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REVIEWS: CURRENT TOPICS

The role of virgin olive oil components in the modulation of endothelial function

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Abstract

The endothelium is involved in many of the processes related to the development of atherosclerosis, which is considered an inflammatory disease. Actually, traditional risk factors for atherosclerosis predispose to endothelial dysfunction, which is manifested as an increase in the expression of specific cytokines and adhesion molecules. There are firm evidence supporting the beneficial effects of olive oil, the most genuine component of the Mediterranean diet. Although the effects of olive oil and other oleic acid-rich dietary oils on atherosclerosis and plasma lipids are well known, the roles of minor components have been less investigated. Minor components constitute only 1–2% of virgin olive oil (VOO) and are composed of hydrocarbons, polyphenols, tocopherols, sterols, triterpenoids and other components usually found in traces. Despite their low concentration, non-fatty acid constituents may be of importance because studies comparing monounsaturated dietary oils have reported different effects on cardiovascular disease. Most of these compounds have demonstrated antioxidant, anti-inflammatory and hypolipidemic properties. In this review, we summarize current knowledge on the effects of these compounds contained in VOO on vascular dysfunction and the mechanisms by which they modulate endothelial activity. Such mechanisms involve the release of nitric oxide, eicosanoids (prostaglandins and leukotrienes) and adhesion molecules, in most cases by activation of nuclear factor κB by reactive oxygen species.

Keywords: Adhesion molecules; Endothelium; Nitric oxide; Reactive oxygen species; Virgin olive oil

1. Endothelial dysfunction in cardiovascular disease

1.1. Nature of the atherosclerotic process

Atherosclerosis remains to be the leading cause of death in developed countries and is on track to become the most common cause of disease-related disability and death by 2020 [1,2]. Although several theories have been proposed for the pathogenesis of atherosclerosis, the current trend is to consider it as a response of the vascular wall to a variety of agents and mechanisms that contribute to the development of the atheromatous plaque [3]. The vascular endothelium is an active and dynamic monolayer of cells that serves as a semipermeable barrier between blood and tissue [4]. Because of this strategic location, it is involved in maintaining homeostasis by sensing changes in hemodynamic forces and signals and responding to them by releasing bioactive substances [5,6].

Traditional risk factors for atherosclerosis, which is considered an inflammatory disease, predispose to endothelial dysfunction and activation of the endothelium [7]. This activation is manifested as an increase in the expression of specific cytokines and adhesion molecules such as intracellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM-1) and E-selectin [8,9]. Circulating monocytes are attracted by these molecules and adhered to the endothelium, from which they transmigrate to the subendothelial space. Once within the endothelium, monocytes differentiate into macrophages, which scavenge oxidized LDL and triglyceride (TG)-rich lipoproteins (TRLs), becoming foam cells and contributing to the formation of the atheroma in the early stages of the atherosclerotic process [7].

1.2. Function of endothelial cells

The endothelium has a major function in thrombotic and coagulant activities, synthesizing several molecules that are released in response to different stimuli [10]. Heparan

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sulfate, nitric oxide (NO) and prostacyclin are vasodilators, whereas thromboxane A_2, prostaglandin H_2 and endothelin 1 are vasoconstrictors [11,12]. NO is the primary endothelial-derived relaxing factor playing a pivotal role in vascular reactivity [13]. It inhibits platelet aggregation [13], alters cell adhesion molecule expression and inhibits proliferation of smooth muscle cells [14]. It is generated in the endothelial cell by NO synthase (eNOS), which converts the amino acid L-arginine to NO and L-citrulline. It diffuses from the endothelial cell to the vascular smooth muscle and increases cyclic guanosine monophosphate, thereby causing relaxation of smooth muscle and dilation of the artery.

Endothelial dysfunction occurs early in the development of atherosclerosis, even before the formation of the plaque [15]. Clinical studies have revealed that risk factors for atherosclerosis, such as smoking, hypercholesterolemia, hypertension, diabetes and hyperhomocystinemia, predispose to endothelial dysfunction [7]. When dysfunctional, the endothelium increases flow disturbances due to improper vasoreactivity. Most importantly, it initiates inflammatory responses by releasing pro-inflammatory cytokines and chemokines. Thus, it increases leukocyte activation, promotes monocyte adhesion molecule expression and facilitates entry of monocytes and lipoproteins into the subendothelial space [16].

1.3. Measurement of endothelial function

Reliable assessment of endothelial function in humans seems to be of major importance and can be achieved by different approaches. The determination of soluble endothelial markers in blood is the most common approach and will be discussed subsequently in this review. However, direct measurement of endothelial function would be desirable. Vasodilation can be measured after intra-arterial pharmacologic stimulation with substances that enhance NO release but is highly invasive. The most promising noninvasive method is the measurement of the flow-mediated dilation (FMD) in the brachial artery by Doppler ultrasonography. This technique is based on the endothelium sensitivity to shear stress, which elicits NO release and dilation of underlying smooth muscle [17]. Shear stress is caused by hyperemia (induced by cuff inflation and then deflation), and FMD appears to be mediated mostly by NO (70%) and prostacyclin (30%) in such a condition [17,18]. Thus, FMD may also serve as an index of NO bioavailability [19]. The endothelial signaling cascade responsible for concerting mechanic stimuli into the release of vasodilatory molecules is not fully known. Although some mechanisms have been suggested, it is probable that it involves phosphorylation of a serine residue of eNOS by shear stress, altering its sensitivity to intracellular calcium levels and hence increasing NO formation [20].

There are ample evidence that FMD is diminished in patients with atherosclerosis and coronary risk factors and that it can detect endothelial dysfunction in hyperlipidemia, hypertension and diabetes [21–23]. FMD has been shown to be decreased after a single high-fat meal and postprandial lipemia [24]; however, there are very scarce data on the effect of olive oil. Vogel et al. [25] found a decrease in FMD after olive oil-rich meals as compared with canola oil and salmon, which was attributed to oxidative stress because the decrease in FMD was reduced by theconcomitant administration of vitamins C and E. However, these results should be confirmed with further investigation.

2. Factors that influence endothelial function

2.1. Reactive oxygen species impair the activity of NO

There are ample evidence indicating that increased vascular free oxygen levels are the prime mechanism for endothelial dysfunction [6]. Reactive oxygen species (ROS) may be produced during normal metabolism or after oxidative processes and include superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) [26]. The contributions of NO and ROS to the vascular tone are inversely proportional to each other and the appearance of one could likely compensate for the absence of the other [27]. The formation of ROS is balanced by a range of antioxidant defenses, but the excess, as in several cardiovascular risk factors such as hypercholesterolemia, hypertriglyceridemia and high postprandial triglyceridemia, can overwhelm these systems, leading to oxidative stress.

2.1.1. Effect of hypercholesterolemia on endothelial dysfunction

Hypercholesterolemia [28,29], diabetes mellitus [30] and hypertension [31,32] are related to increased production of O_2^-. This anion reacts rapidly with NO to form peroxynitrite ($OONO^-$), thus inactivating NO and leading to endothelial dysfunction. ROS can also react with polyunsaturated fatty acids (PUFAs) contained in lipoproteins in the vessel wall, initiating lipid peroxidation. The hydroperoxides formed in this process can in turn react with NO to form $OONO^-$, inactivating NO, and directly decrease the endothelial synthesis of NO [33]. In addition, altered LDLs are not recognized by LDL receptors, which are saturable, and instead are taken up by macrophages through scavenger receptors, which are not saturable, eventually becoming foam cells. Antioxidants constitute a diverse group of compounds, among whose effects include inhibition of LDL oxidation by both reductions in the concentration and reactivity of oxidants and improved resistance of the particle to them [34]. The antioxidants that have received the most attention are vitamin C (ascorbic acid), vitamin E (α-tocopherol) and phenolic compounds. In contrast to LDL, HDL provides an atheroprotective effect by inhibiting cytokine-induced endothelial cell adhesion molecule expression [35] and by enhancing agonist-induced vasodilation in coronary arteries [36].

2.1.2. Effect of hypertriglyceridemia on endothelial dysfunction

There are evidence that hypertriglyceridemia-induced endothelial dysfunction plays a critical role in this pathology. Traditionally, fasting TG concentrations have not been considered as an indicator of coronary artery disease (CAD). The metabolic relation between TRL and HDL, the heterogeneity of TRL and the imprecision by which serum TGs are determined contribute to the erosion of the relationship between TGs and CAD [37,38]. However, the serum TG concentration is often more strongly correlated with future CAD incidence in multivariate analysis than is serum cholesterol [39]. Considering this evidence and that TG metabolism also determines the fate of some other lipoproteins, such as LDL and HDL [40], TGs seem to have a central role in the pathogenesis of atherosclerosis.

Disorders in TG metabolism may promote atherogenesis by increasing the expression of vascular cell adhesion molecules. The concentrations of E-selectin, ICAM-1 and VCAM-1 are increased in the serum of patients with hypertriglyceridemia, independently of other risk factors [41].

2.1.3. Effect of postprandial triglyceridemia on endothelial dysfunction

Postprandial triglyceridemia is also a good predictor of the presence and progression of atherosclerosis [42]. TRLs, comprising TGs contained in chylomicrons (CMs) and VLDL, as well as their remnants, can cross the endothelial barrier and enter the arterial wall [43] and be taken up by macrophages without need for further oxidation of the particles [44]. It has been suggested that the entry of lipoproteins into the arterial wall is inversely related to the size of the particles; for this reason, it was thought for many years that TRLs were not atherogenic because they are too large to penetrate the tissue [45,46]. However, the TRL remnants formed after the hydrolytic activity of lipoprotein lipase (LPL) and endothelial lipase (EL) can enter the arterial intima and thus be more atherogenic than their nascent precursors [47].

In vitro studies on endothelial cells reported that LPL-derived TRL remnants isolated from hypertriglyceridemic subjects have the ability of disrupting endothelial integrity [48,49]. It has been shown that remnant-like lipoprotein particles have a causative role in endothelial vasomotor dysfunction in human coronary arteries and that they directly induce endothelial dysfunction in the isolated rabbit aorta [50,51]. In fact, TRLs and their remnants stimulate endothelial cell plasminogen activator inhibitor-1 production [52], which is a marker of endothelial dysfunction, and cause a profound depression of fibrinolytic activity [53]. TRL remnants also promote an enhanced thrombogenic tendency by increasing circulating factor VII levels [54].

In addition, it has been reported that monocyte adhesion to endothelial surface is enhanced by TRLs [55,56]. CM preparations obtained from human plasma 4 h after a standard fat-containing meal were shown to up-regulate the expression of E-selectin and VCAM-1. Interestingly, TRLs did not require LPL to promote the expression of adhesion molecules, suggesting that these particles may be pro-inflammatory themselves independently of their ability to release fatty acids [57]. Doi et al. [56] confirmed the up-regulation of protein and the mRNA expression of these adhesion molecules when endothelial cells were incubated with TRLs obtained from hypertriglyceridemic patients and extended it to ICAM-1 and tissue factor.

The oxidative mechanism can at least partially explain the adverse effects of TRLs on endothelial cell function. TRL accumulation in plasma leads to increased oxidative stress and decreased NO availability as it is associated with an increase in vascular O_2^- production [58]. In their study, Doi et al. [56] observed increased cellular oxidant levels in the endothelial cell incubation medium containing TRLs and that coincubation with α-tocopherol decreased the concentrations of ICAM-1 and VCAM-1.

Hydrolytic enzymes such as LPL and EL may be directly implicated in endothelial dysfunction as their synthesis is up-regulated in early atherosclerosis [59]. Apart from smooth muscle cells, LPL is produced by monocytes and macrophages [60–63]. There are evidence that LPL activity can enhance the retention of LDL and VLDL to the arterial wall [64,65] but that it also facilitates proteoglycan-mediated monocyte adhesion to the endothelium [66]. Actually, inflammatory cytokines such as TNF-α and interleukin (IL)-1β can up-regulate endothelial-derived LPL mRNA [67]. In contrast to LPL, EL is synthesized in the endothelium and has a primary phospholipase activity [68]. However, its mRNA expression is also increased in endothelial cells by TNF-α and IL-1β [67]. The up-regulation of these enzymes is thought to be mediated via the nuclear factor κB (NFκB) pathway [69]. NFκB can be activated by a number of stimuli, including lipids and ROS. These stimuli cause the phosphorylation of IκB and the subsequent proteolytic degradation of this inhibitor subunit, allowing NFκB to translocate into the nucleus, where it binds to recognition sequences in DNA to induce gene expression [70].

2.2. Beneficial and adverse effects of dietary fatty acids

It has been postulated that as energy needs are increased during acute inflammation, lipolytic enzymes are up-regulated as a way of generating free fatty acids from circulating lipoproteins to be used by the tissues, including the endothelium. The increase in free fatty acid concentrations in the endothelium has been shown to decrease endothelial NO bioactivity due to both superoxide generation and reduction in eNOS activity [71,72]. In vitro studies suggest that PUFAs are more pro-inflammatory than monounsaturated FAs (MUFAs) and saturated FAs (SFAs) [73]. In fact, linoleic acid (18:2, n-6) has greater capacity to induce oxidative and inflammatory stress than other fatty acids. Incubation of this fatty acid with endothelial

cells promotes NFκB activation and transcriptional activity, this effect being attenuated by vitamin E [74]. In addition, exposure of endothelial cells to linoleic acid can lead to production of cytokines such as IL-6 and IL-8 [75], which are involved in the initiation and progression of atherosclerosis [76,77].

