Eur J Nutr (2013) 52:1223–1231
DOI 10.1007/s00394-012-0433-2

ORIGINAL CONTRIBUTION

Beneficial effects of polyphenol-rich olive oil in patients with early atherosclerosis

R. J. Widmer · M. A. Freund · A. J. Flammer · J. Sexton · R. Lennon · A. Romani · N. Mulinacci · F. F. Vinceri · L. O. Lerman · A. Lerman

Received: 11 April 2012 / Accepted: 29 July 2012 / Published online: 8 August 2012

Abstract
Purpose Diets rich in plant-derived polyphenols such as olive oil (OO) and/or catechins such as epigallocatechin 3-gallate (EGCG) have been shown to reduce the incidence of cardiovascular diseases, potentially by improving endothelial function, an important surrogate for atherosclerosis. The possible augmentation of endothelial function with the combined efforts of OO and EGCG is intriguing, yet unknown.
Methods Eighty-two patients with early atherosclerosis (presence of endothelial dysfunction) were enrolled in this double-blind, randomized trial with 52 completing the study. The aim of the study was to compare the effect of a daily intake of 30 ml simple OO, with 30 ml of EGCG-supplemented OO, on endothelial function as well as on inflammation and oxidative stress after a period of 4 months. Endothelial function was assessed noninvasively via peripheral arterial tonometry (Endo-PAT®).
Results After 4 months, when OO and EGCG-supplemented OO groups were combined, OO significantly improved endothelial function (RHI, 1.59 ± 0.25–1.75 ± 0.45; $p < 0.05$). However, there were no significant differences in results between the two olive oil groups. Interestingly, with OO supplementation there was a significant reduction in inflammatory parameters: sICAM (196 to 183 ng/mL, $p = < 0.001$); white blood cells (WBCs) (6.0×10^9/L–5.8×10^9/L, $p < 0.05$); monocytes (0.48×10^9/L to 0.44×10^9/L, $p = 0.05$); lymphocytes (1.85×10^9/L to 1.6×10^9/L, $p = 0.01$); and platelets (242–229 $\times 10^9$/L, $p = 0.047$).
Conclusions Improvement in endothelial dysfunction in patients with early atherosclerosis in association with significant reduction in leukocytes may suggest an important role of early cellular inflammatory mediators on endothelial function. The current study supports one potential mechanism for the role of olive oil, independent of EGCG, modestly supplemented to a healthy cardiovascular diet.

Keywords Endothelial function · Olive oil · Inflammation · Oxidative stress · Atherosclerosis

Introduction

Olive oil is an important source of polyphenols in the Mediterranean diet [11]. Consumption of diets rich in polyphenols has been shown to enhance nitric oxide (NO) bioavailability as well as decrease oxidative stress and inflammation [2, 14, 29]. Recent randomized trials have pointed out the important antioxidant effect of extra virgin olive oil (OO) in humans [6, 33]. The Italian Virgin Olive Oil Study [33] demonstrated a significant decrease in total antioxidative capacity (and platelet activation) in mildly dyslipidemic patients. This trial studied the effects of extra virgin OO compared to highly refined olive oil, which is much lower in polyphenols. The Eurolive study [6], a randomized trial, showed a linear decrease in markers of oxidative stress with increasing polyphenolic content of the olive oils.

R. J. Widmer and M. A. Freund contributed equally.

R. J. Widmer · M. A. Freund · A. J. Flammer · J. Sexton · R. Lennon · L. O. Lerman · A. Lerman (✉)
Division of Cardiovascular Diseases, Department of Internal Medicine, Mayo Clinic and College of Medicine, 200 1st Street SW, Rochester, MN 55905, USA
e-mail: lerman.amir@mayo.edu

A. Romani · N. Mulinacci · F. F. Vinceri
Dipartimento di Scienze Farmaceutiche Via Ugo Shiff 6, 50019, Sesto Fiorentino, Florence, Italy

