Review

Vasculoprotective potential of olive oil components

Maria Annunziata Carluccio[1,2], Marika Massaro[1,2], Egeria Scoditti[1,2] and Raffaele De Caterina[1,3]

[1] C.N.R. Institute of Clinical Physiology, Pisa, Italy
[2] C.N.R. Institute of Clinical Physiology, Lecce, Italy
[3] Institute of Cardiology and Center of Excellence on Aging, "G. d'Annunzio" University, Chieti, Italy

Epidemiological and clinical studies found that the traditional Mediterranean-style diet is associated with significantly lower mortality from coronary artery disease. Although it is difficult to isolate individual dietary factors, cumulative evidence suggests that olive oil, used as primary source of fat by Mediterranean populations, may play a key role in the observed cardiovascular benefit. Olive oil is a priceless source of vitamins and polyphenolic antioxidants, and has a balanced ratio of monounsaturated and polyunsaturated fatty acids. There are multiple mechanisms by which olive oil might impact the development of atherosclerosis. Olive oil decreases LDL-cholesterol and increases HDL-cholesterol, and also reduces oxidative stress due to polyphenols, which are able to scavenge free radicals and protect LDL from oxidation. In addition, olive oil components may interfere with the inflammatory response within atherosclerotic lesion, by inhibiting endothelial activation involved in monocyte recruitment during early atherogenesis and macrophage production of inflammatory cytokines and matrix degrading enzymes, thus improving vascular stability. Other vasculoprotective mechanisms by olive oil components derive from anti-thrombotic and anti-hypertensive actions. The available data support the need to preserve certain dietary traditions, such as olive oil consumption, to counteract the burden of cardiovascular disease.

Keywords: Atherosclerosis / Antioxidants / Vascular inflammation / Olive oil / Polyphenols

Received: Deember 29, 2006; revised: March 2, 2007; accepted: April 5, 2007

1 Introduction

At the beginning of this new millennium atherosclerotic vascular disease continues to be the main cause of death in the world. Despite advancements in interventional and pharmacological strategies for the treatment of cardiovascular diseases, few improvements in cardiovascular prevention have so far been made. A consensus about the role of nutritional factors in the etiology of cardiovascular diseases has gradually emerged [1]. Indeed, on the one hand diets high in saturated and *trans*-fatty acids can lead to a pro-atherogenic lipid profile. On the other hand, a high intake of some unsaturated fatty acid and/or antioxidant compounds (vitamins and non-vitamins) can both reduce pro-atherogenic risk factors and the susceptibility of the vascular wall to pro-inflammatory and pro-atherogenic triggers.

One of the emerging strategies gaining particular credit at this present time is the dietary preventive approach termed "Mediterranean Diet" or, better, "Mediterranean Diets" [2]. This is based on observations carried out since the late '50s, demonstrating a very low incidence of coronary artery disease (CAD) in residents of Mediterranean countries and the importance of the "alimentary factor" in such protection. Traditional diets of people living in the Mediterranean basin are, among other components, very rich in extra-virgin olive oil, the most typical source of visible fat. For centuries olive oil, largely produced in Mediterranean countries, has been thought not only to contribute nutrients to the diet, but also to have a positive impact on health, so that it was traditionally treasured as a functional food. Many authors started to recognize olive oil as one of the key elements in the cardioprotection and longevity of

Correspondence: Dr. Raffaele De Caterina, Institute of Cardiology and Center of Excellence on Aging, "G. d'Annunzio" University – Chieti, C/o Ospedale S. Camillo de Lellis, Via Forlanini, 50 66100 Chieti, Italy
Fax: +39-0871-402-817
E-mail: rdecater@ifc.cnr.it

Abbreviations: BP, blood pressure; **CAD,** coronary artery disease; **ICAM-1,** intercellular adhesion molecule-1; **M-CSF**, macrophage-colony stimulating factor; **MMP**, matrix metalloproteinase; **NO,** nitric oxide; **ROS,** reactive oxygen species; **TNFα**, tumor necrosis factor-a; **VCAM-1,** vascular cell adhesion molecule-1

inhabitants of Mediterranean regions. Among these, Ancel Keys, the instigator and principal investigator of the Seven Countries Study and the first world-known supporter of Mediterranean diets, wrote in 1987: *"… I have the impression that centenarians are common among farmers, whose breakfast is often only a wineglass of olive oil…"* [3]. After Keys' surveys [4, 5], the results from the secondary prevention clinical trial termed the Lyon Diet Hearth Study confirmed the cardioprotective role obtained from the adoption of a Mediterranean regimen, associated with an extraordinary 70% reduction in the risk of a coronary event [6].