Conversely, n-3 PUFAs are believed to exert an endothelial protective effect. Particularly, docosahexaenoic acid (DHA; 22:6, n-3) decreases expression of VCAM-1 on the vascular endothelium and monocyte adhesion [78–80] and eicosapentaenoic acid (EPA; 20:4, n-3) increases NO production. Although these results from in vitro studies are promising, in vivo studies are more controversial. Abe et al. [41] found no reduction in soluble adhesion molecules after 6 weeks in patients receiving n-3 fatty acids but did find reductions in ICAM-1 and E-selectin after 7 months. Seljeflot et al. [81] supplemented male smokers with n-3 fatty acids for 6 weeks, finding reductions in prothrombogenic von Willebrand factor but increases in VCAM-1 and E-selectin. Their results were corroborated by Johansen et al. [82].

Among the key inflammatory mediators released by the endothelium are the eicosanoids derived from the n-6 PUFA arachidonic acid (AA; 20:4, n-6). Prostaglandin E_2 (PGE_2) can cause pain and vasodilation and leukotriene B_4 (LTB_4) is a chemoattractant and activator of neutrophils. PGE_2 and LTB_4 are formed from AA via the cyclooxygenase (COX) and 5-lipooxygenase (LOX) pathways, respectively. However, EPA is also a potential COX substrate and can compete with AA for this enzyme, leading to formation of PGE_3, which is synthesized with very low efficiency [83]. EPA is also a substrate for LOX, forming LTB_5, with less inflammatory activity as compared with LTB_4 [84,85]. Thus, by increasing the n-3 content in the diet, the balance of the eicosanoids produced can be shifted to a less inflammatory mixture.

3. Evidence of the benefits of olive oil on cardiovascular disease

A number of epidemiologic studies developed in different countries constitute a firm and reliable experimental base supporting the beneficial effects of the Mediterranean diet, rich in olive oil, with regard to the reduction of CAD [86].

The Seven Countries Study, initiated by Keys et al. [87] in 1970, was designed to investigate relationships between diet and CAD by comparing different populations. The results of that study showed that the population of the Mediterranean island of Crete had the lowest rates of CAD and cancer, concluding that the cause might be the low-saturated fat and high-oleic acid intake, in terms of olive oil, of the Mediterranean diet. Subsequently, the Lyon Diet Heart Study [88], developed on patients recovering from a myocardial infarction, was the first clinical evidence in support of the health benefits of a Mediterranean-style diet, similar to that of Crete. The protective effects were ascribed to higher intakes of oleic acid and α-linolenic acid (18:3, n-3) and lower intakes of SFAs and linoleic acid (18:2, n-6). Trichopoulou et al. [89] demonstrated that adherence to a Mediterranean diet reduced mortality from cancer and coronary heart disease (CHD) in a Greek population of more than 22,000 individuals. The results were more recently confirmed in subjects diagnosed with CHD [90].

Cumulative evidence suggests that MUFAs may be key components in the protective role of the Mediterranean diet [91]. In fact, MUFAs are believed to be as effective as n-6 PUFAs in lowering total and LDL-cholesterol when replacing SFAs, as supported by two meta-analyses [92,93]. In addition, it has been reported that a controlled olive oil-based diet can even lower plasma TG levels [94], although there are some controversies in this regard [95–98].

It is probable that arterial hypertension resulted to be *quantitatively* the most important risk factor for CHD due to its repercussion on cardiovascular mortality [99]. Diets enriched with n-3 PUFAs are known to reduce blood pressure in humans [100,101] and in spontaneously hypertensive rats (SHRs) [102], which has been related to increased synthesis of series n-3 eicosanoids, with a stronger vasodilator effect than their series n-6 homologous [103]. However, there are increasing evidence showing that olive oil reduces systolic and diastolic blood pressure in normotensive [104,105] and hypertensive [106] individuals. Recently, another study including participants who had never received a diagnosis of hypertension confirmed these results. It concluded that the Mediterranean diet is inversely associated with arterial blood pressure and that olive oil intake per se is inversely associated with both systolic and diastolic blood pressure [107]. The modification in blood pressure by dietary olive oil has been related to changes in the fatty acid composition of the cell membrane, which affects its functionality [106,108].

Oxidative modification of LDL is an important determinant in the development of atherosclerosis as it accelerates the uptake of LDL by macrophages, which is the beginning of the formation of a fatty streak. LDL may be protected against attacks of free radicals by antioxidants in plasma and in the particle itself. Lipoproteins rich in MUFAs after long-term consumption of olive oil have been shown to be less susceptible to oxidation as compared with particles enriched with PUFAs [109–113].

An increasing number of studies point out that the content of oleic acid alone cannot fully explain the impact on health of olive oil. This conclusion has been drawn from studies comparing the effects of diets enriched with different monounsaturated oils [114], among which virgin olive oil (VOO) and high-oleic sunflower oil (HOSO) have been most widely used.

Unlike VOO, HOSO is not able to reduce blood pressure in hypertensive patients [106]. Further studies on humans

Table 1
Minor component composition of VOO

Subfraction	Component	Concentration (mg/kg)
Unsaponifiable		
Hydrocarbons	Squalene	200–7500
	β-Carotene	0.3–0.7
	Polycyclic aromatic hydrocarbons	Traces
Sterols	β-Sitosterol	1800–2600
	Campesterol	<4.0% of total sterols
	Δ7-Stigmasterol	<0.5% of total sterols
	Brassicasterol	<0.1% of total sterols
Terpenic dialcohols	Erythrodiol + uvaol	6–10+18
Tocopherols	α-Tocopherol	60–200
	β+γ-Tocopherol	3% of total tocopherols
	Δ-Tocopherol	<2% of total tocopherols
Phenolic compounds	Tyrosol	
	Hydroxytyrosol	
	Caffeic acid	50–800 (total phenols)
	Oleuropein	
Others	Flavor components	Traces

revealed that this differential effect was related to the composition and functionality of the cell membrane of hypertensive patients [106,115]. In SHRs, VOO and HOSO yield different liver, adipose tissue and myocardium lipid compositions [116–118] and vascular reactivities [119]. These differences may be a consequence of a differential incorporation of the components of VOO and HOSO into TRLs [120–122]. Actually, postprandial lipoproteins obtained after ingestion of VOO or HOSO are differentially incorporated into vascular cells [123], from which release of eicosanoid substances is also affected [124]. Furthermore, it has been reported that LDLs from olive oil-fed rats were more resistant to oxidation in vitro than those isolated from the plasma of triolein-fed rats [125]. The differential effects of different oleic acid-rich oils on LDL oxidation were confirmed in 10 normolipidemic subjects by Nicolaiew et al. [126] after the administration of VOO or HOSO. These data support the idea that the protective effects of olive oil against CAD must be attributed not only to oleic acid but also to other components of the oil.

Among the differential characteristics of VOO, the quantitatively most important is the TG molecular species composition. Compared with HOSO, which contains mainly triolein, VOO contains also important amounts of dioleoyl–palmitoyl–glycerol. In much lower concentrations, but with growing evidence of important biologic effects, minor components of olive oil can be also used to differentiate this oil from others (Table 1).

4. Chemical composition of olive oil

In contrast to most dietary oils that are obtained from the seeds of plants by means of solvent extraction and refined before being edible, olive oil is obtained from the whole fruit of *Olea europaea* L. only by physical pressure. This procedure makes olive oil unique because some compounds that cannot be found in other dietary oils are transferred from the leaves and skin of the fruit to the oil.

Olive oil can be classified into two fractions from a quantitative point of view. The major fraction constitutes 98–99% of the oil and is mainly composed of saponifiable glyceridic compounds as TGs. The abundance of oleic acid is peculiar to olive oil and ranges from 60% to 84% of total fatty acids in TGs, whereas linoleic acid, the major essential fatty acid and the most abundant polyunsaturated acid in our diet, is present in concentrations between 3% and 21%.

Minor compounds account for 1–2%, comprising unsaponifiable compounds, phenolics and waxes; in addition, despite the fact that they are of limited proportion, they confer important biologic activities. The minor compounds of VOO, classified in growing order of polarity after being developed by thin-layer chromatography, are hydrocarbons, tocopherols, fatty alcohols, 4-methylesterols, sterols, triterpenic dialcohols, polar-colored pigments and phenolic compounds [127].

One of the greatest differences between VOO and the rest of edible oils is the composition in hydrocarbons [128]. Among them, the most important is squalene [129], a polyunsaturated triterpene that appears at high concentration, makes up 60–75% of the unsaponifiable fraction of the olive oil [130] and is a precursor in the biosynthesis of cholesterol and steroid hormones. β-Carotene is also found within this group, a triterpenic polyunsaturated hydrocarbon that plays an important role as precursor of vitamin A and, along with lycopene, confers the yellowish color to the oil. The analysis of the sterol fraction is of importance because it helps in the characterization of the species from which the oil had been extracted [131]. The main sterol found in VOO is β-sitosterol [95%], but campesterol, Δ7-stigmastenol, -stigmasterol, -spinasterol and -avenasterol are also present. VOO contains α-, β-, γ- and Δ-tocopherols, but α-tocopherol typically accounts for more than 85% of the total tocopherols. Triterpenic dialcohols and acids, from the skin of the fruit and from the leaves, are incorporated in second pressing oil; the final concentration is higher than that in VOO. The main dialcohol is known as erythrodiol, which in some cases is accompanied by another triterpenic–tetracyclic diol identified as uvaol. Among acids, oleanolic and maslinic have recently revealed some pharmacologic properties, which will be discussed subsequently.

Phenolic compounds are rarely determined in routine analysis because of their solubility in water and diluted bleach, which explains their absence from both unsaponifiable and glyceridic fractions. These substances constitute the polar fraction in VOO and underlie its exceptional thermal stability [132–134], contribute to its characteristic flavor and taste and prevent its auto-oxidation, contributing to the resistance of VOO to oxidative rancidity [135]. Phenolic compounds have emerged as potent antioxidants present in VOO. Among these compounds, oleuropein itself and its derivatives, tyrosol and hydroxytyrosol, have been reported to have a protective role against LDL oxidation in

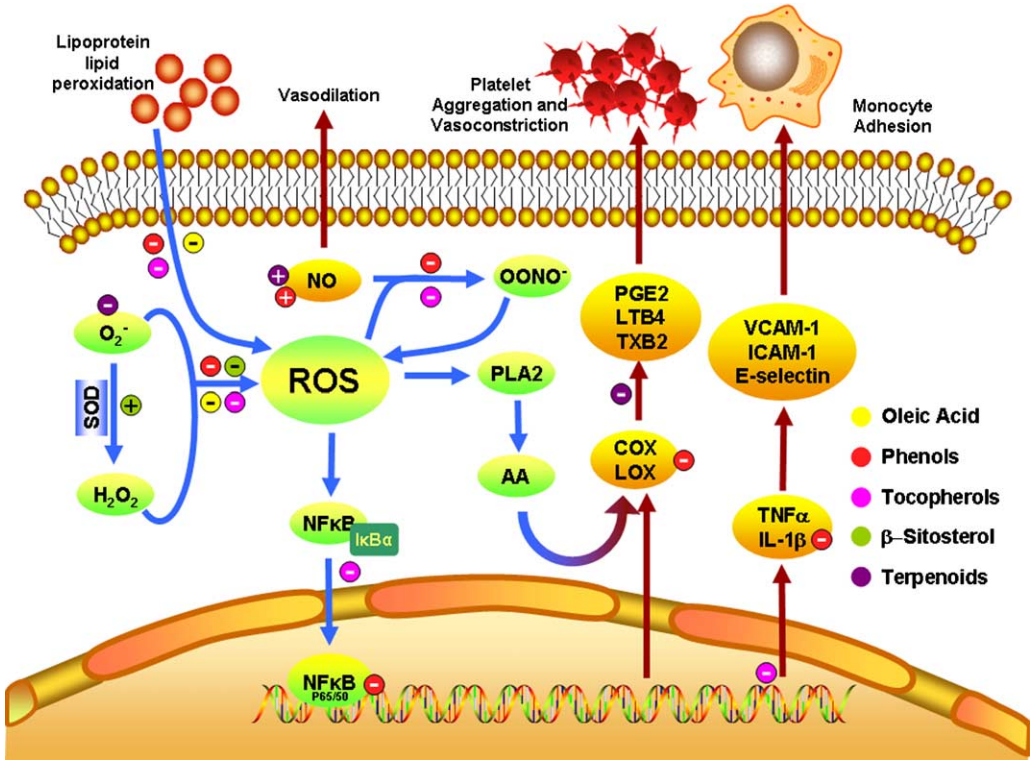

Fig. 1. Proposed model for the action mechanisms of oleic acid and minor compounds from olive oil based on the literature gathered for the present review. Despite the number of studies contributing to this model, several gaps that should be filled with further investigation are still present. The main mechanism by which the components of olive oil influence endothelial activation involves inhibition and/or scavenging of ROS. Oleic acid and β-sitosterol may reduce intracellular ROS by creating a less-oxidant environment through inhibition of intracellular ROS production. β-Sitosterol may also enhance SOD activity, hence decreasing O_2^- levels. This reduction has also been observed for the terpenoid oleanolic acid, although the mechanism is not presently known. Tocopherols and phenolic compounds are potent antioxidants that may help reduce lipid peroxidation and scavenge intracellular ROS and free NO, reducing the formation of $OONO^-$. ROS can activate the NFκB, which is then translocated into the nucleus, where it binds to recognition sequences in DNA to induce gene expression. This mobilization of NFκB is blocked by α-tocopheryl succinate but not by α-tocopherol. In contrast, phenolic compounds have been proposed to act blocking the formation of NFκB/DNA binding complexes. NFκB modulates the expression of cytokines, LOX and COX, thereby affecting the levels of adhesion molecules and eicosanoids. However, some of the minor compounds of olive oil may act directly on these enzymes and cytokines. LOX and COX activities are inhibited at different points by phenolics and triterpenoids whereas IL-1β expression is inhibited by phenolics and tocopherols, contributing to protect the endothelium against vasoconstriction, platelet aggregation and monocyte adhesion. Vasodilation is also suggested to be enhanced by oleuropein and oleanolic acid through an increase in the production of NO.

vitro, equivalent to vitamin E [136]. Although it has been demonstrated that VOO phenolics are dose dependently absorbed in humans [137], they are mostly present as conjugates of glucuronic acid in plasma. The in vivo antioxidant effect of VOO phenolics and the antioxidant capacity of the glucuronidates are scarce and controversial. However, interventional studies administering increasing doses of phenolic compounds in VOO have reported reduced oxidative statuses in healthy [138,139] and dyslipidemic [140] subjects.