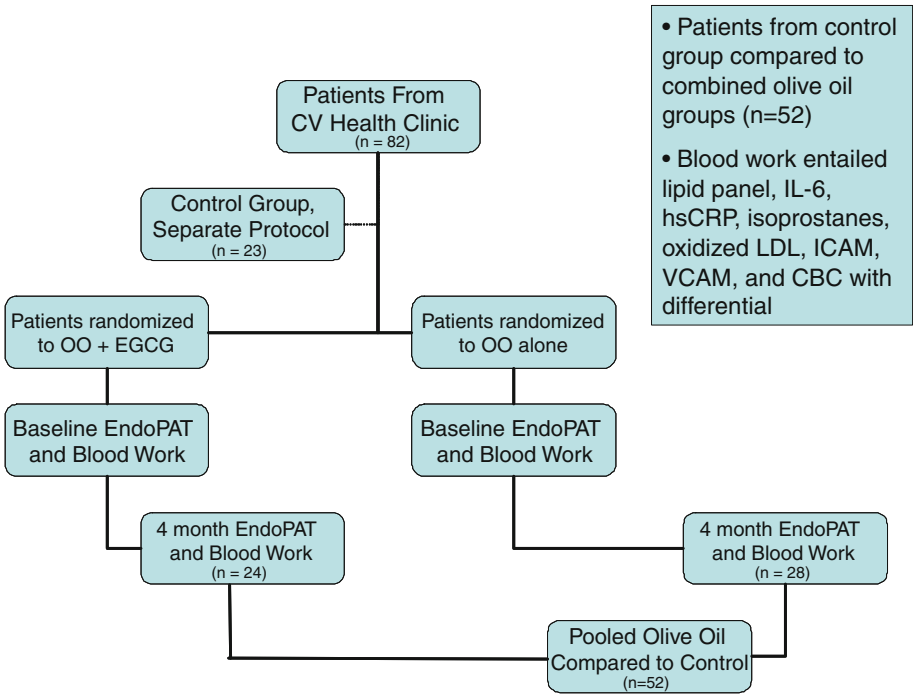

Fig. 1 Schematic representation of the patient selection and trial execution

Epigallocatechin 3-gallate (EGCG) is a green tea-derived phenol that has been described as a biologically potent fraction of the polyphenol family and is thought to be the active ingredient in green tea [1, 5]. OO and EGCG might potentiate antioxidant power when combined; however, the effects on cardiovascular health of dietary supplementation with either olive oil alone or in combination with EGCG are unknown.

Endothelial dysfunction is regarded as an early stage of atherosclerosis leading to subsequent cardiovascular morbidity and mortality [4]. Endothelial dysfunction is associated with most cardiovascular risk factors and is an independent predictor of cardiovascular events [18]. Recently, studies showed conflicting results of the effect of olive oil on endothelial function, potentially because of the low polyphenol content and the brief period of OO ingestion [15, 28, 31].

Definitive evidence regarding the effect of long-term (4 month) supplementation with OO on endothelial function is lacking. Additionally, there are no data regarding the effect of long-term OO and/or EGCG supplementation on endothelial function.

The aim of our study was to demonstrate the effect of OO on endothelial function over the long term (4 month), and to study whether OO with additional EGCG is more effective than OO alone in augmenting endothelial function and surrogate markers for cardiovascular disease. Thus, we utilized a double-blind, randomized controlled study to compare the effect of 4-month supplementation of OO versus EGCG-enriched OO (OO + EGCG) on endothelial function, as well as on oxidative stress and inflammation in patients with low to intermediate cardiovascular risk.

Methods

Study population

Subjects over the age of 18 years were enrolled regardless of previous history of cardiovascular events. Patients were recruited from the Division of Cardiovascular Diseases at Mayo Clinic in Rochester, MN (ClinicalTrials.gov Identifier: NCT00865787), as well as by intra-institutional advertising seeking research participants. Participants underwent endothelial function testing with the EndoPAT® device. Those with normal EndoPAT® score (>2.0) and/or uncontrolled hypertension (blood pressure, >180/100) were excluded from the study. Other exclusion criteria were history of renal or liver failure and relevant food allergies. Informed consent was obtained and signed by all participants.

Experimental protocol

This was a controlled, randomized double-blind study conducted in accordance with the ethical standards of the responsible institutional or regional committee on human experimentation or in accordance with the Helsinki Declaration of 1975 as revised in 1983 and in accordance with the policies and procedures of the local ethics committee and Mayo Clinic Institutional Review Board. Participants were instructed not to change their diets despite olive oil supplementation and were not given any special dietary instructions so as to have olive oil as the sole added variable in their diet. Details are in Fig. 1.

All participants were instructed to fast for at least 4 h prior to each appointment and to abstain from caffeine and tobacco products for 24 h. In addition, participants were instructed to hold ACE inhibitors and calcium channel blockers for 24 h, and nitrates for 6 h prior to the appointment. Examinations were performed in the morning. During a short clinical examination, blood pressure, body weight, and body height were obtained. EndoPAT® testing was performed. Participants were then randomized to receive a once-daily serving of 30 mL of either EGCG-containing OO or OO alone for a total duration of 4 months. Participants were instructed to consume a single dose of the uncooked study product during one of their daily meals so as to simplify the regimen and ensure a total of 30 mL OO taken daily. Measurements were repeated after 4 months to assess long-term effects. Participants were also contacted by phone at 1 and 3 months to assess compliance and any changes in medications or symptoms.

Endothelial function assessment

Studies were performed in a designated quiet, temperature-controlled, and uniformly lit room. EndoPAT® signals were obtained using the Endo-PAT® 2000 device (Itamar Medical Inc. Ltd, Caesarea, Israel.), which has been used previously by our laboratory [4, 12, 17] with a high level of reproducibility [27]. Endothelial function was measured by a reactive hyperemia-peripheral arterial tonometry (RH-PAT) as previously described [3, 4], typically in the dominant hand of the individual unless a contraindication existed and on the arm originally used at baseline for follow-up for each returning subject. After 5-min baseline PAT recording, a blood pressure cuff was inflated on one arm to 60 mmHg (or at least 200 mmHg) above baseline systolic blood pressure for 5 min. Occlusion of pulsatile arterial flow was confirmed via the PAT tracing. After 5 min, the cuff was deflated and the PAT tracing recorded for another 6 min. The ratio (RH-PAT index) of the PAT signal after cuff release compared to baseline was calculated through a computer algorithm automatically normalizing for baseline RH-PAT signal and indexed to the contralateral arm. Reactive hyperemia responses were recorded at baseline and after the 4-month treatment period.