The healthful properties of olive oil have been often attributed to its high content of monounsaturated fatty acids, namely in the form of oleic acid (18:1 n-9) [7]. However it should be underlined that olive oil, unlike other vegetable oils, contains high amounts of several micronutrient constituents, including polyphenolic compounds (100 – 1000 mg/Kg), such as hydroxytyrosol and oleuropein. The amounts of these minor components in olive oil vary, depending on a number of several factors including the cultivars, the degree of maturation, possible infestations, the climate, as well as the means of olive oil production and storage [8]. Polyphenolic compounds are important to plant physiology, contributing to resistance to microorganisms and insects, pigmentation and organoleptic characteristics (odor and flavor). They display relevant antioxidant properties, preserving plant integrity against environmental stress, including the ultra-violet radiation and relatively high temperatures (common in the Mediterranean basin). In addition, they contribute to olive oil stability, protecting it from auto-oxidation [9]. The availability of pure compounds has spurred investigations on antioxidants and other biological activities of olive oil polyphenols. Thus, a large mass of research has been accumulating in the area of olive oil, in the attempt to provide evidence for the health benefits of olive oil consumption and to scientifically support the widespread adoption of traditional Mediterranean diet as a model of healthy eating.

2 The pathogenesis of atherosclerosis

Atherosclerosis is a process with relevant inflammatory components in its inception, progression and complications [10, 11]. In most animal models atherosclerosis begins with the accumulation of monocyte-derived macrophages in the arterial intima. These cells, by taking up lipid droplets (mostly oxidized or otherwise modified LDL), become foam cells, which are typical cellular elements of the "fatty streak", the earliest detectable atherosclerotic lesion [12, 13]. Animal observations have also shown that fatty streaks precede the development of "intermediate lesions" [14, 15], which are composed of macrophages and smooth muscle cells. The activation of these cell types leads to the release of hydrolytic enzymes (metalloproteinases and other proteases), cytokines, chemokines, and growth factors, which can induce focal necrosis or apoptosis. Cycles of mononuclear cell accumulation, migration and proliferation of smooth muscle cells, as well as the formation of fibrous tissue, lead to the enlargement and restructuring of the lesion, with the formation of a fibrous cap and other morphological changes. Plaques range from those with a prevailing lipid component (fatty lesions) to those with prevailing fibrous tissue (fibrous plaques). The increase in plaque size, by limiting blood flow in a coronary artery, may cause ischemia in the myocardium in conditions of increased myocardial oxygen demand, a condition believed to be the main mechanism behind stable effort angina. However, it is now believed that the physical rupture of a more or less advanced plaque – and not lumen stenosis – is responsible for the majority of acute clinical manifestations of atherosclerosis, including acute myocardial infarction, stroke and sudden cardiovascular death [16, 17].

Over the past 150 years, there have been numerous efforts to explain the complex intracellular events associated with the onset, progression and complication of atherosclerosis. There is now a consensus that atherosclerosis represents a state of heightened oxidative stress, characterized by lipid and protein oxidation in the vascular wall [18]. The "oxidative modification hypothesis" of atherosclerosis predicts that the oxidation of LDL is the earliest biochemical event in atherosclerosis[18]. In support of this hypothesis are the findings according to which oxidized LDL support foam cell formation *in vitro* [19], stimulate monocytes chemotaxis [20], prevent the egress of monocytes [21], induce endothelial dysfunction and activation [22], leading to the expression of adhesion molecule for monocytes [23 – 25]. Several structurally unrelated antioxidants inhibit atherosclerosis in animals [26 – 28], although human data are much more problematic. This is probably due to the use of weak antioxidants, single-molecule interventions, the mainly extracellular mechanism of action of the most common antioxidant used in clinical trials, vitamin E, and attempts at intervening late in the phenomenon (for a review on this, see [18]). An emerging consensus underscores the contribution of vascular wall cells in the generation of the oxidative events: smooth muscle cells, endothelial cells and macrophages all possess the enzymatic machinery to produce reactive oxygen and nitrogen species, which not only oxidatively modify the LDL entrapped in the subendothelial space, but also directly determine the other oxidative modifications, mostly occurring intracellularly, contributing to endothelial dysfunction and, later, plaque complications.