5. Effects of the components of olive oil on endothelial function

Very few studies have addressed the effects of long-term olive oil consumption on endothelial function. Mediterranean diet, rich in olive oil, has been shown to improve endothelial function in diabetic [141] and hypercholesterolemic [142] patients, as assessed by measuring endothelium-dependent vasoreactivity. Fuentes et al. [142] also observed a reduction in the plasma levels of P-selectin. In a randomized crossover trial, Ros et al. [143] confirmed an improvement on endothelial function in 22 hypercholesterolemic subjects receiving a Mediterranean diet. However, the results were more evident when part of the dietary olive oil was replaced with walnuts. Sondergaard et al. [144] observed a greater improvement in FMD in patients receiving fluvastatin when they were advised of following a Mediterranean diet. Esposito et al. [145] carried out a randomized trial among 180 subjects with metabolic syndrome who were instructed to follow a Mediterranean-style diet, including olive oil. After 2 years of follow-up, they observed improved endothelial function as a measure of blood pressure and platelet aggregation response to L-arginine, the natural precursor of NO. They also reported a significant reduction of markers of systemic vascular inflammation, such as C-reactive protein and IL-6, IL-7 and 18 IL-18, apart from total and LDL-cholesterol. However,

the mechanisms by which dietary olive oil elicits these effects and that are the actual components of the oil responsible for the effects are poorly elucidated. Below, we show the current knowledge on the effects of the components of olive oil on endothelial activation. A graphic summary of the mechanisms is shown in Fig. 1.

5.1. Effects of major components of olive oil on endothelial function

5.1.1. Effects of oleic acid

Oleic acid, the main fatty acid contained in olive oil, accounts for approximately 29% of the daily caloric intake in some Mediterranean countries. Recent studies testing the role of different UFAs in endothelial cell activation and injury suggest that oleic acid does not activate endothelial cells, confirming its benefits on early events in atherosclerosis as compared with other UFAs [146]. Carluccio et al. [147] reported that the plasma concentrations of oleate under conditions of high olive oil consumption are likely to be fully in the range of concentrations exerting biologic effects in our system, likely between 10 and 100 μmol/L.

Tsimikas et al. [148] assessed the pro-inflammatory potential of LDL isolated from Greek subjects consuming a diet naturally rich in olive oil. Oleic acid content in LDL was significantly higher in Greeks as compared with Americans and was inversely correlated with the extent of in vitro LDL oxidation and the induction of monocyte adhesion by mildly oxidized LDLs. Native LDLs were also isolated from American subjects after consumption of a liquid diet supplemented with either oleic or linoleic acid to confirm that dietary fatty acids influence the pro-inflammatory properties of mildly oxidized LDLs. The oleic acid-supplemented group had higher oleic and lower linoleic acid content in LDLs as compared with the linoleate-supplemented group. When exposed to oxidative stress, the LDLs enriched with oleic acid promoted less monocyte chemotaxis and reduced monocyte adhesion, showing a strong negative correlation between oleic acid LDL content and monocyte adhesion. This study demonstrated that the level of dietary enrichment with oleic acid necessary to obtain these benefits is realistic and readily achieved by using diets currently in use in Mediterranean countries. Also, this study suggested that LDLs enriched with oleic acid and reduced in PUFAs may be less easily converted to pro-inflammatory, minimally modified LDLs having the ability to enhance monocyte chemotaxis and adhesion.

The main challenge after these results is to discover which are the underlying mechanisms implicated in oleic acid beneficial effects. Initially, Tsimikas et al. [148] explained the mechanism by which oleic acid-enriched diets decreased lipoprotein susceptibility to oxidation, presumably, as a result of the decreased linoleic acid content within lipoproteins. However, previous experiments with liposomes progressively enriched with oleic acid but with constant amounts of linoleic acid showed that particles with higher oleic acid concentrations were less susceptible to oxidation and that monocyte chemotaxis and adhesion were nearly completely inhibited when exposed to mild oxidative stress, suggesting that oleic acid may have an additional independent mechanism of action [149].

Studies on endothelial cells in vitro have shown that the main dietary PUFAs and oleic acid may prevent endothelium activation either by inhibiting the expression of adhesion molecules or by improving NO production [150]. Supplementation of endothelial cells with oleic acid in vitro reduces endothelial cell sensitivity to oxidants, creating a reduced pro-oxidant environment as a consequence of reduced intracellular ROS [151,152]. In this environment, oleate reduces the activation and mRNA expression of NFκB and AP-1, thereby interfering with the endothelial expression of adhesion molecules for circulating monocytes and contributing to a direct vascular atheroprotective effect [153–156]. In addition, cellular treatment with this fatty acid protects endothelial cells against cytokine-induced VCAM-1, ICAM-1 or E-selectin overexpression [147].

This condition might occur by two mechanisms: reduced enzymatic production of ROS or increased scavenging after their production. It has been suggested that the inhibitory potency of UFAs is directly proportional to their number of double bonds [153,156]. According to this, DHA, with six double bonds, would be six times more potent than oleic acid. However, addition of oleic acid to the culture medium significantly increases the unsaturation index [147], probably through selective displacement of SFAs, but not PUFAs, in cell membrane phospholipids, with a consequent modulation of gene expression for molecules involved in monocyte recruitment [153]. Actually, the kinetics of VCAM-1 inhibition by oleic acid resembles that of DHA. Similar to what has been previously shown for DHA, the effect is totally independent of the stimulus used: cytokines acting on totally different receptors, such as IL-1, TNF-α, IL-4 or LPS, and inducing different responses [78].

Oxidation of fatty acids might help in the scavenging of ROS, thus reducing the formation of superoxides that can be dismutated to H_2O_2, which is in turn responsible for NFκB activation [157,158]. Therefore, an alteration of H_2O_2 metabolism in vascular cells may contribute to the ability of fatty acids to modulate cellular oxidant susceptibility [159].

Experiments with porcine artery endothelial cells supplemented with oleic acid and exposed to oxidant conditions by means of H_2O_2 treatment showed an attenuated increase in intracellular H_2O_2 [160]. The consequence was an increase in resistance to derangements caused by oxidized LDL and a reduction in oxidant-mediated dysfunction [159,161]. Massaro et al. [162] showed that the incubation of oleate with cytokine-stimulated endothelial cells prevents the depletion of glutathione (GSH) and partially prevents stimuli-induced increase of intracellular ROS. This occurred

without any change in the activity of GSH-related antioxidant enzymes, superoxide dismutase (SOD) and catalase. These authors suggested that oleate may exert direct vascular atheroprotective effects by inhibiting endothelial activation through quenching of ROS. In contrast, incubation with linoleic and stearic acids reduces GSH levels and increases NFκB [146,155]. In addition, coincubation of linoleic acid with TNF-α doubled the production of IL-6 as compared with TNF-α alone. This was concomitant to an increment in the content of AA in membrane phosphatidylethanolamine.

In contrast with these results, other authors have suggested negative effects of circulating nonesterified fatty acids such as oleic acid in diabetic patients. Experiments with cultured rat aortic smooth cells maintained in media containing oleic acid concentrations similar to those in diabetic patients showed a significant increase in endothelin-1 receptor amount, suggesting that it may contribute to the acceleration of atherosclerosis in diabetic patients [163,164].

5.2. Effects of minor components of olive oil on endothelial function

5.2.1. Effects of olive oil phenolic compounds

The major phenolic compounds in olive oil — oleuropein, hydroxytyrosol and tyrosol — are strong antioxidants and radical scavengers [165] that can help revert the imbalance between increased oxidative stress and impaired antioxidant defense that affects endothelial function and therefore contributes to atherosclerotic disease progression.

Associations between oxidative stress and impaired endothelial function have been demonstrated in experimental animal models of atherosclerosis, hypertension, hypercholesterolemia and diabetes [28,166,167]. Reduced bioavailability of NO in a setting of increased O_2^- levels seems to be constant biologic changes that occur in the vessel wall under these conditions [168]. In fact, it has been described that endothelial vasomotor dysfunction could be reversed in these patients by the administration of agents capable of scavenging ROS [169–172].

A number of articles have reported in vitro experiments evaluating and confirming the antioxidant activities and the scavenging potencies of olive oil and its isolated constituents [173–177].

The low unsaturation of olive oil fatty acids, in addition to water-soluble antioxidant protection in the form of phenolic compounds, favorably influences a reduced susceptibility to oxidation of olive oil-derived lipoproteins [178]. Recently, it has been reported that an olive oil intake of 25 ml in a single dose does not promote exacerbated hypertriglyceridemia and hyperglycemia, which are linked to postprandial oxidative stress. These authors found a postprandial increase of both tyrosol and hydroxytyrosol, two phenolic compounds frequently used as markers of olive oil intake. The increased concentration of these phenols in plasma may be related to the reduced oxidative stress because their increase was concomitant to a postprandial decrease in plasma oxidized LDLs.

Oleuropein and hydroxytyrosol are potent scavengers of ROS and O_2^- in neutrophils [175,177]. Saija et al. [173] hypothesized that, whereas hydroxytyrosol can serve as a scavenger of aqueous peroxyl radicals near the membrane surface, oleuropein acts also as a scavenger of chain-propagating lipid peroxyl radicals within the membranes. In addition, oleuropein has been shown to increase NO production from LPS-stimulated mouse macrophages [179] and to possess a tonic effect toward the inducible form of NO synthase. Tuck and Hayball [165] reported that these two phenolic compounds, as well as caffeic acid, but not tyrosol, can scavenge free radical NO (NO) and $OONO^-$ in a concentration-dependent fashion. Altogether, these features of oleuropein and hydroxytyrosol might contribute to an increase in NO levels and prevent formation of the powerful oxidant peroxynitrite [180].

Besides the antioxidant properties of the phenolic compounds from extra VOO, anti-inflammatory effects have been demonstrated in several cell types. De la Puerta et al. [181] studied a range of VOO phenolics in rat peritoneal leukocytes, finding inhibition of LTB_4 production by oleuropein glycoside, caffeic acid and tyrosol. This had already been described in human platelets and leukocytes [182,183]. Hydroxytyrosol has also demonstrated inhibitory effects on LOX [183] in leukocytes, presumably by penetrating cell membranes [184]. The inhibition of LTB_4 production from AA may lead to reduced platelet aggregation [182,185].

It has been described that some phenolic compounds may inhibit cytokine and eicosanoid production by inhibiting IL-1β mRNA and protein expression and COX-2 activity and transcription [181,183,187], which may contribute to the antiatherogenic properties ascribed to extra VOO. Miles et al. [186] showed a very strong effect of oleuropein glycoside, but none of the phenolic compounds from olive oil studied was able to affect the production of IL-6 or TNF-α. However, these authors did not use oleuropein aglycone or hydroxytyrosol in their experiments.