Blood tests

Blood measurements of lipids, inflammatory markers, and endogenous oxidative stress markers were performed at baseline and at 4 months as previously described [4]. Inflammatory markers measured included hsCRP, IL-6, soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), fibrinogen, and a complete blood count with differential. Endogenous oxidative stress markers measured included plasma 8-isoprostane and oxidized LDL (oxLDL). Standard lipid profiles (total cholesterol, triglycerides, HDL, and LDL), and lipoprotein particle subfractions by NMR (nuclear magnetic resonance) [10] spectroscopy, were also assessed at baseline and 4 months in both OO groups.

Study olive oil and polyphenols

OO was supplied by Olivi Agri Team Srl (Grosseto, Italy), and the enhanced/enriched sample (OO with EGCG) was prepared according to a patent (pending, Olivi Agri Team Srl). Oleuropein, EGCG, and luteolin were purchased from Extrasynthese (Geney, France), and the OH-Tyr was purchased from Cayman Chemical (SPI-BIO, Europe). The enhanced samples were produced at IMET factory in Italy (Bergamasco, Italy). OO alone (340 mg/kg polyphenol content) and OO plus EGCG (600 mg/kg polyphenol content) were incorporated into participants' diets as previously reported [23].

Statistical analysis

Statistical analysis was performed by an independent statistician blinded to the randomization after completion of the studies. Results are expressed as mean ± standard deviation or median with interquartile ranges if data were not normally distributed. Discrete data are presented as frequency (percentage). Differences between randomized groups were compared using Student's two-sample t test, the Wilcoxon rank sum test, or Pearson's chi-squared test, as appropriate. Among all randomized patients who had a 4-month follow-up study, differences between 4-month and baseline measures were compared using a signed rank test. A two-sample t test of the change in EndoPAT® score at 4 months was used to compare those with baseline scores <1.6 versus those with scores ≥ 1.6 to delineate. Statistical significance was accepted at $p < 0.05$. OO groups, with and without EGCG, were analyzed separately and also subsequently pooled as part of a subgroup analysis. These were compared using a two-way ANOVA between and among groups. Additionally, these patients underwent the same rigorous enrollment and intake process described above.

Results

Olive oil composition

The main difference in the phenol composition of the two OOs (Table 1) is the higher content of secoiridoids in the

Table 1 Phenolic composition of the two OO samples

Compounds	OO + EGCG[a] (mg/L)	OO (mg/L)
Hydroxy tyrosol	26.18	9.91
Tyrosol	17.45	13.09
3,4 Dihydroxyphenylethanol-elenolic acid	23.63	72.72
Oleocanthal	49.51	68.28
Other secoiridoids	15.57	15.77
Lignans	110.79	104.96
Oleuropein aglycone	55.85	41.57
Luteolin	2.03	0.85
Total phenols (TP)	301.00	327.15
% Secoiridoids/TP	48 %	61 %
% Lignans/TP	37 %	33 %

[a] To this sample 280 mg of EGCG was added

OO (61 %) while the OO enriched with EGCG arrived up only to 48 %. At the same time, the lignans, another group of main constituents, have shown almost the same amount in the two oils.

Baseline characteristics

Eighty-two qualified subjects were randomized for the study. Of these, 52 subjects were able to complete the 4-month protocol as a majority of patients voluntarily declined to continue the study usually secondary to side effects of the OO consumption, and 50 participants had follow-up EndoPAT® measurements. Baseline characteristics of the OO and OO + EGCG patients evaluated in the study are displayed in Table 2. No significant differences between the two randomized olive oil groups were detected regarding factors such as comorbidities, medications, sex, risk factors, or other entities known to be associated with early CVD or endothelial dysfunction. Framingham risk scores were not significantly different between the two OO groups (2.0 ± 2.9 vs. 2.2 ± 3.8; $p = 0.81$). Comparisons of participant's medications taken prior to the study were examined and found not to be significantly different.

The effect of OO on hemodynamic data and endothelial function

After 4 months of olive oil supplementation, there was no significant change in heart rate ($p = 0.28$), systolic ($p = 0.16$) or diastolic blood pressure ($p = 0.73$), or BMI ($p = 0.64$) between the two OO groups. The improvement in endothelial function as measured by EndoPAT® was similar in the OO with additional EGCG and in the OO-alone group (1.6 ± 0.2–1.8 ± 0.5 and 1.6 ± 0.2–1.7 ± 0.4, respectively; $p = 0.85$ for delta). However, when OO groups were combined, there was a significant increase in Endo-PAT® from baseline to 4 months (1.595–1.675, $p = 0.03$, Fig. 2). In those patients who had poor endothelial function at baseline (RHI <1.6), there was a significant increase in EndoPAT® score from 1.38 ± 0.15 to 1.60 ± 0.3 (Fig. 3, $p < 0.01$). Those patients who had normal baseline EndoPAT® scores did not have significant improvement in their endothelial function as measured by EndoPAT® (1.79 ± 0.11–1.90 ± 0.52, $p = 0.21$).