3 Olive oil and atherosclerosis

The cardiovascular benefit of olive oil consumption reported by epidemiological and clinical studies has been

confirmed by experimental results in different animal models of atherosclerosis. Indeed diet enrichment with olive oil might interfere with the development and progression of atherosclerosis [29 – 31]. Several biochemical and metabolic results trying to identify the protective factors found that the beneficial effects of olive oil may occur at different levels and with several mechanisms that we summarize below.

3.1 Olive oil, olive oil components, and lipid profile

Experimental studies have demonstrated that one of the most important cardioprotective effects of dietary oleic acid is the quantitative and qualitative regulation of serum cholesterol levels, one the most important atherosclerotic risk factors. While high consumption of food rich in saturated fat increases plasma cholesterol concentrations, controlled metabolic studies on fat metabolism have shown that dietary replacement of saturated fatty acids with monounsaturated or polyunsaturated fatty acids results in a significant cholesterol lowering [32, 33]. In particular, consumption of diets relatively high in monounsaturated – compared to polyunsaturated fatty acids – apparently has the advantage of selectively (although mildly) decreasing LDL-cholesterol without lowering HDL cholesterol [32], the abundance of which is instrumental in removing excess cholesterol from extrahepatic tissues and transporting it to the liver and steroidogenic organs (reverse cholesterol transport) [34].

More recent findings have indicated that the phenolic content of olive oil can provide further benefit for plasma lipid levels compared with the monounsaturated acid content. Olive oil with high phenolic content (366 mg/Kg) increased HDL-cholesterol levels in addition to reducing triglyceride level as compared with olive oils low in polyphenols [35].

Many studies have examined the effect of changes from a carbohydrate-rich to a fat-rich diet [36], or from a saturated- to an unsaturated fat-rich diet in diabetes [37]. Recently, a study comparing the effect of a polyunsaturated fat diet (sunflower oil) with that of an isocaloric Mediterranean-style monounsaturated fat-rich diet (olive oil) on postprandial lipoproteins, found lower levels of postprandial lipoproteins after the oleic acid-rich diet.

Additionally, diabetic rats fed a basal diet supplemented with olive oil showed lower levels of triglycerides when compared with sunflower oil or fish oil, suggesting that olive oil provides a better control of the hypertriglyceridemia accompanying diabetes [38].

3.2 Olive oil, olive oil components, and oxidative stress

Oxidative stress, an overproduction of reactive oxygen species (ROS) not balanced by their sufficient disposal, is now recognized as key mediator of chronic inflammatory diseases, including atherosclerosis [39]. The oxidation of lipoproteins, in particular LDL, is thought to play a fundamental role in the pathogenesis of atherosclerosis [40].

Olive oil supplementation (50 mg/day) to the diet enriched LDL with oleic acid and significantly reduced human LDL susceptibility to *in vitro* oxidation [41, 42], thus making them significantly less atherogenic [43, 44]. In part, this reflects the lesser susceptibility of monounsaturated fatty acids to lipid peroxidation compared with that of polyunsaturated fatty acids, which are particularly prone to peroxidation due to the greater number of double bonds [45].