Monocyte adhesion to endothelial cells can also be modulated by VOO phenolic compounds. Carluccio et al. [187] incubated oleuropein, hydroxytyrosol and tyrosol with LPS or cytokine-stimulated HUVECs and observed inhibition of the expression of VCAM-1, ICAM-1 and E-selectin and the adhesion of monocytes. The oleuropein aglycone and hydroxytyrosol were the most potent phenolic compounds, which is consistent with their higher antioxidant activity. In contrast to the study of Miles et al. [186], the oleuropein glycoside showed a low activity on adhesion molecule expression, which was attributed to its lower lipophilicity and consequent lower incorporation into membranes and interaction with lipids. mRNA expression for VCAM-1 was also affected, indicating a pretranscriptional action of the phenolic compounds. These

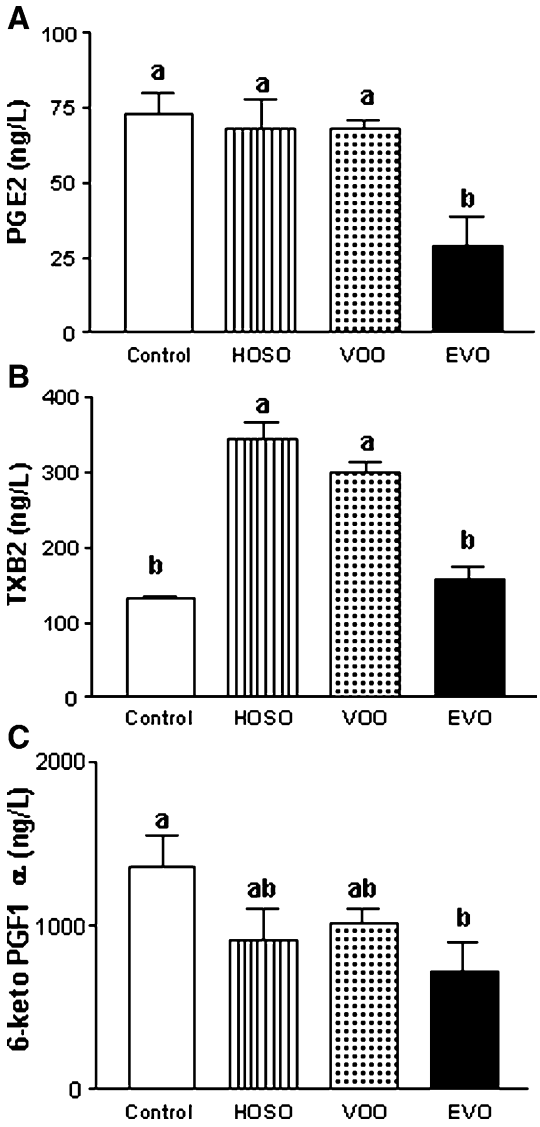

Fig. 2. Effect of the unsaponifiable fraction of olive oil in TRLs on eicosanoid production. HUVECs were incubated for 24 h with TRLs obtained 2 h after the ingestion of HOSO, VOO or EVO. Eicosanoids released to the medium were determined by EIAs. Results corresponding to PGE_2, TXB_2 and 6-keto-$PGF_{1\alpha}$ are shown in Panels A, B and C, respectively. a: $P<.05$ versus control; b: $P<.05$ versus HOSO; c: $P<.05$ versus VOO.

authors observed a repression of the transcription factors NFκB and AP-1, the interaction of which is known to amplify VCAM-1 promoter activation [188]. Very recently, Turner et al. [189] also reported increased VCAM-1 and ICAM-1 production by VOO phenolics (namely, oleuropein, hydroxytyrosol, tyrosol and homovanillic alcohol) but no influence on NO production or platelet aggregation. Activation of NFκB involves complex signal transduction pathways that ultimately result in the activation of a specific IκB kinase (IKK) and translocation of NFκB from the cytoplasm to the nucleus. In an attempt of elucidating the mechanisms by which phenolic compounds inhibit NFκB, Ma et al. [190] showed that these compounds do not inhibit activation of IKK activity, degradation of IκBα, a component of the cytoplasmic NFκB complex, or translocation of activated NFκB to the nucleus but do block the formation of NFκB–DNA binding complexes. These authors suggested that this blockade involves the antioxidant property of the phenolic compounds.

5.2.2. Effects of the unsaponifiable fraction

The unsaponifiable fraction of VOO is also rich in other minor components with antioxidant and anti-inflammatory properties, such as tocopherols, sterols and terpenic compounds [191–193].

The investigation of the effects of the unsaponifiable fraction of olive oil as a whole is almost an unexplored field. Ochoa et al. [194] investigated the influence of VOO and HOSO with different unsaponifiable fractions on the fatty acid composition and lipid peroxidation of LDLs in rabbits. The main outcomes of the study were the lower susceptibility of LDL oxidation and higher antioxidant content in the LDLs of animals fed the diet enriched with VOO, which were attributed to the higher phenolic content in this oil. Unfortunately, as stated above, the phenolic compounds of olive oil are not included in the unsaponifiable fraction.

We recently incubated endothelial cells with postprandial TRLs derived from the intake by healthy subjects of meals containing VOO, HOSO and VOO enriched with its unsaponifiable fraction [enriched VO (EVO)] to a final concentration of 2.4% [124]. The unsaponifiable fraction of VOO was richer in squalene, terpenic compounds and waxes (long-chain fatty alcohols), whereas HOSO presented a higher concentration of tocopherols. The total concentration in sterols was similar between VOO and HOSO but was almost double in EVO. We found a reduction in the production of PGE_2 and TXB_2 after incubation with EVO-TRL, compared with VOO and HOSO (Fig. 2), but no effect on NO production. These results suggest that minor components from VOO that are transported postprandially in TRLs may have favorable effects on endothelial function by improving the balance between vasoprotective and prothrombotic factors released by endothelial cells. Despite the beneficial effects attributed to phenolic compounds, it is very unlikely that they were responsible for the effects observed in this study because of the following: first, their concentrations in VOO and EVO were very similar; second, due to their hydrophilic nature, they are readily transported into plasma and not to TRLs; and third, they appear not to affect COX activity [181]. Therefore, we suggested that tocopherols, sterols or terpenoids might be responsible for the effects observed.

Vitamin E, comprising tocopherols, tocotrienols and some of their derivatives, has a protective role against the attacks of free radicals by acting as lipid-based radical chain-breaking molecules [195]. More recently, non-antioxidant functions of vitamin E have been proposed, in particular as a gene

regulator, which seems to be unrelated to its radical chain-breaking potential [196].

Apart from protecting LDLs from lipid peroxidation [197–200], α-tocopherol has an inhibitory effect on LDL- and cytokine-induced production and expression of adhesion molecules [201–203] and adhesion of monocytes to endothelial cells, probably by inhibition of ICAM-1 expression [202,204]. In cells stimulated with IL-1β, α-tocopherol reduces the up-regulation of ICAM-1, VCAM-1 [205] and E-selectin [204]; more importantly, it can also regulate the production of IL-1β by down-regulating its gene expression [206]. However, the effect on adhesion molecules seems to not be mediated by NFκB mobilization [157,204].

Vitamin E can also modulate eicosanoid metabolism in endothelial cells. Actually, PGI_2 synthesis is impaired in vitamin E-deficient mice [207,208] and can restore reduced PGI_2 synthesis in endothelial cells [209,210]. In addition, there are data indicating that α-tocopherol inhibits LOX [211] and COX-2 [212]. In a series of studies carried out by Meydani et al. [213–215], it was demonstrated that α-tocopherol can eliminate the increase in PGE_2, TXA_2 and TXB_2 by reducing the activity of COX in LPS-stimulated macrophages from aged mice. This effect was found not to be due to regulation of COX transcription or translation but to the effect of α-tocopherol scavenging hydroperoxides and NO, which leads to a lower production of $OONO^-$. There are data suggesting that peroxynitrites may modulate COX activation via Ca^{2+}-dependent phospholipase A_2 activity and AA release [216].

Finally, pretreatment of endothelial cells with vitamin E prevents alterations in the plasma membrane by H_2O_2 [217] and decreases oxidized LDL-mediated degradation of IκB and apoptosis [218].

However, not all tocopherols have the same effects: α-tocopherol but not β-tocopherol can regulate AP-1 [219] and integrin [220] gene expression. Inhibition of the induction of VCAM-1 and E-selectin by IL-1β was time and dose dependent for α-tocopheryl succinate but not for IL-1β [221]. In fact, α-tocopheryl succinate can inhibit cytokine-induced mobilization of NFκB [221] by activating caspase-3 and caspase-6, which cleave the p65 subunit of the transcription factor [222,223]. According to Christen et al. [224], γ-tocopherol can inhibit $OONO^-$-induced peroxidation more effectively than can α-tocopherol. Hence, α-tocopherol isomers might inhibit COX activity more effectively than α-tocopherol itself [212]. Nevertheless, there is controversy regarding the in vivo and long-term effects of vitamin E and it is not clear what amounts or combinations may be beneficial in preventing chronic diseases [195].

There are studies on humans and animals evidencing that plant sterol supplementation, including the most abundant in VOO, β-sitosterol, can cause a decrease in serum cholesterol concentration [225,226]. One of the principal mechanisms by which this effect has been explained is through inhibition of the absorption of cholesterol [227]. Despite this, a recent study carried out by Ho and Pal [228] suggests that the mechanisms also involve decreased production of the apoB-containing lipoproteins from the liver and intestine. However, not much is known about their effect on vascular function. De Jongh et al. [229] administered a mixture of phytosterols (β-sitosterol, campesterol, stigmasterol and others) to 41 children with familial hypercholesterolemia, finding a reduction in LDL-cholesterol but no effect on endothelial dysfunction as measured by FMD. However, de la Puerta et al. [192] had reported that β-sitosterol has anti-inflammatory effects as it can reduce the auricular edema induced by TPA in mice. The effect was as high as that of hydroxytyrosol or oleuropein. Moreno [230] incubated phorbol ester-stimulated RAW 264.7 macrophages with β-sitosterol, which was responsible for a reduction in ROS production and AA release. ROS modulation may regulate the release of AA by phospholipase A_2, as well as the induction of COX-2 through NFκB activation. By this mechanism, β-sitosterol might reduce PGE_2 and LTB_4 production by macrophages, as observed in this study. Subsequently, this author reported that β-sitosterol can regulate the GSH redox cycle, enhancing GSH peroxidase and SOD activities, hence decreasing O_2^- levels although no ROS scavenger activity was found.

The potential therapeutic importance of olive oil triterpenoids, encompassing acids and alcohols, has not been extensively studied. Although their presence in VOO is very low, it is very significant in olive pomace oil, as it can be as high as 120 mg/kg. For this reason, the concentration of triterpenic alcohols is used as a parameter of purity for the presence of pomace oil in VOO [231].

Oleanolic acid has been identified in a multitude of medicinal plants [232] and has been reported to possess a number of biologic pharmacologic activities, including some affecting inflammation [233]. Oleanolic acid inhibits LOX and COX-2 activities [234,235], therefore reducing the production of PGE_2 and LTB_4. In addition, it has been shown that oleanolic acid can inhibit the generation of O_2^- by human neutrophils, which might occur through the protein kinase-independent pathway [236]. Although it has attributed anti-inflammatory activities to the triterpenic alcohol erythrodiol, its mechanism of action is still unknown. Erythrodiol is able to reduce the edema caused by TPA, with a possible action on phospholipase A_2 [192].

We recently developed a study to evaluate properties as vasodilator agents of oleanolic acid and erythrodiol and to determine their mechanism of action [193]. The vaso-relaxant effect induced by these triterpenoids was studied in isolated thoracic rat aorta. Results from this work introduced the first in vitro evidence that oleanolic acid and erythrodiol evoke an endothelium-dependent vasorelaxation in rat aorta and suggested that the mechanism of relaxation is mainly mediated by the endothelial production of NO (Fig. 3). According to the pharmacologic effects obtained, it was concluded that oleanolic acid and erythrodiol may have

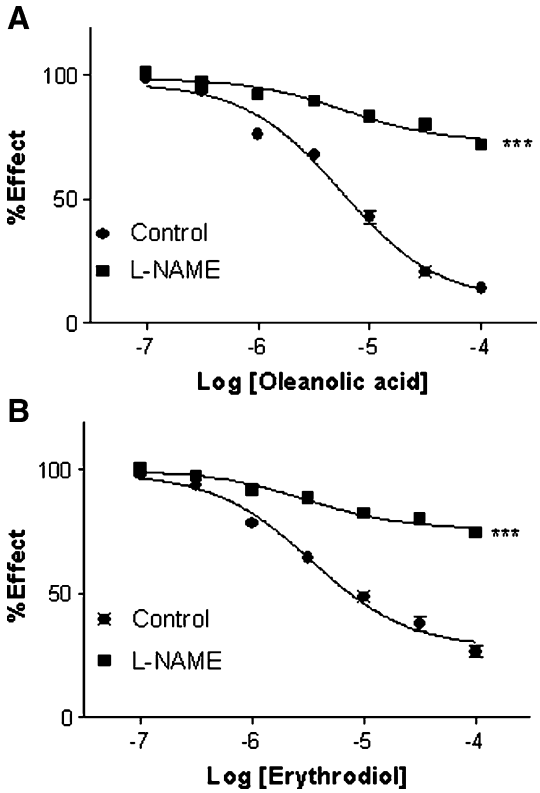

Fig. 3. Relaxant effect of oleanolic acid (A) and erythrodiol (B) in phenylephrine (10^{-6}M) precontracted rat aortic rings. Addition of the NOS inhibitor L-NAME (3×10^{-4}M) produced a significant reduction of the endothelium-dependent relaxation to both triterpenic compounds (circles) as compared with control (squares). ***$P<.001$ versus control.

interesting therapeutic potential as new vasodilator drugs, being able to protect the cardiovascular system.