The effect of OO on laboratory characteristics (inflammation and oxidative stress parameters)

Results and comparisons between OO and supplemented OO are shown in Tables 3 and 4. Among the study

Table 2 Baseline characteristics of the subjects involved in the study

Baseline demographics					
Variable	OO + ECGC (N = 24)		OO alone (N = 28)		*p* value
Age, Years	41.8	±14.4	41.3	±14.7	0.92
Sex, No. (%)					0.73
Male	10	(42 %)	13	(46 %)	
Female	14	(58 %)	15	(54 %)	
Baseline BMI	27.1	±5.7	28.2	±6.1	0.52
Known cardiovascular disease, No. (%)	2	(8 %)	1	(4 %)	0.46
Has had cardiac catheterization, No. (%)	2	(8 %)	1	(4 %)	0.46
Family history of CAD, No. (%)	7	(29 %)	9	(35 %)	0.68
Hyperlipidemia, No. (%)	12	(57 %)	16	(76 %)	0.19
Statins, No. (%)	6	(17 %)	9	(23 %)	0.53
Smoking, No. (%)	0	(0 %)	1	(4 %)	0.35
Hypertension currently drug treated, No.	6	(25 %)	6	(21 %)	0.76
Diabetes mellitus, No. (%)	1	(4 %)	0	(0 %)	0.28

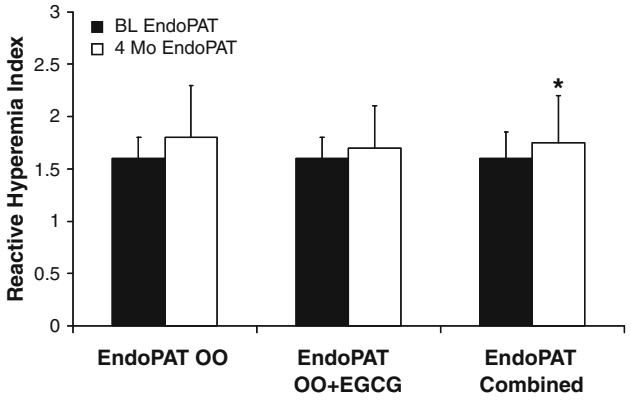

Fig. 2 Comparing Reactive Hyperemia Indices (RHI, ratio) for patients at baseline and after 4 months of treatment. No statistical differences exist when treatment arms of the OO groups are separated. There is a significant difference between baseline and 4-month endothelial function when the groups are combined (A + B), suggesting that OO alone can improve endothelial function (*$p < 0.05$)

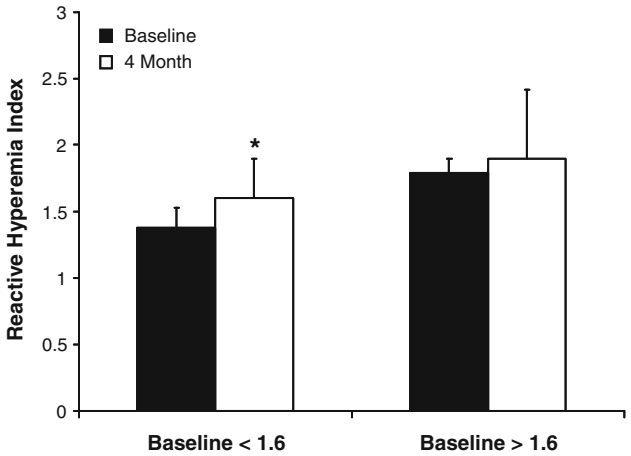

Fig. 3 Graphical representation demonstrating that those with reduced endothelial function (baseline Endo-PAT® reactive hyperemia index (RHI) <1.6) show significant improvements in endothelial function after 4 months of OO treatment ($p = 0.004$)

participants, there were significant reductions in white blood cell counts (WBCs) and sICAM-1 (Tables 3 and 4) in the combined OO group after 4 months. Moreover, the cell types reduced by OO supplementation included lymphocytes, monocytes, and platelets. Neutrophils also tended to be reduced. However, these effects did not differ between the different OO treatment groups (with and without EGCG). Inflammatory and oxidative stress markers such as hsCRP, IL-6, sVCAM-1, as well as oxLDL were not affected significantly. Plasma 8-isoprostane increased with the treatment of olive oil supplementation (Tables 3 and 4).

Serum lipoproteins

Lipid profiles did not change significantly within or between OO groups: HDL (46 mg/dL, baseline, to 51 mg/dL at four months; $p = 0.79$), LDL (102–105 mg/dL; $p = 0.56$), total cholesterol (178–178 mg/dL; $p = 0.79$), and triglycerides (106–103 mg/dL; $p = 0.35$). Lipoprotein particles measured by NMR spectroscopy also showed no significant changes in the OO groups (not reported).

Discussion

The current study demonstrates that longer-term supplementation of olive oil improves endothelial function in individuals with low to intermediate CV risk, an effect likely attributed to reduction in vascular inflammation. This is the first such demonstration of such a permanent endothelial benefit via long-term supplementation of a macronutrient. However, further enrichment with polyphenols (EGCG) does not provide any additional benefit above those attributable to olive oil alone [19, 38]. Although the main goal of the study—demonstrating the superiority of polyphenol-enriched OO—was not achieved, the study nevertheless further supports the role, and potentially ascribes some mechanisms, of the aforementioned benefit of olive oil on cardiovascular health [6, 20, 33]. A concomitant reduction in white blood cells, particularly monocytes and neutrophils, as well as sICAM points to a potential mechanism for improved endothelial function through a reduction in vascular inflammation. Interestingly, patients with low endothelial function at baseline appear to garner the most benefit from OO. Thus, supplementation with OO seems a reasonably easy and relatively cheap dietary measure to improve the endothelial function and perhaps favorably alter the progression of atherosclerotic disease, particularly in patients with already markedly impaired endothelial function.