To isolate the effects of oleic acid from other potentially biologically active compounds in olive oil, extra-virgin olive oil was compared with oleic acid-rich sunflower oil in an attempt to determine whether oleic acid alone was responsible for the ability to prevent LDL-oxidizability [46]. Consumption of either oil produced similar effect on fasting and postprandial plasma lipid levels, but only olive oil diet decreased LDL susceptibility to oxidation (although to a non-statistically-significant extent), suggesting an effect of minor components of extra-virgin olive oil on cardiovascular risk factors. Indeed, recent findings suggest that LDL oxidation is inhibited by polyphenolic compounds found in olive oil, such as the complex phenol oleuropein and its derivative hydroxytyrosol [47 – 49]. Hydroxytyrosol and oleuropein inhibit copper sulphate-induced oxidation of LDL, as indicated by a reduced formation of malondialdeyde adducts and a higher vitamin E content in the residual LDL [50]. In addition, their antioxidant activities, which have be proven to be more effective than that of vitamin E, were further confirmed by the use of metal-independent oxidative systems. These compounds are able to scavenge free radicals, due to the hydrogen-donating capacity of the hydroxyl group in the *ortho*-diphenolic structure [50]. Hydroxytyrosol is able to scavenge peroxynitrite and therefore to prevent peroxinitrite-dependent DNA damage and tyrosine nitration [51].

Olive oil phenolic compounds also counteract ROS-mediated cytotoxicity in human erythrocytes [52], and inhibit passive smoking-induced oxidative stress in human volunteers, as demonstrated by a reduction in the urinary excretion of F_2 isoprostanes, known markers of enhanced oxidative stress [53].

Recent clinical studies in healthy male volunteers have also shown that oxidative stress markers, including cholesterol-conjugated dienes, hydroxy-fatty acids and DNA oxidative damage, all decreased linearly with the increase of the phenolic content of olive oil [35, 54].

3.3 Olive oil, olive oil components, and endothelial activation

The earliest morphologically detectable cellular event in atherogenesis is the adhesion of circulating blood monocytes to the arterial endothelium [55 – 57]. This occurs

because of a modification of the normal functional state of the arterial endothelium, consisting of the appearance of new antigenic and functional properties, in general termed "endothelial activation" [58]. Possible causes of endothelial activation include elevated levels of oxidized and minimally-modified LDL, free radicals caused by cigarette smoking, the advanced glycation endproducts of diabetes, hypertension, elevated plasma homocysteine concentrations, infectious microorganisms and/or inflammatory cytokines, involved in the amplification of local inflammatory responses once monocytes/macrophages have already been recruited. When the vascular endothelium is chronically exposed to the above-mentioned stimuli, leukocyte adhesion to the endothelium occurs because of the selective expression of endothelial leukocyte adhesion molecules. These can slow down the leukocyte run in the circulation, determine the rolling of leukocytes over endothelial cells, tether leukocytes in a labile and then stable fashion, and subsequently induce their trans-endothelial migration [59].

Since monocyte recruitment into the intima of large arteries is specific for atherosclerosis, compared with other forms of leukocyte-endothelial interactions, it was hypothesized that these localized monocyte-endothelium interactions reflect specific molecular changes in the adhesive properties of the endothelial surface, leading to the surface expression of specific"athero-endothelial leukocyte adhesion molecules", *i. e.* endothelium leukocyte adhesion molecules expressed in the early phases of atherosclerosis. The specific endothelial adhesion molecules thought to play a pivotal role in monocyte recruitment are Vascular Cell Adhesion Molecule-1 (VCAM-1), InterCellular Adhesion Molecule-1 (ICAM-1) [60] and E-selectin [10]. In addition, endothelial growth factors and chemoattractants, such as macrophage-colony stimulating factor (M-CSF) and monocyte chemoattractant protein-1, cooperate to the leukocyte accumulation into the arterial wall. In particular, VCAM-1 binds a heterodimeric integrin receptor, very late antigen 4, whose leukocyte selectivity of expression, on monocytes and lymphocytes, but not on neutrophils, can explain the selectivity of monocyte recruitment in early atherogenesis [13]. Endothelial cells express VCAM-1 early during cholesterol feeding in the rabbit, before the appearance of macrophages/foam cells in the intima of developing fatty streak, in a temporal pattern consistent with its pathogenetic role in lesion development. A causal role of VCAM-1 in atherosclerosis is shown by the fact that a hypomorphic variant with reduced expression of VCAM-1 protects against atherosclerosis in the LDL receptor knockout mouse model [61].