6. Conclusion

It is becoming clear that the content of oleic acid alone cannot fully explain the impact on health of olive oil and that, being a unique fruit-derived oil, VOO is rich in a number of minor compounds with relevant physiological and pharmacologic functions. Among these compounds, tocopherols and phenolic compounds have demonstrated antioxidant properties that may improve endothelial function by reducing levels of ROS in the endothelium and, consequently, the production of eicosanoids and adhesion molecules. However, there are other less studied compounds that have been proven to exert important effects on endothelial function. Phytosterols and triterpenoids have anti-inflammatory and vasorelaxant effects, respectively, but their roles in the endothelium need to be further studied. The increasing investigations on the properties of these minor compounds help explain not only some of the classic beneficial effects of the Mediterranean diet and VOO itself but also the emergence of other olive-derived oils, such as pomace olive oil, which, being more enriched with these minor components, might be helpful in preventing CVD.

References

[1] Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990–2020: Global Burden of Disease Study. Lancet 1997;349:1498–504.
[2] Fuster V. Epidemic of cardiovascular disease and stroke: the three main challenges. Presented at the 71st scientific sessions of the American Heart Association. Dallas, Texas. Circulation 1999; 99(9):1132–7.
[3] Hennig B, Toborek M, McClain CJ. High-energy diets, fatty acids and endothelial cell function: implications for atherosclerosis. J Am Coll Nutr 2001;20(2 Suppl):97–105.
[4] Cooke JP. The endothelium: a new target for therapy. Vasc Med 2000;5:49–53.
[5] Vane JR, Born GVR, Welzel D, editors. The endothelial cell in health and disease. Stuttgart (Germany): Schattauer; 1995. p. 75–83.
[6] Duvall WL. Endothelial dysfunction and antioxidants. Mt Sinai J Med 2005;72(2):71–80.
[7] Ross R. Atherosclerosis — an inflammatory disease. N Engl J Med 1999;340:115–49.
[8] Davies MJ, Gordon JL, Gearing AJ, Pigott R, Woolf N, Katz D, et al. The expression of adhesion molecules ICAM-1, VCAM-1, PECAM, and E-selectin in human atherosclerosis. J Pathol 1993;171:223–9.
[9] Hennig B, Toborek M. Nutrition and endothelial cell function: implications in atherosclerosis. Nutr Res 2001;21(1–2):279–93.
[10] Vogel RA. Coronary risk factors, endothelial function and atherosclerosis: a review. Clin Cardiol 1997;20:426–32.
[11] Ross R. Cell biology of atherosclerosis. Annu Rev Physiol 1995; 57:791–804.
[12] Shimokawa H. Primary endothelial dysfunction: atherosclerosis. J Mol Cell Cardiol 1999;31:23–7.
[13] Moncada S, Higgs A. The L-arginine–nitric oxide pathway. N Engl J Med 1993;329(27):2002–12.
[14] Tsao PS, Buitrago R, Chan JR, Cooke JP. Fluid flow inhibits endothelial adhesiveness: nitric oxide and transcriptional regulation of VCAM-1. Circulation 1996;94:1682–9.
[15] Kinlay S, Ganz P. Relation between endothelial dysfunction and the acute coronary syndrome: implications for therapy. Am J Cardiol 2000;86:10J–4J.
[16] Gimbrone Jr MA, Topper JN, Nagel T, Anderson KR, Garcia-Cardena G. Endothelial dysfunction, hemodynamic forces, and atherogenesis. Ann N Y Acad Sci 2000;902:230–9.
[17] Vogel RA. Measurement of endothelial function by brachial artery flow-mediated vasodilation. Am J Cardiol 2001;88(2A):31E–4E.
[18] Kelm M. Flow-mediated dilatation in human circulation: diagnostic and therapeutic aspects. Am J Physiol Heart Circ Physiol 2002; 282(1):H1–H5.
[19] Faulx MD, Wright AT, Hoit BD. Detection of endothelial dysfunction with brachial artery ultrasound scanning. Am Heart J 2003; 145(6):943–51.
[20] Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher A. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. Nature 1999;399(6736):601–5.
[21] Moens AL, Goovaerts I, Claeys MJ, Vrints CJ. Flow-mediated vasodilation: a diagnostic instrument, or an experimental tool? Chest 2005;127(6):2254–63.
[22] Schnell GB, Robertson A, Houston D, Malley L, Anderson TJ. Impaired brachial artery endothelial function is not predicted by elevated triglycerides. J Am Coll Cardiol 1999;33(7):2038–43.
[23] Vogel RA, Corretti MC, Gellman J. Cholesterol, cholesterol lowering, and endothelial function. Prog Cardiovasc Dis 1998;41(2):117–36.
[24] Vogel RA, Corretti MC, Plotnick GD. Effect of a single high-fat meal on endothelial function in healthy subjects. Am J Cardiol 1997; 79(3):350–4.
[25] Vogel RA, Corretti MC, Plotnick GD. The postprandial effect of components of the Mediterranean diet on endothelial function. J Am Coll Cardiol 2000;36(5):1455–60.

[26] Voetsch B, Jin RC, Loscalzo J. Nitric oxide insufficiency and atherothrombosis. Histochem Cell Biol 2004;122(4):353–67.
[27] Triggle CR, Hollenberg M, Anderson TJ, Ding H, Jiang Y, Ceroni L, et al. The endothelium in health and disease — a target for therapeutic intervention. J Smooth Muscle Res 2003;39(6):249–67.
[28] Ohara Y, Peterson TE, Harrison DG. Hypercholesterolemia increases endothelial superoxide anion production. J Clin Invest 1993;91: 2546–51.
[29] Stokes KY, Cooper D, Tailor A, Granger DN. Hypercholesterolemia promotes inflammation and microvascular dysfunction: role of nitric oxide and superoxide. Free Radic Biol Med 2002;33: 1026–36.
[30] Guzik TJ, Mussa S, Gastaldi D, Sadowski J, Ratnatunga C, Pillai R, et al. Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxide synthase. Circulation 2002;105:1656–62.
[31] Just A. Nitric oxide and renal autoregulation. Kidney Blood Press Res 1997;20:201–4.
[32] Kerr S, Brosnan MJ, McIntyre M, Reid JL, Dominiczak AF, Hamilton CA. Superoxide anion production is increased in a model of genetic hypertension: role of the endothelium. Hypertension 1999; 33:1353–8.
[33] Chin JH, Azhar S, Hoffman BB. Inactivation of endothelial derived relaxing factor by oxidized lipoproteins. J Clin Invest 1992;89:10–8.
[34] Quinn MT, Parthasarathy S, Fong LG, Steinberg D. Oxidatively modified low density lipoproteins: a potential role in recruitment and retention of monocyte/macrophages during atherogenesis. Proc Natl Acad Sci U S A 1987;84(9):2995–8.
[35] Kuhn FE, Mohler ER, Salter LF, Reagan K, Lu DY, Rackley CE. Effects of high-density lipoprotein on acetylcholine-induced coronary vasoreactivity. Am J Cardiol 1991;68:1425–30.
[36] Verges BL. Dyslipidemia in diabetes mellitus. Diabetes Metab 1999; 25:32–40.
[37] Gotto AM. Triglyceride as a risk factor for coronary artery disease. Am J Cardiol 1998;5:22Q–5Q.
[38] Rapp RJ. Hypertriglyceridemia: a review beyond low-density lipoprotein. Cardiol Rev 2002;10(3):163–72.
[39] Durrington PN. Triglycerides are more important in atherosclerosis than epidemiology has suggested. Atherosclerosis 1998;(141 Suppl 1): S57–S62.
[40] Roche HM, Gibney MJ. Effect of long-chain n-3 polyunsaturated fatty acids on fasting and postprandial triacylglycerol metabolism. Am J Clin Nutr 2000;71:232S–7S.
[41] Abe Y, El-Masri B, Kimball KT, Pownall H, Reilly CF, Osmundsen K, et al. Soluble cell adhesion molecules in hypertriglyceridemia and potential significance on monocyte adhesion. Arterioscler Thromb Vasc Biol 1998;18:723–31.
[42] Patsch JR, Miesenbock G, Hopferwieser T, Muhlberger V, Knapp JK, Dunn JK, et al. Relation of triglyceride metabolism and cardiovascular disease. Arterioscler Thromb Vasc Biol 1992;12: 1336–45.
[43] Mamo JC, Wheeler JR. Chylomicrons or their remnants penetrate rabbit thoracic aorta as efficiently as do smaller macromolecules, including low-density lipoprotein, high-density lipoprotein, and albumin. Coron Artery Dis 1994;5:695–705.
[44] Napolitano M, Avella M, Botham KM, Bravo E. Chylomicron remnant induction of lipid accumulation in J774 macrophages is associated with up-regulation of triacylglycerol synthesis which is not dependent on oxidation of the particles. Biochim Biophys Acta 2003;1631(3):255–64.
[45] Stender S, Zilversmit DB. Transfer of plasma lipoprotein components and of plasma proteins into aortas of cholesterol-fed rabbits. Molecular size as a determinant of plasma lipoprotein influx. Arteriosclerosis 1981;1(1):38–49.
[46] Nordestgaard BG, Zilversmit DB. Large lipoproteins are excluded from the arterial wall in diabetic cholesterol-fed rabbits. J Lipid Res 1988;29(11):1491–500.
[47] Cohn JS, Marcoux C, Davignon J. Detection, quantification, and characterization of potentially atherogenic triglyceride-rich remnant lipoproteins. Arterioscler Thromb Vasc Biol 1999;19(10):2474–86.
[48] Hennig B, Shasby DM, Fulton AB, Spector AA. Exposure to free fatty acid increases the transfer of albumin across cultured endothelial monolayers. Arteriosclerosis 1984;4(5):489–97.
[49] Hennig B, Chung BH, Watkins BA, Alvarado A. Disruption of endothelial barrier function by lipolytic remnants of triglyceride-rich lipoproteins. Atherosclerosis 1992;95(2–3):235–47.
[50] Doi H, Kugiyama K, Ohgushi M, Sugiyama S, Matsumura T, Ohta Y, et al. Remnants of chylomicron and very low density lipoprotein impair endothelium-dependent vasorelaxation. Atherosclerosis 1998; 137(2):341–9.
[51] Kugiyama K, Doi H, Motoyama T, Soejima H, Misumi K, Kawano H, et al. Association of remnant lipoprotein levels with impairment of endothelium-dependent vasomotor function in human coronary arteries. Circulation 1998;97(25):2519–26.
[52] Eriksson P, Nilsson L, Karpe F, Hamstem A. Very-low-density lipoprotein response element in the promoter region of the human plasminogen activator inhibitor-1 gene implicated in the impaired fibrinolysis of hypertriglyceridemia. Arterioscler Thromb Vasc Biol 1998;18:20–6.
[53] Wilhelm MG, Cooper AD. Induction of atherosclerosis by human chylomicron remnants: a hypothesis. J Atheroscler Thromb 2003; 10(3):132–9.
[54] Saigo M, Abe S, Ogawa M, Biro S, Minagoe S, Maruyama I, et al. Plasma level of triglyceride-rich lipoprotein remnants is closely associated with the activation of coagulation factor VII in patients with myocardial infarction. Thromb Res 2000;100:9–17.
[55] Dart AM, Chin-Dusting JP. Lipids and the endothelium. Cardiovasc Res 1999;43:308–22.
[56] Doi H, Kugiyama K, Oka H, Sugiyama S, Ogata N, Koide SI, et al. Remnant lipoproteins induce proatherothrombogenic molecules in endothelial cells through a redox-sensitive mechanism. Circulation 2000;102:670–6.
[57] Moers A, Fenselau S, Schrezenmeir J. Chylomicrons induce E-selectin and VCAM-1 expression in endothelial cells. Exp Clin Endocrinol Diabetes 1997;(105 Suppl 2):35–7.
[58] Kusterer K, Pohl T, Fortmeyer HP, Marz W, Scharnagl H, Oldenburg A, et al. Chronic selective hypertriglyceridemia impairs endothelium-dependent vasodilatation in rats. Cardiovasc Res J 1999;42:783–93.
[59] Van Eck M, Zimmermann R, Groot PH, Zechner R, Van Berkel TJ. Role of macrophage-derived lipoprotein lipase in lipoprotein metabolism and atherosclerosis. Arterioscler Thromb Vasc Biol 2000; 20:E53–E62.
[60] Khoo JC, Mahoney EM, Witztum JL. Secretion of lipoprotein lipase by macrophages in culture. J Biol Chem 1981;256:7105–8.
[61] Ylä-Herttuala S, Lipton BA, Rosenfeld ME, Goldberg IJ, Steinberg D, Witztum JL. Macrophages and smooth muscle cells express lipoprotein lipase in human and rabbit atherosclerotic lesions. Proc Natl Acad Sci U S A 1991;88:10143–7.
[62] O'Brien KD, Gordon D, Deeb S, Ferguson M, Chait A. Lipoprotein lipase is synthesized by macrophage-derived foam cells in human coronary atherosclerotic plaques. J Clin Invest 1992;89: 1544–50.
[63] Sofer O, Fainaru M, Schafer Z, Goldman R. Regulation of lipoprotein lipase secretion in murine macrophages during foam cell formation in vitro. Effect of triglyceride-rich lipoproteins. Arterioscler Thromb 1992;12:1458–66.
[64] Saxena U, Klein MG, Vanni TM, Goldberg IJ. Lipoprotein lipase increases low density lipoprotein retention by subendothelial cell matrix. J Clin Invest 1992;89:373–80.
[65] Edwards IJ, Goldberg IJ, Parks JS, Xu H, Wagner WD. Lipoprotein lipase enhances the interaction of low density lipoproteins with artery-derived extracellular matrix proteoglycans. J Lipid Res 1993; 34:1155–63.