Olive oil, a major component of the Mediterranean diet, is a rich source of a variety of polyphenols. Indeed, OO has been shown to positively and acutely impact endothelial function as soon as 2 h after consumption [22]; however, the mechanism for this improvement and the responsible ingredient has not yet clearly been identified [26]. The current study is in accord with—and expounds upon—previously observed data and is also the first to show a sustained effect on endothelial function after long-term OO supplementation. We also show that those with baseline endothelial dysfunction benefit the greatest from OO supplementation, perhaps offering yet another treatment modality for those patients at greatest risk of future cardiac events. Because the bioavailability of plant-derived polyphenols in the blood is rather short [36], ingredients in

Table 3 Comparison OO vs. OO + EGCG lipid profiles, inflammatory markers, white blood cell components, and endothelial function after 4 months of supplementation

Variable	OO (N = 24) 4-month change	OO + EGCG (N = 24) 4-month change	*p* value
HDL Chol	0.5 (−5.0, 2.0)	−1.5 (−4.0, 4.0)	0.78
LDL Chol	2.5 (−6.0, 12.0)	−4.5 (−16.0, 13.0)	0.18
Total Chol	2.0 (−9.0, 13.0)	−2.0 (−16.0, 18.0)	0.36
Triglyceride	−1.0 (−10.0, 0.1)	7.5 (−12.0, 27.0)	0.70
HsCRP	0.0 (−0.0, 0.1)	−0.0 (−0.2, 0.0)	0.12
Plasma 8-isoprostanes	25.1 (−7.4, 152.3)	17.3 (−41.6, 59.3)	0.09
sICAM-1	−10.0 (−34, −3.5)	11.5 (−36.5, −2.0)	0.76
Hemoglobin	−0.1 (−0.5, 0.3)	−0.2 (−0.6, 0.0)	0.26
Lymphocytes	−0.1 (−0.4, 0.1)	−0.1 (−0.3, 0.1)	0.84
Monocytes	0.0 (−0.1, 0.0)	−0.0 (−0.1, 0.0)	0.98
Neutrophils	0.0 (−0.8, 0.4)	0.3 (−0.9, 0.0)	0.37
Plasma count	−11.0 (−32.0, 18.0)	−7.0 (−27.0, 12.0)	0.79
White blood cells	−0.1 (−1.1, 0.4)	−0.6 (−1.0, −0.1)	0.46

Values are reported in medians and inter-quartile ranges (*parenthesis*). *P* values denote the comparison in differences after 4 months

Table 4 Baseline, 4 month, and the absolute changes in values given for the entire group of participants exposed to OO during the 4 months

Variable	N	Baseline (IQR)	4 Mo (IQR)	Delta (IQR)	*p* value
EndoPAT score	50	1.595 (1, 2)	1.675 (1, 3.16)	0.09 (−0.72, 1.37)	#0.029
HDL Chol (md/dL)	49	46 (29, 97)	51 (29, 97)	0 (−24, 23)	0.786
LDL Chol (mg/dL)	49	102 (35, 244)	105 (43, 222)	1 (−40, 38)	0.564
Total Chol (mg/dL)	49	178 (94, 312)	178 (109, 302)	0 (−63, 45)	0.789
Triglyceride (mg/dL)	49	106 (25, 311)	103 (38, 407)	5 (−229, 219)	0.346
Oxidized LDL (mg/dL)	49	4.29 (2.86)	4.31 (2.77)	0.02 (0.85)	0.79
Hemoglobin ($\times 10^9$/L)	49	13.7 (11.9, 16.6)	13.6 (11.6, 16.9)	−0.2 (−1.7, 3.1)	0.119
HsCRP (mg/L)	51	0.101 (0.01, 2.81)	0.09 (0.01, 1.21)	0 (−2.68, 0.639)	0.220
sICAM-1 (ng/mL)	53	196 (84, 298)	183 (91, 239)	−11 (−168, 130)	# < .001
IL-6 (pg/mL)	51	1.3 (0.3, 33)	1.3 (0.5, 5.1)	−0.1 (−30.9, 2.7)	0.518
White Blood Cells ($\times 10^9$/L)	49	6 (3.8, 11.7)	5.8 (3.7, 10.4)	−0.4 (−5.2, 2.8)	#0.009
Lymphocytes ($\times 10^9$/L)	48	1.845 (0.47, 3.63)	1.6 (0.64, 3.07)	−0.13 (−0.81, 1.12)	#0.005
Monocytes ($\times 10^9$/L)	48	0.48 (0.27, 0.94)	0.44 (0.26, 0.97)	−0.03 (−0.51, 0.34)	#0.047
Neutrophils ($\times 10^9$/L)	48	3.69 (1.37, 8.94)	3.38 (2.11, 6.4)	−0.15 (−5.02, 2.91)	0.072
Platelet Count ($\times 10^9$/L)	49	242 (124, 367)	229 (146, 338)	−11 (−128, 51)	#0.047
VCAM-1 (ng/mL)	52	575 (368, 1054)	560 (346, 1166)	−26 (−284, 200)	0.068
Plasma 8-isoprostanes (ng/mL)	52	130.8 (28.71, 310.6)	164.4 (55.37, 491.6)	22.39 (−113, 324.7)	#0.003