Since endothelial activation is an essential step of atherosclerosis, each factor able to reduce endothelial activation is a candidate to have favorable effects on the development of atherosclerotic vascular disease. We devised a series of experiments to investigate whether oleic acid and natural antioxidants obtained from extra-virgin olive oil influence the endothelial response to pro-inflammatory stimuli triggering endothelial activation. Oleic acid, when added to endothelial cells in culture and under conditions yielding significant incorporation in total cell lipids, reduced the stimulated surface expression of endothelial leukocyte adhesion molecules as well as M-CSF release in the culture medium, in a concentration- and time-dependent fashion [62, 63]. The effect appears to be a generalized"damping" of endothelial activation, is specific for cytokine-inducible molecules, is independent of the stimulus used to elicit endothelial activation, and is accompanied by a functional counterpart in the inhibition of monocytoid cell adhesion to the endothelium. The analysis of fatty acid incorporation in total cellular lipids pointed out that oleate addition to culture medium significantly increases the unsaturation index, because of a relatively selective replacement of saturated fatty acids by oleate, leaving higher unsaturated fatty acid pools relatively unaffected [63]. This was the first report of a direct, vascular effect of oleate on vascular cells, and is a candidate explanation for direct anti-atherogenic properties of diets rich in this compound, adding to other beneficial effects of oleate on the lipid profile or other cardiovascular risk factors.

Because the transcriptional activation of adhesion molecules is sensitive to the intracellular redox status [64], we investigated the effects of olive oil polyphenols endowed with antioxidant activity, on monocyte adhesion and the expression of endothelial leukocyte adhesion molecules. Among olive oil polyphenols tested, oleuropein and hydroxytyrosol significantly inhibited the stimulated expression of VCAM-1 in a concentration-dependent fashion. The efficacy of oleuropein and hydroxytyrosol appears strictly related to the antioxidant activity of their ortho-diphenolic structure. Indeed, no significant effects were obtained, in our experimental system, with the two other tested phytochemicals from olive oil, elenolic acid and tyrosol, devoid of antioxidant activity. Similarly to VCAM-1, hydroxytyrosol and oleuropein reduced the stimulated expression of E-selectin and ICAM-1, indicating a generalized effect on endothelial activation [65].

The concerted endothelial expression of VCAM-1, ICAM-1 and E-Selectin, as well as of M-CSF, monocyte chemoattractant protein-1, and other inflammatory genes, is regulated by few transcription factors including the early response genes (c-*jun*, c-*fos*), GATA, and nuclear factor-κB (NF-κB) [66]. Recent evidence suggests that some overproduction of ROS is involved in the endothelial activation of NF-κB and, consequently, in the induced expression of NF-κB-dependent genes [67, 68]. There is evidence that NF-κB activation can be effectively blocked by antioxidants [68, 69], ultimately leading to a decreased activation of the inflammatory response. Both oleic acid and olive oil polyphenols reduced NF-κB activation, as demonstrated by electrophoretic mobility shift assays[62, 65], likely acting through a quenching of ROS [63].

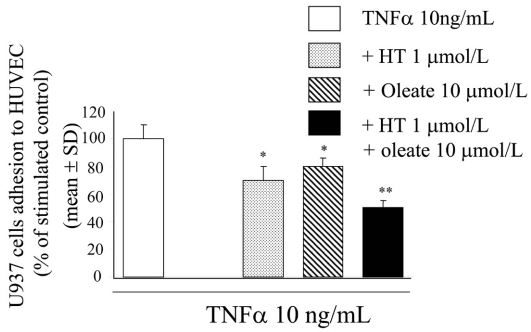

Figure 1. Effects of hydroxytyrosol, oleic acid or both on cytokine-induced monocytoid cell adhesion to human umbilical vein endothelial cells (HUVEC). Olive oil components hydroxytyrosol (HT, 1 μmol/L), oleate (10 μmol/L), or both, significantly decrease U937 monocytoid cell adhesion to HUVEC stimulated with 10 ng/mL TNFα for 20 h (100% adhesion). HUVEC were pre-treated with 1 μmol/L HT for 1 h, or oleate 10 μmol/L for 48 hours, or their combination before the stimulation with TNFα, after which U937 cell adhesion to the endothelium was evaluated as described [62]. Monocyte adhesion was measured as the number of U937 cells adhering within a high-power (0.09 mm^2) field, and expressed as percent of stimulated control; each concentration point is the mean ± S.D. of adhering cells from three experiments, each consisting of eight counts per condition. *$p < 0.05$ and **$p < 0.01$ *vs.* stimulated control (unpublished data).