[66] Mamputu JC, Desfaits AC, Renier G. Lipoprotein lipase enhances human monocyte adhesion to aortic endothelial cells. J Lipid Res 1997;38:1722–9.
[67] Hirata K, Ishida T, Matsushita H, Tsao PS, Quertermous T. Regulated expression of endothelial cell-derived lipase. Biochem Biophys Res Commun 2000;272:90–3.
[68] McCoy MG, Sun GS, Marchadier D, Maugeais C, Glick JM, Rader DJ. Characterization of the lipolytic activity of endothelial lipase. J Lipid Res 2002;43(6):921–9.
[69] Jin W, Sun GS, Marchadier D, Octtaviani E, Glick JM, Rader DJ. Endothelial cells secrete triglyceride lipase and phospholipase activities in response to cytokines as a result of endothelial lipase. Circ Res 2003;92(6):644–50.
[70] Traenckner EB, Pahl HL, Henkel T, Schmidt KN, Wilk S, Baeuerle PA. Phosphorylation of human I kappa B-alpha on serines 32 and 36 controls I kappa B-alpha proteolysis and NF-kappa B activation in response to diverse stimuli. EMBO J 1995;14:2876–83.
[71] Steinberg HO, Tarshoby M, Monestel R, Hook G, Cronin J, Johnson A, et al. Elevated circulating free fatty acid levels impair endothelium-dependent vasodilation. J Clin Invest 1997;100(5): 1230–9.
[72] Inoguchi T, Li P, Umeda F, Yu HY, Kakimoto M, Imamura M, et al. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C-dependent activation of NAD(P)H oxidase in cultured vascular cells. Diabetes 2000;49(11): 1939–45.
[73] Williams CM, Maitin V, Jackson KG. Triacylglycerol-rich lipoprotein–gene interactions in endothelial cells. Biochem Soc Trans 2004; 32:994–8.
[74] Toborek M, Barger SW, Mattson MP, Barve S, McClain CJ, Hennig B. Linoleic acid and TNF-alpha cross-amplify oxidative injury and dysfunction of endothelial cells. J Lipid Res 1996;37(1):123–35.
[75] Young VM, Toborek M, Yang F, McClain CJ, Hennig B. Effect of linoleic acid on endothelial cell inflammatory mediators. Metabolism 1998;47:565–6.
[76] Rus HG, Vlaicu R, Niculescu F. Interleukin-6 and interleukin-8 protein and gene expression in human arterial atherosclerotic wall. Atherosclerosis 1996;127:263–71.
[77] Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis 2000;148:209–14.
[78] De Caterina R, Cybulsky MI, Clinton SK, Gimbrone MA, Libby P. The omega-3 fatty acid docosahexaenoate reduces cytokine-induced expression of proatherogenic and proinflammatory proteins in human endothelial cells. Arterioscler Thromb 1994;14:1829–36.
[79] Weber C, Erl W, Pietsch A, Danesch U, Weber P. Docosahexaenoic acid selectively attenuates induction of vascular cell adhesion molecule-1 and subsequent monocytic adhesion to human endothelial cells stimulated by tumor necrosis factor-alpha. Arterioscler Thromb Vasc Biol 1995;15:622–8.
[80] Khalfoun B, Thibault G, Bardos P, Lebranchu Y. Docosahexaenoic and eicosapentaenoic acids inhibit in vitro human lymphocyte–endothelial adhesion. Transplantation 1996;62:1649–57.
[81] Seljeflot I, Arnesen H, Brude IR, Nenseter MS, Drevon CA, Hjermann I. Effects of omega-3 fatty acids and/or antioxidants on endothelial cell markers. Eur J Clin Invest 1998;28:629–35.
[82] Johansen O, Seljflot I, Hostmark AT, Arnesen H. The effect of supplementation with omega-3 fatty acids on soluble markers of endothelial function in patients with coronary heart disease. Arterioscler Thromb Vasc Biol 1999;19:1681–6.
[83] Hawkes JS, James MJ, Cleland LG. Biological activity of prostaglandin E_3 with regard to oedema formation in mice. Agents Actions 1992;35:85–7.
[84] Goldman DW, Pickett WC, Goetzl EJ. Human neutrophil chemotactic and degranulating activities of leukotriene B_5 (LTB_5) derived from eicosapentaenoic acid. Biochem Biophys Res Commun 1983; 117:282–8.
[85] James MJ, Cleland LG, Gibson RA, Hawkes JS. Interaction between fish and vegetable oils in relation to rat leucocyte leukotriene production. J Nutr 1991;121:631–7.
[86] Wahrburg U, Kratz M, Cullen P. Mediterranean diet, olive oil and health. Eur J Lipid Sci Technol 2002;104:698–705.
[87] Keys A, Menotti A, Karvonen MJ, Aravanis C, Blackburn H, Buzina R, et al. The diet and 15-year death rate in the Seven Countries Study. Am J Epidemiol 1986;124(6):903–15.
[88] de Lorgeril M, Renaud S, Mamelle N, Salen P, Martin JL, Monjaud I, et al. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. Lancet 1994;343:1454–9.
[89] Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med 2003;348(26):2599–608.
[90] Trichopoulou A, Bamia C, Trichopoulos D. Mediterranean diet and survival among patients with coronary heart disease in Greece. Arch Intern Med 2005;165(8):929–35.
[91] Hu FB. The Mediterranean diet and mortality — olive oil and beyond. N Engl J Med 2003;348(26):2595–6.
[92] Mensink RP, Katan MB. Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. Arterioscler Thromb 1992;12(8):911–9.
[93] Gardner CD, Kraemer HC. Monounsaturated versus polyunsaturated dietary fat and serum lipids. A meta-analysis. Arterioscler Thromb Vasc Biol 1995;15(11):1917–27.
[94] Kris-Etherton PM, Pearson TA, Wan Y, Hargrove RL, Moriarty K, Fishell V, et al. High-monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations. Am J Clin Nutr 1999;70(6):1009–15.
[95] Mata P, Garrido JA, Ordovas JM, Blazquez E, Alvarez-Sala LA, Rubio MJ, et al. Effect of dietary monounsaturated fatty acids on plasma lipoproteins and apolipoproteins in women. Am J Clin Nutr 1992;56(1):77–83.
[96] Perona JS, Canizares J, Montero E, Sanchez-Dominguez JM, Ruiz-Gutierrez V. Plasma lipid modifications in elderly people after administration of two virgin olive oils of the same variety (*Olea europaea* var. *hojiblanca*) with different triacylglycerol composition. Br J Nutr 2003;89(6):819–26.
[97] Perona JS, Canizares J, Montero E, Sanchez-Dominguez JM, Catala V, Ruiz-Gutierrez V. Virgin olive oil reduces blood pressure in hypertensive elderly subjects. Clin Nutr 2004;23(5):1113–21.
[98] Aguilera CM, Mesa MD, Ramirez-Tortosa MC, Nestares MT, Ros E, Gil A. Sunflower oil does not protect against LDL oxidation as virgin olive oil does in patients with peripheral vascular disease. Clin Nutr 2004;23(4):673–81.
[99] Aranda P. Arterial hypertension in Spain: the experience of the Spanish League. J Hum Hypertens 1996;(10 Suppl 1):S73–5.
[100] Coulston AM, Hollenbeck CB, Swislocki AL, Reaven GM. Persistence of hypertriglyceridemic effect of low-fat high-carbohydrate diets in NIDDM patients. Diabetes Care 1989;12(2):94–101.
[101] Appel LJ, Miller III ER, Seidler AJ, Whelton PK. Does supplementation of diet with 'fish oil' reduce blood pressure? A meta-analysis of controlled clinical trials. Arch Intern Med 1993;153(12):1429–38.
[102] Lam BK, Marcinkiewicz E, Quilley J, Hirai A, Yoshida S, Tamura Y, et al. Hypotensive effects of eicosapentaenoic acid (EPA) and its influence on eicosanoid metabolism in spontaneously hypertensive rats. J Hypertens Suppl 1986;4(3):S453–5.
[103] Perez-Jimenez F, Lopez-Miranda J, Mata P. Protective effect of dietary monounsaturated fat on arteriosclerosis: beyond cholesterol. Atherosclerosis 2002;163(2):385–98.
[104] Espino A, López-Miranda J, Castro P, Rodríguez M, López F, Blanco A, et al. Monounsaturated fatty acids enriched diets lower plasma insulin levels and blood pressure in healthy young men. Nutr Metab Cardiovasc Dis 1996;6:147–54.
[105] Lahoz C, Alonso R, Ordovas JM, Lopez-Farre A, de Oya M, Mata P. Effects of dietary fat saturation on eicosanoid production, platelet aggregation and blood pressure. Eur J Clin Invest 1997;27(9):780–7.

[106] Ruiz-Gutierrez V, Muriana FJ, Guerrero A, Cert AM, Villar J. Plasma lipids, erythrocyte membrane lipids and blood pressure of hypertensive women after ingestion of dietary oleic acid from two different sources. J Hypertens 1996;14(12):1483–90.

[107] Psaltopoulou T, Naska A, Orfanos P, Trichopoulos D, Mountokalakis T, Trichopoulou A. Olive oil, the Mediterranean diet, and arterial blood pressure: the Greek European Prospective Investigation into Cancer and Nutrition (EPIC) Study. Am J Clin Nutr 2004;80(4): 1012–8.

[108] Mills DE, Ward RP, Mah M, DeVette L. Dietary N-6 and N-3 fatty acids and salt-induced hypertension in the borderline hypertensive rat. Lipids 1989;24(1):17–24.

[109] Berry EM, Eisenberg S, Haratz D, Friedlander Y, Norman Y, Kaufmann NA, et al. Effects of diets rich in monounsaturated fatty acids on plasma lipoproteins — the Jerusalem Nutrition Study: high MUFAs vs high PUFAs. Am J Clin Nutr 1991;53(4):899–907.

[110] Reaven P, Parthasarathy S, Grasse BJ, Miller E, Almazan F, Mattson FH, et al. Feasibility of using an oleate-rich diet to reduce the susceptibility of low-density lipoprotein to oxidative modification in humans. Am J Clin Nutr 1991;54(4):701–6.

[111] Bonanome A, Pagnan A, Biffanti S, Opportuno A, Sorgato F, Dorella M, et al. Effect of dietary monounsaturated and polyunsaturated fatty acids on the susceptibility of plasma low density lipoproteins to oxidative modification. Arterioscler Thromb 1992;12(4):529–33.

[112] Reaven P, Grasse B, Barnett J. Effect of antioxidants alone and in combination with monounsaturated fatty acid-enriched diets on lipoprotein oxidation. Arterioscler Thromb Vasc Biol 1996;16(12): 1465–72.

[113] Hargrove RL, Etherton TD, Pearson TA, Harrison EH, Kris-Etherton PM. Low fat and high monounsaturated fat diets decrease human low density lipoprotein oxidative susceptibility in vitro. J Nutr 2001; 131(6):1758–63.

[114] Truswell AS, Choudhury N. Monounsaturated oils do not all have the same effect on plasma cholesterol. Eur J Clin Nutr 1998;52(5): 312–5.

[115] Muriana FJ, Villar J, Ruiz-Gutierrez V. Intake of olive oil can modulate the transbilayer movement of human erythrocyte membrane cholesterol. Cell Mol Life Sci 1997;53(6):496–500.

[116] Perona JS, Ruiz-Gutierrez V. Effect of two high-oleic oils on the liver lipid composition of spontaneously hypertensive rats. Life Sci 2000; 66(6):521–31.

[117] Perona JS, Ruiz-Gutierrez V. Virgin olive oil normalizes the altered triacylglycerol molecular species composition of adipose tissue in spontaneously hypertensive rats. J Agric Food Chem 2004;52(13): 4227–33.

[118] Perona JS, Ruiz-Gutierrez V. Triacylglycerol molecular species are depleted to different extents in the myocardium of spontaneously hypertensive rats fed two oleic acid-rich oils. Am J Hypertens 2005; 18(1):72–80.

[119] Herrera MD, Perez-Guerrero C, Marhuenda E, Ruiz-Gutierrez V. Effects of dietary oleic-rich oils (virgin olive and high-oleic-acid sunflower) on vascular reactivity in Wistar–Kyoto and spontaneously hypertensive rats. Br J Nutr 2001;86(3):349–57.

[120] Ruiz-Gutierrez V, Perona JS, Pacheco YM, Muriana FJ, Villar J. Incorporation of dietary triacylglycerols from olive oil and high-oleic sunflower oil into VLDL triacylglycerols of hypertensive patients. Eur J Clin Nutr 1999;53(9):687–93.