Significant reductions (#) in ICAM-1, lymphocytes, monocytes, platelets, and WBCs are seen after 4 months of OO treatment. There was a trend toward reduction in neutrophils as well. No significant reductions existed with regard to lipid profiles or inflammatory markers. Plasma-8 isoprostane actually increased (#) after OO treatment

olive oil likely have altered the expression of certain long-term endothelial modulators, such as nitric oxide synthase, to see this sustained effect. Contrary to other studies [6, 24, 33], we were not able to demonstrate a sustained antioxidant effect from OO supplementation as neither oxLDL nor the isoprostanes (markers of lipid peroxidation) were reduced by OO. The significant increase in the plasma 8-isoprostane within the EGCG group might also raise the concern of a possible pro-oxidant effect of EGCG as previous animal work has shown deleterious effects of high-dose vitamin supplementation on myocardial perfusion and coronary endothelial function [32]. Thus, it remains likely that over-supplementation with nearly any substance may not be beneficial and may even pose greater harm to the patient. Moreover, we were unable to appreciate a significant improvement with EGCG supplementation as seen in the previous studies [36]. This, again, could be due to the possible pro-oxidant effect of EGCG negating the beneficial effects of OO alone, or the fact that long-term supplementation with both substances offers no endothelial function benefit.

We observed a simultaneous reduction in WBCs and soluble cellular adhesion molecules sICAM-1, thus

pointing toward the possible role of olive oil consumption on reducing, specifically, vascular inflammation. This mirrors the previous studies that have shown an inverse correlation between WBC counts and cardiovascular disease [21, 30, 35]. Moreover, increased leukocyte counts have been found in both smokers [16] and diabetics [37], and reducing WBCs has been shown to be associated with endothelium-dependent vasodilatation [8, 34]. Our data indicate that CRP is not reduced with OO supplementation. Diets high in monounsaturated fats have been known to increase NO bioavailability, and it is possible that there could be further enhancement of NO-mediated vasodilation via reduction in inflammatory markers associated with WBCs. Myeloperoxidase has also been shown to interfere with tetrahydrobiopterin production and subsequent NO release, bioavailability, and mechanism of action [13, 25]. Therefore, cellular inflammatory mediators plausibly reduce production or bioavailability of NO, or the production of reactive oxygen species that interfere with the ability of tetrahydrobiopterin to aid in the production and synthesis of NO [25]. The reduced leukocytic inflammatory mediations would allow for increased NO-mediated vasodilation and improved endothelial function and could underlie the vascular endothelial improvement seen with OO.

Importantly, both the OO + EGCG and OO alone had polyphenol concentrations that were two to five times more potent than typical commercialized olive oil [6]. This could account for the lack of difference in outcomes between the two variants of OO. The high levels of polyphenols and fats (an extra 30 g of lipids ingested daily by the patients) could also explain the high levels of isoprostanes, as the overabundance of OO could have reached the levels that caused an increase in oxidative stress. Notably, however, the participants did not gain weight with the additional daily caloric intake of the olive oil. Nevertheless, there could be a target level of polyphenols in certain foods such as olive oil that provide maximum cardiovascular benefit without side effects.

Our study did have limitations that must be taken into account. One limitation involved the lack of a control group. As OO supplementation is already known to improve endothelial function (as is green tea, of which the active ingredient is EGCG), we used OO alone as the "control" group. While this could be seen as a weakness, repeated and recent work has demonstrated that the "control" group has no change in endothelial function [7, 9]. Regardless, the addition of a "control" group would have no bearing on the fact that there was no further improvement with the addition of EGCG. Also, noted was the attrition of the subjects, albeit evenly divided, in this study mainly due to gastrointestinal discomfort and inability to incorporate the uncooked study product into their daily diet. Coupling with this, our study, while an RCT over an extended period of time, was limited in patient size and diversity somewhat limiting generalizability yet augmenting internal validity. Another limitation involves not measuring polyphenol plasma concentration, introducing the possibility that our supplementation did not achieve therapeutic concentrations. However, there were no statistical differences in fasting plasma glucose levels between the OO groups. Also of note, our sample population was of low to intermediate risk (mean Framingham 10-year risk percentage between 0.5 and 4 %); thus, we were examining primary prevention characteristics. Had we examined secondary prevention or a higher risk population, we might have seen further improvement in endothelial function, inflammatory markers, lipids, and WBCs.

In conclusion, OO supplementation in patients with low to intermediate risk improves endothelial function through mechanisms possibly related to improvements in inflammation. We did not observe any additive benefit of EGCG, the main component of green tea. Therefore, olive oil supplementation may be beneficial for most individuals and might theoretically reduce cardiovascular events. These benefits are consistent with the prominent role of olive oil in the Mediterranean diet.

Acknowledgments This study was partly supported by Olivi Agri Team Srl, Grosetto, Italy, and the University of Florence. However, the study was investigator initiated and investigator driven. The clinical trial was conducted using Olive Oil and EGCG from Olivi Agri Company. This work was also partly supported by NIH grant #HL085307. AJF received a scholarship from the Walter and Gertrud Siegenthaler Foundation, Zurich, and is supported by a "Nachwuchsförderungskredit" of the young academics Support Committee of the University of Zurich, Switzerland, and the Swiss National Science Foundation (PASMP3_132551).

