.53 mg/dl after 6 h for EVOO; from 166.16 ± 37.57 mg/dl at T_0 to 193.42 ± 52.48 mg/dl after 6 h for CO; $P = 0.001$. HDL-C: from 55.85 ± 10.53 mg/dl at T_0 to 56.28 ± 18.11 mg/dl at 6 h for EVOO; from 52.26 ± 7.82 mg/dl at T_0 to 62.16 ± 13.93 mg/dl at 6 h for CO; $P = 0.027$)

Glycemia was also unaffected by the consumption of either OO or CO (Table 2). Plasma FA also did not change significantly after oil administrations (data not shown).

The analysis of covariance showed significant differences in serum TXB_2 production among the three oils after 2 h from the ingestion ($P = 0.0167$). In particular, significant differences between EVOO and OO ($P = 0.0065$) and between EVOO and CO ($P = 0.0173$) were recorded. Concerning EVOO, this effect was noted in all 12 subjects (Table 3).

Conversely, TXB_2 production was not affected by either OO and CO consumption. Moreover, the random effects regression analysis from T_0 to 6 h demonstrated significant differences between EVOO and OO administration ($P = 0.021$) and between EVOO and CO administration ($P = 0.05$).

The production of LTB_4 was also affected by EVOO consumption (Table 4) and at 2 h, was 31.6% lower than that of T_0 in blood from volunteers who were given EVOO, as compared with OO and CO. In particular, the analysis of covariance established a significant difference after 2 h for three oil ($P = 0.0016$). The analysis of covariance found significant differences between EVOO and OO ($P = 0.0004$) and between EVOO and CO ($P = 0.0275$). The random effects regression analysis from T_0 to 6 h showed a significant difference between EVOO and OO ingestion ($P = 0.008$).

Table 5 shows the serum antioxidant capacity (expressed as μmol/l Cu^{++} reduced) at T_0 and 1, 2, and 6 h after the meal. The consumption of different oils by the subjects induced significant differences between the oils, as established with analysis of covariance (at 2 h) ($P = 0.006$). The analysis of covariance also showed an increase of antioxidant capacity after consumption of EVOO. In particular, significant

Table 5
Effects of different oils on plasma antioxidant capacity (μmol/l Cu^{++} reduced)

	Time after oil ingestion (μmol/l Cu^{++} reduced)			
	Baseline	1 h	2 h	6 h
EVOO	83.18 ± 8.72	84.82 ± 9.59	95.45 ± 7.85	83.07 ± 10.39
OO	87.31 ± 10.24	88.10 ± 9.80	94.48 ± 15.43	92.80 ± 13.99
CO	96.26 ± 18.73	93.88 ± 19.91	96.40 ± 20.83**	96.15 ± 22.76*

Values are means ± S.D., $n = 12$ for each group. Differences between EVOO and other oils were assessed through analysis of covariance at time 2 and random effects regression for the entire period.
* $P < 0.05$.
** $P < 0.01$ as compared with EVOO.

Table 6
Concentrations of hydrogen peroxide (μmol/mol creatinine) in freshly voided human urine

	Time after oil ingestion (μmol/mol creatinine)	
	Baseline	2 h
EVOO	330.38 ± 201.35	529.61 ± 844.37
OO	270.49 ± 117.37	513.39 ± 248.40
CO	494.92 ± 385.71	587.10 ± 678.23

Urine samples were collected and immediately assayed. Data are means ± S.D. of triplicate determinations for each sample, $n = 12$ for each group.

differences were noted after 2 h between EVOO and CO ($P = 0.0014$) and between OO and CO ($P = 0.047$). The random effects regression analysis from T_0 to 6 h showed a significant difference in serum antioxidant capacity between the EVOO and CO groups ($P = 0.013$).

We have also assessed the concentrations of H_2O_2, a putative marker of in vivo oxidative stress [10], in freshly voided (form T_0 to 2 h) urine from the volunteers. No significant modifications in the urinary output of hydrogen peroxide were recorded (Table 6), regardless of the kind of oil ingested.