[121] Ruiz-Gutierrez V, Morgado N, Prada JL, Perez-Jimenez F, Muriana FJ. Composition of human VLDL triacylglycerols after ingestion of olive oil and high oleic sunflower oil. J Nutr 1998;128(3):570–6.

[122] Abia R, Pacheco YM, Perona JS, Montero E, Muriana FJ, Ruiz-Gutierrez V. The metabolic availability of dietary triacylglycerols from two high oleic oils during the postprandial period does not depend on the amount of oleic acid ingested by healthy men. J Nutr 2001;131(1):59–65.

[123] Pacheco YM, Abia R, Perona JS, Reina M, Ruiz-Gutierrez V, Montero E, et al. Triacylglycerol-rich lipoproteins interact with human vascular cells in a lipid-dependent fashion. J Agric Food Chem 2001;49(11):5653–61.

[124] Perona JS, Martinez-Gonzalez J, Sanchez-Dominguez JM, Badimon L, Ruiz-Gutierrez V. The unsaponifiable fraction of virgin olive oil in chylomicrons from men improves the balance between vasoprotective and prothrombotic factors released by endothelial cells. J Nutr 2004;134(12):3284–9.

[125] Scaccini C, Nardini M, D'Aquino M, Gentili V, Di Felice M, Tomassi G. Effect of dietary oils on lipid peroxidation and on antioxidant parameters of rat plasma and lipoprotein fractions. J Lipid Res 1992;33(5):627–33.

[126] Nicolaiew N, Lemort N, Adorni L, Berra B, Montorfano G, Rapelli S, et al. Comparison between extra virgin olive oil and oleic acid rich sunflower oil: effects on postprandial lipemia and LDL susceptibility to oxidation. Ann Nutr Metab 1998;42(5):251–60.

[127] Ruiz-Gutiérrez V, De la Puerta R, Perona JS. Beneficial effects of olive oil on health. In: Pandalai SG, editor. Recent research and development in nutrition. India: Researchsignpost. Trivandrum; 2000. p. 173–97.

[128] Lanzón A, Albi T, Cert A, Gracián J. The hydrocarbon fraction of virgin olive oil and changes resulting from refining. J Am Oil Chem Soc 1994;71(3):285–91.

[129] Ginda A, Lanzón A, Albi T. Differences in hydrocarbons of virgin olive oils obtained from several olive varieties. J Agric Food Chem 1993;44(7):1723–6.

[130] Tiscornia EFM, Evangelisti F. Chemical composition of olive oil and its variations induced by refining. Riv Ital Sostanze Grasse 1982; 59:519–56.

[131] Zamora R, Navarro JL, Hidalgo FJ. Identification and classification of olive oils by high-resolution C-13 nuclear-magnetic-resonance. J Am Oil Chem Soc 1994;71(4):361–4.

[132] Gutfinger T. Polyphenols in olive oils. J Am Chem Soc 1981; 58(11):966–8.

[133] Vazquez-Roncero A. Les polyphénoles de l'huile d'olive et leur influence sur les caractéristiques de l'huile. Rev Fr Corps Gras 1978; 25:21–6.

[134] Tsimidou M, Papadopoulos G, Boskou D. Phenolic compounds and stability of virgin olive oil: Part I. Food Chem 1992;45:141–4.

[135] Boskov D. Olive oil chemistry and technology. Illinois (USA): AOCS Press, 1996, 1996. p. 115–7.

[136] Visioli F, Galli C. The effect of minor constituents of olive oil on cardiovascular disease: new findings. Nutr Rev 1998;56(5 Pt 1): 142–7.

[137] Visioli F, Galli C, Bornet F, Mattei A, Patelli R, Galli G, et al. Olive oil phenolics are dose-dependently absorbed in humans. FEBS Lett 2000;468(2–3):159–60.

[138] Weinbrenner T, Fito M, de la Torre R, Saez GT, Rijken P, Tormos C, et al. Olive oils high in phenolic compounds modulate oxidative/ antioxidative status in men. J Nutr 2004;134(9):2314–21.

[139] Weinbrenner T, Fito M, Farre Albaladejo M, Saez GT, Rijken P, Tormos C, et al. Bioavailability of phenolic compounds from olive oil and oxidative/antioxidant status at postprandial state in healthy humans. Drugs Exp Clin Res 2004;30(5–6):207–12.

[140] Visioli F, Caruso D, Grande S, Bosisio R, Villa M, Galli G, et al. Virgin Olive Oil Study (VOLOS): vasoprotective potential of extra virgin olive oil in mildly dyslipidemic patients. Eur J Nutr 2005; 44(2):121–7.

[141] Ryan M, McInerney D, Owens D, Collins P, Johnson A, Tomkin GH. Diabetes and the Mediterranean diet: a beneficial effect of oleic acid on insulin sensitivity, adipocyte glucose transport and endothelium-dependent vasoreactivity. QJM 2000;93(2):85–91.

[142] Fuentes F, Lopez-Miranda J, Sanchez E, Sanchez F, Paez J, Paz-Rojas E, et al. Mediterranean and low-fat diets improve endothelial function in hypercholesterolemic men. Ann Intern Med 2001; 134(12):1115–9.

[143] Ros E, Nunez I, Perez-Heras A, Serra M, Gilabert R, Casals E, et al. A walnut diet improves endothelial function in hypercholesterolemic

subjects: a randomized crossover trial. Circulation 2004;109(13): 1609–14.
[144] Sondergaard E, Moller JE, Egstrup K. Effect of dietary intervention and lipid-lowering treatment on brachial vasoreactivity in patients with ischemic heart disease and hypercholesterolemia. Am Heart J 2003;145(5):E19.
[145] Esposito K, Marfella R, Ciotola M, Di Palo C, Giugliano F, Giugliano G, et al. Effect of a Mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. JAMA 2004;292(12):1440–6.
[146] Hennig B, Meerarani P, Ramadass P, Watkins BA, Toborek M. Fatty acid-mediated activation of vascular endothelial cells. Metabolism 2000;49(8):1006–13.
[147] Carluccio MA, Massaro M, Bonfrate C, Siculella L, Maffia M, Nicolardi G, et al. Oleic acid inhibits endothelial activation: direct vascular antiatherogenic mechanism of a nutritional component in the Mediterranean diet. Arterioscler Thromb Vasc Biol 1999; 19(2):220–8.
[148] Tsimikas S, Philis-Tsimikas A, Alexopoulos S, Sigari F, Lee C, Reaven PD. LDL isolated from Greek subjects on a typical diet or from American subjects on an oleate-supplemented diet induces less monocyte chemotaxis and adhesion when exposed to oxidative stress. Arterioscler Thromb Vasc Biol 1999;19(1):122–30.
[149] Lee C, Barnett J, Reaven PD. Liposomes enriched in oleic acid are less susceptible to oxidation and have less proinflammatory activity when exposed to oxidizing conditions. J Lipid Res 1998;39(6):1239–47.
[150] Christon RA. Mechanisms of action of dietary fatty acids in regulating the activation of vascular endothelial cells during atherogenesis. Nutr Rev 2003;61(8):272–9.
[151] Spolarics Z. Endotoxin stimulates gene expression of ROS eliminating pathways in rat hepatic endothelial and Kupffer cells. Am J Physiol 1996;270:660–6.
[152] Baeuerle PA, Henkel T. Function and activation of NF-kB in the immune system. Annu Rev Immunol 1994;12:141–79.
[153] Massaro M, Carluccio MA, Paolicchi A, Bosetti F, Solaini G, De Caterina R. Mechanisms for reduction of endothelial activation by oleate: inhibition of nuclear factor-kappaB through antioxidant effects. Prostaglandins Leukot Essent Fatty Acids 2002; 67(2–3):175–81.
[154] Massaro M, Carluccio MA, De Caterina R. Direct vascular antiatherogenic effects of oleic acid: a clue to the cardioprotective effects of the Mediterranean diet. Cardiologia 1999;44(6):507–13.
[155] Toborek M, Lee YW, Garrido R, Kaiser S, Hennig B. Unsaturated fatty acids selectively induce an inflammatory environment in human endothelial cells. Am J Clin Nutr 2002;75(1):119–25.
[156] Massaro M, Carluccio MA, Bonfrate C, Siculella L, Lazzerini G, Bernini W, et al. The double bond in unsaturated fatty acids is the necessary and sufficient requirement for the inhibition of expression of endothelial leukocyte adhesion molecules through interference with nuclear factor-kappaB activation. Lipids 1999;(34 Suppl): S213–4.
[157] Thanos D, Maniatis T. NF-kB: a lesson in family values. Cell 1995; 80:529–32.
[158] Marui N, Offermann MK, Swerlick R, Kunsch C, Rosen CA, Ahmad M, et al. Vascular cell adhesion molecule (VCAM-1) gene transcription and expression are regulated through an antioxidant-sensitive mechanism in human vascular endothelial cells. J Clin Invest 1993;92:1866–74.
[159] Hart CM, Gupta MP, Evanoff V. Oleic acid reduces oxidant stress in cultured pulmonary artery endothelial cells. Exp Lung Res 1997; 23(5):405–25.
[160] Karman RJ, Garcia JG, Hart CM. Endothelial cell monolayer dysfunction caused by oxidized low density lipoprotein: attenuation by oleic acid. Prostaglandins Leukot Essent Fatty Acids 1997; 56(5):345–53.
[161] Hart CM, Andreoli SP, Patterson CE, Garcia JG. Oleic acid supplementation reduces oxidant-mediated dysfunction of cultured porcine pulmonary artery endothelial cells. J Cell Physiol 1993; 156(1):24–34.
[162] Massaro M, Basta G, Lazzerini G, Carluccio MA, Bosetti F, Solaini G, et al. Quenching of intracellular ROS generation as a mechanism for oleate-induced reduction of endothelial activation and early atherogenesis. Thromb Haemost 2002;88(2):335–44.
[163] Kwok CF, Shih KC, Hwu CM, Ho LT. Linoleic acid and oleic acid increase the endothelin-1 binding and action in cultured rat aortic smooth muscle cells. Metabolism 2000;49(11):1386–9.
[164] Oram JF, Bornfeldt KE. Direct effects of long-chain non-esterified fatty acids on vascular cells and their relevance to macrovascular complications of diabetes. Front Biosci 2004;9:1240–53.
[165] Tuck KL, Hayball PJ. Major phenolic compounds in olive oil: metabolism and health effects. J Nutr Biochem 2002;13(11):636–44.
[166] Keaney Jr JF, Vita JA. Atherosclerosis, oxidative stress, and antioxidant protection in endothelium-derived relaxing factor action. Prog Cardiovasc Dis 1995;38:129–54.
[167] Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res 2000;87:840–4.
[168] Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. Circulation 2001;104(22): 2673–8.
[169] Ting HH, Timimi FK, Haley EA, Roddy MA, Ganz P, Creager MA. Vitamin C improves endothelium-dependent vasodilation in forearm resistance vessels of humans with hypercholesterolemia. Circulation 1997;95(12):2617–22.
[170] Levine GN, Frei B, Koulouris SN, Gerhard MD, Keaney Jr JF, Vita JA. Ascorbic acid reverses endothelial vasomotor dysfunction in patients with coronary artery disease. Circulation 1996;93(6):1107–13.
[171] Heitzer T, Just H, Munzel T. Antioxidant vitamin C improves endothelial dysfunction in chronic smokers. Circulation 1996;94:6–9.
[172] Jackson TS, Xu A, Vita JA, Keaney Jr JF. Ascorbate prevents the interaction of superoxide and nitric oxide only at very high physiological concentrations. Circ Res 1998;83(9):916–22.
[173] Saija A, Trombetta D, Tomaino A, Lo Cascio R, Princi P, Ucella N, et al. In vitro evaluation of the antioxidant activity and biomembrane interaction of the plant phenols oleuropein and hydroxy-tyrosol. Int J Pharm 1998;166:123–33.
[174] Gordon MH, Paiva-Martins F, Almeida M. Antioxidant activity of hydroxytyrosol acetate compared with that of other olive oil polyphenols. J Agric Food Chem 2001;49(5):2480–5.
[175] Briante R, La Cara F, Tonziello MP, Febbraio F, Nucci R. Antioxidant activity of the main bioactive derivatives from oleuropein hydrolysis by hyperthermophilic beta-glycosidase. J Agric Food Chem 2001;49(7):3198–203.
[176] Tuck KL, Hayball PJ, Stupans I. Structural characterization of the metabolites of hydroxytyrosol, the principal phenolic component in olive oil, in rats. J Agric Food Chem 2002;50(8):2404–9.
[177] Visioli F, Bellomo G, Galli C. Free radical-scavenging properties of olive oil polyphenols. Biochem Biophys Res Commun 1998;247(1): 60–4.
[178] Sutherland WH, de Jong SA, Walker RJ, Williams MJ, Murray Skeaff C, Duncan A, et al. Effect of meals rich in heated olive and safflower oils on oxidation of postprandial serum in healthy men. Atherosclerosis 2002;160(1):195–203.
[179] Visioli F, Bellosta S, Galli C. Oleuropein, the bitter principle of olives, enhances nitric oxide production by mouse macrophages. Life Sci 1998;62(6):541–6.
[180] Beckman JS, Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. Am J Physiol 1996; 271(5 Pt 1):C1424–37.
[181] de la Puerta R, Ruiz Gutierrez V, Hoult JR. Inhibition of leukocyte 5-lipoxygenase by phenolics from virgin olive oil. Biochem Pharmacol 1999;57(4):445–9.
[182] Petroni A, Blasevich M, Salami M, Papini N, Montedoro GF, Galli C. Inhibition of platelet aggregation and eicosanoid production