The combination of low concentration of hydroxytyrosol and oleic acid, which better reproduces the olive oil composition, resulted in a more-than-additive inhibitory effect on endothelial activation and the subsequent functional adhesion of monocytes to the endothelium (Fig. 1). Most relevant, such effects occurred at low micromolar concentrations [62, 65, 70], within the plasma range expected to be achieved with a classical Mediterranean diet [71]. It is likely that such anti-inflammatory effects would be amplified *in vivo* because of the continuous exposure of vascular endothelial cells to these compounds. Thus, both oleic acid and olive oil antioxidant polyphenols, at nutritionally relevant concentrations, inhibit endothelial activation and monocyte recruitment, thus partially explaining atheroprotection observed in animal models and the healthy properties of Mediterranean diets.

3.4 Olive oil, olive oil components, and macrophage function

Atherosclerosis is characterized by the recruitment of monocyte-derived macrophages and lymphocytes to the arterial wall. These cellular elements critically contribute to the amplification and exacerbation of local inflammation by producing a number of cytokines, growth factors and matrix-degrading enzymes [11]. Olive oil seems to be a molecular modulator of the inflammatory/immune response.

In animal model, tumor necrosis factor (TNF)-α production by rat peritoneal macrophages was reduced after 8 weeks of olive oil feeding [72]. Similar results were obtained by Wallace et al. who investigated the effects of dietary fats on macrophage-mediated cytotoxicity [73]. These authors found that lipopolysaccharide-stimulated TNFα production decreased with the increasing unsaturated fatty acid content of the oil tested, with olive oil producing less effects than fish oil (rich in n-3 polyunsaturated fatty acid) or sunflower oil (rich in linoleic acid), but more than coconut oil or low-fat diet. They also found that macrophage nitric oxide (NO) production was higher in animals fed with olive oil compared with sunflower oil, but lower than the low fat diet and the fish oil diet [73]. An *in vivo* study confirmed the NO increase and also documented a decrease in arachidonic acid mobilization and prostaglandin E_2 production in macrophages from animals fed olive oil [74].

The phenol fraction of olive oil has been found to modulate inflammation. Visioli *et al.* showed that oleuropein increased NO production in macrophages challenged with lipopolysaccharide, through an induction of the inducible form of the enzyme nitric oxide synthase, thus increasing the functional activity of these immunocompetent cells [75]. In fact, NO is an important bactericidal and cytostatic factor with vaso-relaxing and anti-aggregating properties [76].

3.5 Olive oil, olive oil components, and plaque stability

Atherosclerotic plaques show enhanced expression and activity of many proteolytic enzymes, in particular matrix metalloproteinases (MMPs) [77, 78]. From early stages of vascular disease up to the formation of advanced plaques, increased levels of MMPs, through multiple and complex mechanisms, contribute to plaque remodeling, cell invasion, migration and proliferation, as well as angiogenesis, all phenomena linked to plaque growth and destabilization [79]. The shoulder regions of the plaque, located at the edges of the overlying fibrous cap, are the most common sites of plaque rupture, and characteristically contain numerous macrophages, which are an important source of MMPs [77, 80].