4. Discussion

Hypercholesterolemia is associated with a higher incidence of atherosclerosis [5]. It is also well known that inflammation plays a key role in the onset of atherosclerosis-associated endothelial dysfunction [4]. LDL oxidation is further responsible for fat deposition and for the cell-mediated inflammatory response that increases plaque instability [4]. Consequently, reductions in both circulating lipids and inflammatory markers are independently associated with a reduction in cardiovascular incidents [11]. This partially explains why a diet rich in antioxidants, such as polyphenols, and unsaturated fatty acids correlates with a significant reduction of serum inflammatory markers [12] and with lower cardiovascular mortality [13]. As an example, a recent study showed that an olive oil meal did not induce the postprandial activation of an inflammatory parameter, namely NF-κB, and that this effect was associated with MUFA and antioxidant components of extra virgin olive oil [14]. Finally, a recent paper by Ruano et al. demonstrated that high-phenolic virgin olive oil improves ischemic reactive hyperemia during the postprandial state of hypercholesterolemic patients [15]. This effect was associated with a reduction in oxidative stress markers.

In the context of the Mediterranean diet and CHD, it has also been shown that extra virgin olive oil rich in polyphenols increases the resistance of LDL to oxidation, both in vitro and ex vivo [16]. Also, the Lyon Diet Heart Study [17] and the Attica Study [18] demonstrated how a Mediterranean-like diet is able to lower the degree of inflammation and could thus reduce the risk for CHD, without significantly affecting the serum lipid profile.

For these reasons, we focused our attention on the antithrombotic and anti-inflammatory potential of EVOO, previously shown in vitro [19] and in vivo [20]. The results show significant reductions in serum TXB_2 and LTB_4 concentrations at 2 and 6 h after consumption of EVOO, but not after consumption of either OO or CO (Tables 3 and 4). Hence, this study confirms the antithrombotic and anti-inflammatory effects of EVOO phenolic components, in a postprandial setting. Bartoli et al. [21] have also demonstrated EVOO beneficial activities on inflammatory parameters in districts other than the vascular bed. In rats with colon cancer, an EVOO-rich diet led to a significant reduction in mucosal arachidonic acid concentration and these changes were associated with a lower production of AA metabolites, namely PGE_2, TXB_2 and LTB_4, involved in inflammatory processes. The anti-inflammatory potential of EVOO has been recently corroborated by the discovery of an olive oil component (termed oleocanthal), which inhibits COX-1 and COX-2 [22]. Moreover, our data are in line with other in vitro studies [19] that point to a role of phenolic compounds in the anti-inflammatory reaction and to their modulation of the enzymatic activities related to eicosanoid metabolism. Finally, olive oil phenolics have been also shown to inhibit endothelial activation [23], which is sensitive to intracellular redox status and is linked to the inflammatory response of the arterial wall [24].

We also evaluated the effects of the different oils on some in vivo indexes of oxidative stress (plasma antioxidant capacity and urinary hydrogen peroxide levels). Even though there is no consensus on the in vivo antioxidant actions of EVOO phenolics [25], there is increasing evidence that shows how these compounds retain their antioxidant activity after ingestion [2]. We now report increased plasma antioxidant capacity after 2 h of EVOO consumption, in agreement with previous observations obtained with grape seed anthocyanins [5]. Also, in agreement with our data, Fito et al. [26] showed that EVOO modulates postprandial oxidant/antioxidant status; however, the results of our study (which included olive oil as a control group) allow us to attribute these positive changes to its phenolic fraction. Another potential biomarker of oxidative stress is hydrogen peroxide in urine, which is widely regarded as a cytotoxic agent whose levels must be minimized by antioxidant enzymes. These are regulated by metabolic mechanisms which control the level of H_2O_2 in the human body [27]. Recent experiments have demonstrated that coffee drinking increases urinary hydrogen peroxide levels, suggesting that H_2O_2 levels are influenced by diet [27]. For this reason, we have analyzed the effects of different kinds of oils with diverse polyphenol contents on urinary hydrogen peroxide concentration. This value did not change significantly 2 h after oil consumption and this result might be attributed to the high inter and intra-subject variability, as also shown by recent studies [27].

Concerning the effects on circulating lipids, we recorded significant differences in TC and HDL-C after ingestion of EVOO as compared with CO (Table 2). The effects of olive oil and MUFA on plasma lipids are, to date, still equivocal [28]: some authors [29,30] reported that long term (3–6 weeks) treatment with EVOO exerts beneficial effects on HDL-C, in part associated with its phenolic composition. Conversely, other investigators reported differential variations in lipid parameters depending on the kind of fat ingested. For example, after consumption of carbohydrates accompanied by a high amount (80 g) of olive oil, the magnitude of postprandial lipemia and remnant lipoprotein was reported to be lower than after ingestion of 100 g of butter [3].

In conclusion, this study suggests that EVOO may exert cardioprotective activities (though to a limited extent) in the postprandial status. These data add further evidence to the hypothesis that the cardioprotective properties of the Mediterranean diet are in part due to the consumption of antioxidant-rich olive oil.

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