by phenolic components of olive oil. Thromb Res 1995;78(2): 151–160.
[183] Petroni A, Blasevich M, Papini N, Salami M, Sala A, Galli C. Inhibition of leukocyte leukotriene B_4 production by an olive oil-derived phenol identified by mass-spectrometry. Thromb Res 1997; 87(3):315–22.
[184] Kohyama N, Nagata T, Fujimoto S, Sekiya K. Inhibition of arachidonate lipoxygenase activities by 2-(3,4-dihydroxyphenyl)e-thanol, a phenolic compound from olives. Biosci Biotechnol Biochem 1997;61(2):347–50.
[185] Petroni A, Blasevich M, Salami M, Servili M, Montedoro GF, Galli C. A phenolic antioxidant extracted from olive oil inhibits platelet aggregation and arachidonic acid metabolism in vitro. World Rev Nutr Diet 1994;75:169–72.
[186] Miles EA, Zoubouli P, Calder PC. Differential anti-inflammatory effects of phenolic compounds from extra virgin olive oil identified in human whole blood cultures. Nutrition 2005;21(3):389–94.
[187] Carluccio MA, Siculella L, Ancora MA, Massaro M, Scoditti E, Storelli C, et al. Olive oil and red wine antioxidant polyphenols inhibit endothelial activation: antiatherogenic properties of Mediter-ranean diet phytochemicals. Arterioscler Thromb Vasc Biol 2003; 23(4):622–9.
[188] Ahmad M, Theofanidis P, Medford RM. Role of activating protein-1 in the regulation of the vascular cell adhesion molecule-1 gene expression by tumor necrosis factor-alpha. J Biol Chem 1998;273(8): 4616–21.
[189] Turner R, Etienne N, Alonso MG, de Pascual-Teresa S, Minihane AM, Weinberg PD, et al. Antioxidant and anti-atherogenic activities of olive oil phenolics. Int J Vitam Nutr Res 2005;75(1):61–70.
[190] Ma Q, Kinneer K, Ye J, Chen BJ. Inhibition of nuclear factor kappaB by phenolic antioxidants: interplay between antioxidant signaling and inflammatory cytokine expression. Mol Pharmacol 2003; 64(2):211–9.
[191] Azzi A, Ricciarelli R, Zingg JM. Non-antioxidant molecular functions of alpha-tocopherol (vitamin E). FEBS Lett 2002; 519(1–3):8–10.
[192] de la Puerta R, Martinez-Dominguez E, Ruiz-Gutierrez V. Effect of minor components of virgin olive oil on topical antiinflammatory assays. Z Naturforsch [C] 2000;55(9–10):814–9.
[193] Rodriguez-Rodriguez R, Herrera MD, Perona JS, Ruiz-Gutierrez V. Potential vasorelaxant effects of oleanolic acid and erythrodiol, two triterpenoids contained in 'orujo' olive oil, on rat aorta. Br J Nutr 2004;92(4):635–42.
[194] Ochoa JJ, Quiles JL, Ramirez-Tortosa MC, Mataix J, Huertas JR. Dietary oils high in oleic acid but with different unsaponifiable fraction contents have different effects in fatty acid composition and peroxidation in rabbit LDL. Nutrition 2002;18(1):60–5.
[195] Azzi A, Gysin R, Kempna P, Ricciarelli R, Villacorta L, Visarius T, et al. The role of alpha-tocopherol in preventing disease: from epidemiology to molecular events. Mol Aspects Med 2003;24(6): 325–36.
[196] Azzi A, Gysin R, Kempnà P, Ricciarelli R, Villacorta L, Visarius T, et al. Regulation of gene and protein expression by vitamin E. Free Radic Res 2002;36:30–6.
[197] Jialal I, Fuller CJ, Huet BA. The effect of alpha-tocopherol supplementation on LDL oxidation. A dose–response study. Arte-rioscler Thromb Vasc Biol 1995;15(2):190–8.
[198] Princen HM, van Duyvenvoorde W, Buytenhek R, van der Laarse A, van Poppel G, Gevers Leuven JA, et al. Supplementation with low doses of vitamin E protects LDL from lipid peroxidation in men and women. Arterioscler Thromb Vasc Biol 1995;15(3):325–33.
[199] Devaraj S, Adams-Huet B, Fuller CJ, Jialal I. Dose–response comparison of RRR-α-tocopherol and all-racemic alpha-tocopherol on LDL oxidation. Arterioscler Thromb Vasc Biol 1997;17(10): 2273–9.
[200] Cominacini L, Garbin U, Cenci B, Davoli A, Pasini C, Ratti E, et al. Predisposition to LDL oxidation during copper-catalyzed oxidative modification and its relation to alpha-tocopherol content in humans. Clin Chim Acta 1991;204(1–3):57–68.
[201] Offermann MK, Medford RM. Antioxidants and atherosclerosis: a molecular perspective. Heart Dis Stroke 1994;3(1):52–7.
[202] Martin A, Foxall T, Blumberg JB, Meydani M. Vitamin E inhibits low-density lipoprotein-induced adhesion of monocytes to human aortic endothelial cells in vitro. Arterioscler Thromb Vasc Biol 1997; 17(3):429–36.
[203] Zapolska-Downar D, Zapolski-Downar A, Markiewski M, Ciechanowicz A, Kaczmarczyk M, Naruszewicz M. Selective inhibition by alpha-tocopherol of vascular cell adhesion molecule-1 expression in human vascular endothelial cells. Biochem Biophys Res Commun 2000;274(3):609–15.
[204] Faruqi R, de la Motte C, Di Corleto PE. α-Tocopherol inhibits agonist-induced monocytic cell adhesion to cultured human endo-thelial cells. J Clin Invest 1994;94(2):592–600.
[205] Yoshikawa T, Yoshida N, Manabe H, Terasawa Y, Takemura T, Kondo M. α-Tocopherol protects against expression of adhesion molecules on neutrophils and endothelial cells. Biofactors 1998;7 (1–2):15–9.
[206] Akeson AL, Woods CW, Mosher LB, Thomas CE, Jackson RL. Inhibition of IL-1 beta expression in THP-1 cells by probucol and tocopherol. Atherosclerosis 1991;86(2–3):261–70.
[207] Okuma M, Takayama H, Uchino H. Generation of prostacyclin-like substance and lipid peroxidation in vitamin E-deficient rats. Prostaglandins 1980;19(4):527–36.
[208] Chan AC, Leith MK. Decreased prostacyclin synthesis in vitamin E-deficient rabbit aorta. Am J Clin Nutr 1981;34(11):2341–7.
[209] Kunisaki M, Umeda F, Inoguchi T, Nawata H. Vitamin E binds to specific binding sites and enhances prostacyclin production by cultured aortic endothelial cells. Thromb Haemost 1992;68(6):744–51.
[210] Kunisaki M, Umeda F, Inoguchi T, Nawata H. Vitamin E restores reduced prostacyclin synthesis in aortic endothelial cells cultured with a high concentration of glucose. Metabolism 1992;41(6):613–21.
[211] Jialal I, Devaraj S, Kaul N. The effect of alpha-tocopherol on monocyte proatherogenic activity. J Nutr 2001;131(2):389S–94S.
[212] Wu D, Hayek MG, Meydani SN. Vitamin E and macrophage cyclooxygenase regulation in the aged. J Nutr 2001;131:382S–388S.
[213] Meydani SN, Meydani M, Verdon CP, Shapiro AA, Blumberg JB, Hayes KC. Vitamin E supplementation suppresses prostaglandin $E_{1(2)}$ synthesis and enhances the immune response of aged mice. Mech Ageing Dev 1986;34(2):191–201.
[214] Meydani SN, Lipman R, Blumberg JB, Taylor A. Dietary energy restriction decreases ex vivo spleen prostaglandin E_2 synthesis in Emory mice. J Nutr 1990;120(1):112–5.
[215] Meydani SN, Shapiro AC, Meydani M, Blumberg JB. Lung eicosanoid synthesis is affected by age, dietary fat and vitamin E. J Nutr 1992;122(8):1627–33.
[216] Kim JY, Lee KH, Lee BK, Ro JY. Peroxynitrite modulates release of inflammatory mediators from guinea pig lung mast cells activated by antigen–antibody reaction. Int Arch Allergy Immunol 2005; 137(2):104–14.
[217] Hennig B, Boissonneault GA, Chow CK, Wang Y, Matulionis DH, Glauert HP. Effect of vitamin E on linoleic acid-mediated induction of peroxisomal enzymes in cultured porcine endothelial cells. J Nutr 1990;120(4):331–7.
[218] Li D, Saldeen T, Mehta JL. Effects of alpha-tocopherol on ox-LDL-mediated degradation of IkappaB and apoptosis in cultured human coronary artery endothelial cells. J Cardiovasc Pharmacol 2000; 36(3):297–301.
[219] Fazzio A, Marilley D, Azzi A. The effect of alpha-tocopherol and beta-tocopherol on proliferation, protein kinase C activity and gene expression in different cell lines. Biochem Mol Biol Int 1997; 41(1):93–101.
[220] Breyer I, Azzi A. Differential inhibition by alpha- and beta-tocopherol of human erythroleukemia cell adhesion: role of integrins. Free Radic Biol Med 2001;30(12):1381–9.

[221] Weber C, Erl W, Pietsch A, Weber PC. Aspirin inhibits NF-κB mobilization and monocyte adhesion in stimulated human endothelial cells. Circulation 1995;91:1914–7.
[222] Neuzil J, Svensson I, Weber T, Weber C, Brunk UT. Tocopheryl succinate-induced apoptosis in Jurkat T cells involves caspase-3 activation and both lysosomal and mitochondrial destabilisation. FEBS Lett 1999;445:295–300.
[223] Neuzil J, Weber T, Gellert N, Weber C. Selective cancer cell killing by α-tocopheryl succinate. Br J Cancer 2001;84:87–9.
[224] Christen S, Woodall AA, Shigenaga MK, Southwell-Keely PT, Duncan MW, Ames BN. Gamma-tocopherol traps mutagenic electrophiles such as NO(X) and complements alpha-tocopherol: physiological implications. Proc Natl Acad Sci U S A 1997;94(7):3217–22.
[225] Jones PJ, MacDougall DE, Ntanios F, Vanstone CA. Dietary phytosterols as cholesterol-lowering agents in humans. Can J Physiol Pharmacol 1997;75(3):217–27.
[226] Vanstone CA, Raeini-Sarjaz M, Jones PJ. Injected phytosterols/stanols suppress plasma cholesterol levels in hamsters. J Nutr Biochem 2001;12(10):565–74.
[227] Quilez J, García-Lorda P, Salas-Salvado J. Potential uses and benefits of phytosterols in diet: present situation and future directions. Clin Nutr 2003;22(4):343–51 [review].
[228] Ho SS, Pal S. Margarine phytosterols decrease the secretion of atherogenic lipoproteins from HepG2 liver and Caco2 intestinal cells. Atherosclerosis 2005;182(1):29–36.
[229] de Jongh S, Vissers MN, Rol P, Bakker HD, Kastelein JJ, Stroes ES. Plant sterols lower LDL cholesterol without improving endothelial function in prepubertal children with familial hypercholesterolaemia. J Inherit Metab Dis 2003;26(4):343–351.
[230] Moreno JJ. Effect of olive oil minor components on oxidative stress and arachidonic acid mobilization and metabolism by macrophages RAW 264.7. Free Radic Biol Med 2003;35(9):1073–81.
[231] Cert A, Moreda W, Garcia-Moreno J. Determination of sterols and triterpenic dialcohols in olive oils using HPLC separation and GC analysis. Standardization of the analytical method. Grasas Aceites 1997;48(4):207–18.
[232] Liu J. Pharmacology of oleanolic acid and ursolic acid. J Ethnopharmacol 1995;49(2):57–68.
[233] Herrera MD, Rodriguez-Rodriguez R, Ruiz-Gutierrez V. Functional properties of pentacyclic triterpenes contained in "orujo" olive oil. Curr Nutr Food Sci [in press].
[234] Simon A, Najid A, Chulia AJ, Delage C, Rigaud M. Inhibition of lipoxygenase activity and HL60 leukemic cell proliferation by ursolic acid isolated from heather flowers (*Calluna vulgaris*). Biochim Biophys Acta 1992;1125(1):68–72.
[235] Ringbom T, Segura L, Noreen Y, Perera P, Bohlin L. Ursolic acid from *Plantago major*, a selective inhibitor of cyclooxygenase-2 catalyzed prostaglandin biosynthesis. J Nat Prod 1998;61(10):1212–5.
[236] Leu YL, Kuo SM, Hwang TL, Chiu ST. The inhibition of superoxide anion generation by neutrophils from *Viscum articulactum*. Chem Pharm Bull (Tokyo) 2004;52(7):858–60.