Up to now, very little investigation has been devoted to the effect of minor Mediterranean dietary components on MMP production and release. However, after the demonstration that green tea polyphenols modulate the activity and expression of MMPs [81], we decided to investigate whether hydroxytyrosol also shared the ability to regulate MMP activity and/or expression in vascular wall cells pathophysiologically relevant in atherosclerosis. In particular, given the critical role played by MMP-9 in plaque instabilization, we examined the ability of resveratrol and hydroxytyrosol to reduce MMP-9 activity and expression in monocyte-derived macrophages. To this regard, human mononuclear phagocytes at various stages of differentiation provide an appropriate system to study mechanisms regulating

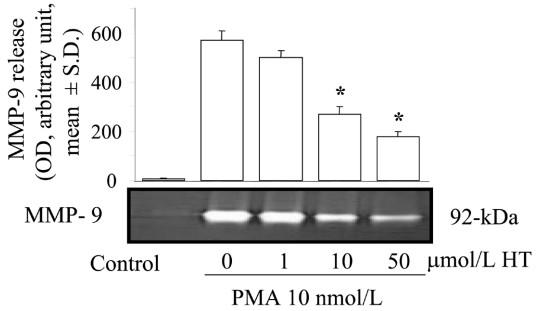

Figure 2. Effects of hydroxytyrosol on MMP-9 activity in monocytoid cells. Hydroxytyrosol (HT) inhibits the activity of MMP-9 induced by phorbol-myristate acetate (PMA) in U937 cells. HT (0 – 50 μmol/L) was added to U937 60 min before the addition of 10 nmol/L PMA for further 24 h, after which MMP-9 released in the conditioned medium was assessed by zymography. To determine the relative MMP-9 levels, MMP-9 bands detected as clear zones of gelatin lysis were scanned, and digitized images were submitted to densitometry analysis using the ScionImage software. Densitometric analysis of bands are reported as OD units in the upper panel. The results reported are representative of three different experiments. (**$p < 0.05$ *vs.* PMA) (unpublished data).

inflammatory protease production [82]. To this aim, U937 monocytoid cells, once stimulated with phorbol ester, highly express MMP-9, thus mimicking the differentiation of monocytes under pro-atherogenic conditions *in vivo*. Under such experimental conditions, monocytes pretreatment with hydroxytyrosol reduced MMP-9 activity, as assessed by gelatin zymography (Fig. 2), without inducing cellular toxicity (not shown). Thus, Mediterranean diet polyphenols, by preventing matrix degradation, might improve vascular stability, further contributing to the prevention of CAD manifestations.

3.6 Olive oil, olive oil components, and hypertension

Elevated blood pressure (BP) remains an extraordinarily common and important risk factor for both cardiovascular and renal diseases, including stroke, coronary heart disease, heart failure, and kidney failure. Of the environmental factors that affect BP (diet, physical inactivity, toxins, and psychosocial factors), dietary factors have a prominent, and likely predominant, role in BP homeostasis. Results of epidemiological studies and feeding trials indicated that olive oil (as reflected in the high ratio of monounsaturated-to-saturated fatty acids and a high content of phenolic compounds) could favorably affect BP control.

In a cross-sectional study of 2 282 residents of the Attica area in Greece (which surrounds and includes Athens), it was reported that adherence to a Mediterranean diet increases the likelihood of having the arterial BP under control [83]. In the Greek arm of the European Prospective Investigation into Cancer and Nutrition (EPIC) study, examining the effects of the Mediterranean diet, as an entity, and olive oil, in particular, on arterial BP, it was found that adherence to the Mediterranean diet is inversely associated with both systolic and diastolic BP, and olive oil intake *per se* may be important in the apparent beneficial effect of Mediterranean diets [84]. The prospective cohort Seguimiento Universidad de Navarra (SUN) study assessed the association of olive oil consumption with the incidence of hypertension. After a follow-up of 28.5 months, a lower risk of hypertension was observed among male participants with a higher olive oil consumption at baseline [85]. Moreover, a recent study showed that a moderate consumption of olive oil may be effective to reduce systolic BP in healthy men who do not typically consume a Mediterranean diet [86]. The molecular mechanisms and contribution of olive oil different components to BP lowering are not fully understood. A high intake of monounsaturated fatty acids, especially oleic acid [87 – 89], as well as a high intake of oleuropein [90] have been associated with a reduced risk of hypertension. A Spanish study compared two diets rich in monounsaturated fatty acids, from high-oleic acid sunflower oil and olive oil, for a period of 4 weeks in hypertensive women. Despite both vegetable oils provided a similar concentration of monounsaturated fatty acids, only olive oil consumption resulted in an increase in n-3 fatty acids and a decrease in the n-6/n-3 fatty acids ratio in the erythrocyte membranes and, importantly, also resulted in a significant reduction in systolic and diastolic BP [91]. A small controlled clinical trial compared the effects of a diet rich in oleic acid (from extra-virgin olive oil) with another oil rich in polyunsaturated fatty acid (sunflower oil) in pharmacologically treated hypertensive patients [92]. After 6 months, systolic and diastolic BP were significantly lower at the end of the olive oil diet compared with the sunflower oil diet. In addition, the olive oil diet, but not the sunflower oil diet, significantly decreased the required daily dosage of antihypertensive drugs [92]. The mechanism behind such BP reductions by olive oil are so far unclear, since the effects on lipid profiles were similar for both diets and independent of possible confounding variables investigated (*i.e.* potassium levels). Further support to the anti-hypertensive effect of olive oil however also derives from animal studies. In spontaneously hypertensive and Wistar-Kyoto rats, olive oil feeding significantly reduced the maximum contraction of aortic ring preparations compared with high-oleic acid sunflower oil feeding, and enhanced the relaxant responses to acetylcholine [93].