Conflict of interest The authors report no actual or potential conflict of interest in connection with this study.

References

1. Basu A, Sanchez K, Leyva MJ, Wu M, Betts NM, Aston CE, Lyons TJ (2010) Green tea supplementation affects body weight, lipids, and lipid peroxidation in obese subjects with metabolic syndrome. J Am Coll Nutr 29:31–40
2. Bazzano L, He J, Ogden LG, Loria CM, Vupputuri S, Myers L, Whelton PK (2002) Fruit and vegetable intake and risk of cardiovascular disease in US adults: the first National Health and Nutrition Examination Survey Epidemiologic Follow-up Study. Am J Clin Nutr 76:93–99
3. Bonetti P, Holmes DR Jr, Lerman A, Barsness GW (2003) Enhanced external counterpulsation for ischemic heart disease: what's behind the curtain? J Am Coll Cardiol 41:1918–1925
4. Bonetti P, Pumper GM, Higano ST, Holmes DR Jr, Kuvin JT, Lerman A (2004) Noninvasive identification of patients with early coronary atherosclerosis by assessment of digital reactive hyperemia. J Am Coll Cardiol 44:2137–2141

5. Brown A, Lane J, Coverly J, Stocks J, Jackson S, Stephen A, Bluck L, Coward A, Hendrickx H (2009) Effects of dietary supplementation with the green tea polyphenol epigallocatechin-3-gallate on insulin resistance and associated metabolic risk factors: randomized controlled trial. Br J Nutr 101:886–894
6. Covas M, Nyyssönen K, Poulsen HE, Kaikkonen J, Zunft HJ, Kiesewetter H, Gaddi A, de la Torre R, Mursu J, Bäumler H, Nascetti S, Salonen JT, Fitó M, Virtanen J, Marrugat J, EUROLIVE Study Group (2006) The effect of polyphenols in olive oil on heart disease risk factors: a randomized trial. Ann Intern Med 145:333–341
7. Dohadwala M, Holbrook M, Hamburg NM, Shenouda SM, Chung WB, Titas M, Kluge MA, Wang N, Palmisano J, Milbury PE, Blumberg JB, Vita JA (2011) Effects of cranberry juice consumption on vascular function in patients with coronary artery disease. Am J Clin Nutr 93:934–940
8. Elkind M, Sciacca RR, Boden-Albala B, Tondella ML, Feikin DR, Fields BS, Sacco RL, Di Tullio MR, Homma S (2005) Leukocyte count is associated with reduced endothelial reactivity. Atherosclerosis 181:329–338
9. Flammer A, Martin EA, Gössl M, Widmer RJ, Lennon RJ, Sexton JA, Loeffler D, Khosla S, Lerman LO, Lerman A (2012) Polyphenol-rich cranberry juice has a neutral effect on endothelial function but decreases the fraction of osteocalcin-expressing endothelial progenitor cells. Eur J Nutr [Epub ahead of print]. PMID 22382203
10. Ford M, McConnell JP, Lavi S, Rihal CS, Prasad A, Sandhu GS, Hartman SJ, Lerman LO, Lerman A (2009) Coronary artery endothelial dysfunction is positively correlated with low density lipoprotein and inversely correlated with high density lipoprotein subclass particles measured by nuclear magnetic resonance spectroscopy. Atherosclerosis 207:111–115
11. Fung T, Rexrode KM, Mantzoros CS, Manson JE, Willett WC, Hu FB (2009) Mediterranean diet and incidence of and mortality from coronary heart disease and stroke in women. Circulation 119:1093–1100
12. Goor D, Sheffy J, Schnall RP, Arditti A, Caspi A, Bragdon EE, Sheps DS (2004) Peripheral arterial tonometry: a diagnostic method for detection of myocardial ischemia induced during mental stress tests: a pilot study. Clin Cardiol 27:137–141
13. Higashi Y, Sasaki S, Nakagawa K, Kimura M, Noma K, Hara K, Jitsuiki D, Goto C, Oshima T, Chayama K, Yoshizumi M (2006) Tetrahydrobiopterin improves aging-related impairment of endothelium-dependent vasodilation through increase in nitric oxide production. Atherosclerosis 186:390–395
14. Hu F, Willett WC (2002) Optimal diets for prevention of coronary heart disease. JAMA 288:2569–2578
15. Jiménez-Morales A, Ruano J, Delgado-Lista J, Fernandez JM, Camargo A, López-Segura F, Villarraso JC, Fuentes-Jiménez F, López-Miranda J, Pérez-Jiménez F (2011) NOS3 Glu298Asp polymorphism interacts with virgin olive oil phenols to determine the postprandial endothelial function in patients with the metabolic syndrome. J Clin Endocrinol Metab 96:1694–1702
16. Lavi S, Prasad A, Yang EH, Mathew V, Simari RD, Rihal CS, Lerman LO, Lerman A (2007) Smoking is associated with epicardial coronary endothelial dysfunction and elevated white blood cell count in patients with chest pain and early coronary artery disease. Circulation 115:2621–2627
17. Lavie P, Shlitner A, Sheffy J, Schnall RP (2000) Peripheral arterial tonometry: a novel and sensitive non-invasive monitor of brief arousals during sleep. Isr Med Assoc J 2:246–247