Overall, human and experimental data therefore suggest that the effects of olive oil on hypertension cannot be attributed exclusively to the content of oleic acid. Other components, such as antioxidant polyphenols, not present in sunflower oil, may be responsible for the vascular protective effect of olive oil consumption. The ability of antioxidants to prevent LDL oxidation and to enhance NO bioavailabil-

ity may explain the observed reduction of BP [94]. Ferrara *et al.* [92] observed that 15 – 20 mg/day of polyphenols obtained from olive oil are an amount similar to the total flavonoid intake associated with a lower incidence of CAD in the Zutphen Elderly Study [95].

3.7 Olive oil, olive oil components, and thrombosis

Other putative mechanisms involved in the beneficial effects of olive oil on cardiovascular system are protective changes against thrombogenesis, thus reducing the risk of acute thrombotic cardiovascular events. Olive oil has been shown to decrease levels of some blood coagulation factors in healthy men [96, 97]. Consumption of a Mediterranean-type diet rich in mono-unsaturated fatty acids decreases plasma levels of endothelial products, such as von Willebrand Factor and Tissue Factor Pathway Inhibitor, and also favorably affects Plasminogen Activator Inhibitor-1 in young healthy males [96]. Factor VII levels were also lower after olive oil consumption when compared with consumption of rapeseed and sunflower oils [97]. Rapeseed and olive oil have very similar oleic acid content, thus again suggesting that non-fatty acid-related effect may be responsible for this anti-thrombotic action.

In *in vitro* studies, polyphenolic components of olive oil not only inhibited platelet aggregation and thromboxane production [98], but also leukotriene B_4 generation by an action at the 5-lipoxygenase level in rat peritoneal leukocytes [99].

Finally, recent evidence in mildly dyslipidemic patients given either extra-virgin phenol-rich olive oil or refined phenol-poor olive oil found a marked decrease in serum thromboxane B_2 production in the extra-virgin olive oil group, thus confirming the anti-thrombotic potential suggested for extra-virgin olive oil polyphenols in *in vitro* studies [100].

4 Conclusions

Compelling evidence from *in vitro*, experimental animal and humans studies demonstrate that olive oil, the main fat source of the traditional Mediterranean diets, can be regarded as a healthy food for its anti-inflammatory, anti-thrombotic, anti-atherogenic and anti-hypertensive properties, warranting its designation as a functional food. In this respect, converging evidence indicates that the protective effects of olive oil can be ascribed not only to its high content of oleic acid, but also to its content in polyphenolic compounds, absent in seed oil. Olive oil polyphenols are absorbed and metabolized in humans [71]. The consumption of olive oils rich in polyphenols, in the typical amount of Mediterranean typical dishes (about 25 mL), yields an amount of polyphenolic compounds providing peak plasma concentrations of hydroxytyrosol in the micromolar range, which has been found to be biologically active in most studies.

Cardiovascular prevention and treatment strategies should consider this simple, direct and inexpensive dietary approach as a first-line approach to the burgeoning burden of cardiovascular diseases, alone or in combination with pharmacologic tools.

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