18. Lerman A, Zeiher AM (2005) Endothelial function: cardiac events. Circulation 111:363–368
19. Lorenz M, Wessler S, Follmann E, Michaelis W, Düsterhöft T, Baumann G, Stangl K, Stangl V (2004) A constituent of green tea, epigallocatechin-3-gallate, activates endothelial nitric oxide synthase by a phosphatidylinositol-3-OH-kinase-, cAMP-dependent protein kinase-, and Akt-dependent pathway and leads to endothelial-dependent vasorelaxation. J Biol Chem 279:6190–6195
20. Perez-Jimenez F et al (2005) International conference on the healthy effect of virgin olive oil. Eur J Clin Invest 35:421–424
21. Prentice R, Szatrowski TP, Fujikura T, Kato H, Mason M, Hamilton HH (1982) Leukocyte counts and coronary heart disease in a Japanese cohort. Am J Epidemiol 116:496–509
22. Ridker P, Hennekens CH, Roitman-Johnson B, Stampfer MJ, Allen J (1998) Plasma concentration of soluble intercellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men. Lancet 35:88–92
23. Romani A, Pinelli P, Mulinacci N, Galardi C, Vincieri FF, Liberatore L, Cichelli A (2001) HPLC and HRGC analyses of polyphenols and secoiridoids in olive oil. Chromatographia 53:279–284
24. Ruano J, Lopez-Miranda J, Fuentes F, Moreno JA, Bellido C, Perez-Martinez P, Lozano A, Gómez P, Jiménez Y, Pérez Jiménez F (2005) Phenolic content of virgin olive oil improves ischemic reactive hyperemia in hypercholesterolemic patients. J Am Coll Cardiol 46:1864–1868
25. Schmidt K, Werner ER, Mayer B, Wachter H, Kukovetz WR (1992) Tetrahydrobiopterin-dependent formation of endothelium-derived relaxing factor (nitric oxide) in aortic endothelial cells. Biochem J 281:297–300
26. Schmitt C, Dirsch VM (2009) Modulation of endothelial nitric oxide by plant-derived products. Nitric Oxide 21:77–91
27. Selamet-Tierney E, Newburger JW, Gauvreau K, Geva J, Coogan E, Colan SD, Ferranti SD (2009) Endothelial pulse amplitude testing: feasibility and reproducibility in adolescents. J Pediatr 154:901–905
28. Siqueira J, Smiley D, Newton C, Le NA, Gosmanov AR, Spiegelman R, Peng L, Osteen SJ, Jones DP, Quyyumi AA, Ziegler TR, Umpierrez GE (2011) Substitution of standard soybean oil with olive oil-based lipid emulsion in parenteral nutrition: comparison of vascular, metabolic, and inflammatory effects. J Clin Endocrinol Metab 96:3207–3216
29. Stocker R (1999) Dietary and pharmacological antioxidants in atherosclerosis. Curr Opin Lipidol 10:589–597
30. Sweetnam P, Thomas HF, Yarnell JW, Baker IA, Elwood PC (1997) Total and differential leukocyte counts as predictors of ischemic heart disease: the caerphilly and speedwell studies. Am J Epidemiol 145:416–421
31. Tousoulis D, Papageorgiou N, Antoniades C, Giolis A, Bouras G, Gounari P, Stefanadi E, Miliou A, Psaltopoulou T, Stefanadis C (2010) Acute effects of different types of oil consumption on endothelial function, oxidative stress status and vascular inflammation in healthy volunteers. Br J Nutr 103:43–49
32. Versari D, Daghini E, Rodriguez-Porcel M, Sattler K, Galili O, Pilarczyk K, Napoli C, Lerman LO, Lerman A (2006) Chronic antioxidant supplementation impairs coronary endothelial function and myocardial perfusion in normal pigs. Hypertension 47:475–481
33. Visioli F, Caruso D, Grande S, Bosisio R, Villa M, Galli G, Sirtori C, Galli C (2005) Virgin Olive Oil Study (VOLOS): vasoprotective potential of extra virgin olive oil in mildly dyslipidemic patients. Eur J Nutr 44:121–127
34. Walker AE, Seibert SM, Donato AJ, Pierce GL, Seals DR (2010) Vascular endothelial function is related to white blood cell count and myeloperoxidase among healthy middle-aged and older adults. Hypertension 55:363–369
35. Wheeler J, Mussolino ME, Gillum RF, Danesh J (2004) Associations between differential leucocyte count and incident coronary heart disease: 1764 incident cases from seven prospective studies of 30 374 individuals. Eur Heart J 25:1287–1292

36. Widlansky M, Hamburg NM, Anter E, Holbrook M, Kahn DF, Elliott JG, Keaney JF, Vita JA (2007) Acute EGCG supplementation reverses endothelial dysfunction in patients with coronary artery disease. J Am Coll Nutr 26:95–102
37. Woodman R, Watts GF, Puddey IB, Burke V, Mori TA, Hodgson JM, Beilin LJ (2002) Leukocyte count and vascular function in Type 2 diabetic subjects with treated hypertension. Atherosclerosis 163:175–181
38. Yokozawa T, Nakagawa T, Kitani K (2002) Antioxidative activity of green tea polyphenol in cholesterol-fed rats. J Agric Food Chem 50:3549–3552