REVIEW

Efficacy of bioactive compounds from extra virgin olive oil to modulate atherosclerosis development

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As olive oil is the main source of calories in the Mediterranean diet, a great deal of research has been devoted to characterizing its role in atherosclerosis. Virgin olive oil is an oily matrix that contains hydrocarbons, mainly squalene; triterpenes such as uvaol, erythrodiol, oleanolic, and maslinic acid; phytosterols; and a wide range of phenolic compounds comprising simple phenols, flavonoids, secoiridoids, and lignans. In this review, we analyze the studies dealing with atherosclerosis and olive oil in several species. A protective role of virgin olive oil against atherosclerosis has been shown in ApoE-deficient mice and hamsters. In the former animal, sex, dose, and dietary cholesterol are modulators of the outcome. Contradictory findings have been reported for rabbits, a circumstance that could be due to the profusion of experimental designs, differing in terms of doses and animal strains, as well as sources of olive oils. This role has yet to be fully validated in humans. Minor components of olive oil have been shown to be involved in atherosclerosis protection. Nevertheless, evidence of the potential of isolated compounds or the right combination of them to achieve the antiatherosclerotic effect of virgin olive oil is inconclusive and will undoubtedly require further experimental support.

Keywords:
Atherosclerosis / Hydroxytyrosol / Phenolic compounds / Squalene / Virgin olive oil

1 Introduction

The "Seven Countries" study evidenced low cardiovascular mortality in the Mediterranean region in comparison with other countries after a 25-year follow-up of 11 579 men, and it was proposed that diet could be one of the reasons. The same study also showed that a high intake of fat, mainly from olive oil, was not associated with elevations of plasma cholesterol [1]. This and other epidemiological studies [2] and several intervention trials indicate that the Mediterranean diet decreases the risk of coronary heart disease in a number of populations [3]. These studies are raising an enormous interest in identifying the components and mechanisms involved in the protection against cardiovascular disease. In traditional Mediterranean diets, the main source of fat was olive oil accompanied by a high consumption of plant foods (fruits, vegetables, cereals, legumes, nuts, etc.). Dairy products, mainly yogurt and cheese, were eaten in low-to-moderate amounts, as were fish and poultry. Red meat eaten sparsely and moderate wine only consumed with meals complete the description of this diet. In terms of nutrient composition, this pattern provides an interesting balance of undesirable (saturated and trans fatty acids, cholesterol) and desirable nutrients (dietary fiber, complex carbohydrates, monounsaturated fatty acids (MUFAs), vitamins, minerals, minor components), and a low energy density [4]. Due to the favorable effects observed with this dietary pattern and since olive oil is the main source of fat calories, a great deal of research has been devoted to characterizing its role in the development of different diseases [5]. Since atherosclerosis is the culprit in the majority of cardiovascular diseases [6], several experiments, mainly in

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Abbreviations: MA, maslinic acid; **NF**, nuclear factor; **NO**, nitric oxide; **OA**, oleanolic acid; **TNF**, tumor necrosis factor; **VOO**, virgin olive oil

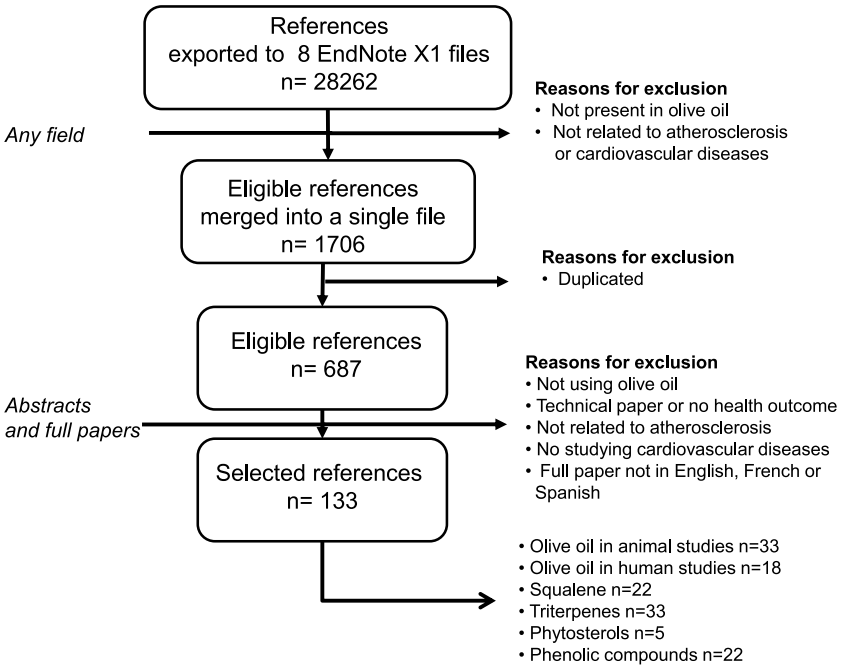

Figure 1. Flow chart displaying the stages used to select the references considered. EndNote X1 (Thomson Reuters, New York, NY).

Table 1. Composition of virgin olive oils

Component	Content (g%)
Triglyceride fatty acids	
Myristic (14:0)	0.0–0.05
Palmitic (16:0)	7.5–20
Palmitoleic (16:1*n*7)	0.3–3.5
Margaric (17:0)	0–0.3
Heptadecenoic (17:1)	0.0–0.3
Stearic (18:0)	0.5–5.0
Oleic (18:1*n*9)	55–83
Linoleic (18:2*n*6)	3.5–21
α-linolenic (18:3*n*3)	0.0–0.9
Arachidic (20:0)	0.0–0.6
Eicosenoic (20:1*n*9)	0.0–0.4
Behenic (22:0)	0.0–0.2
Lignoceric (24:0)	0.0–0.2
Minor components	
Hydrocarbons	
Squalene	0.1–0.8
Carotenes	0.05–0.1
Triterpenes	
Oleanolic acid	0.0008–0.01
Maslinic acid	0.0004–0.005
Erythrodiol	0.0006–0.008
Uvaol	0.0001–0.002
Phytosterols	0.1–0.2
Phenolic compounds	0.05-0.2

Adapted from Refs. [9–12, 153–155].

ApoE-deficient mice and rabbits, as well as in other animal models and in humans, have addressed the effect of olive oil on this entity. The present report has tried to adhere to systematic review guidelines [7]. As displayed in Fig. 1, a search in PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) using the eight keywords (monounsaturated oils, olive oil, oleanolic acid (OA), maslinic acid (MA), squalene, erythrodiol, uvaol, phenolic compounds, and phytosterols) identified 28 262 hits from November 1945 to December 2011. The search was refined by adding "atherosclerosis" or "olive oil" when required, and the resulting database was purged by eliminating duplicate documents. The 687 papers obtained were critically reviewed to verify that olive oil was used, that atherosclerosis was evaluated, or that cellular or molecular mechanisms involved in atherosclerosis were studied. Documents that failed to meet any or all of these criteria were discarded. Thus, this review covers the works related to the effects of olive oil and its components on atherosclerosis in 133 papers.

2 Virgin olive oil (VOO) composition

The first-press juice from the fruit of *Olea europea* is referred to as VOO. When its acidity is below 0.8%, its peroxide content lower than 20 meq O_2/kg, and it has certain organoleptic characteristics reaching a score higher than 6.5, verified by professional tasters, it is classified as extra VOO [8]. VOO is composed of two fractions, saponifiable and nonsaponifiable. The saponifiable fraction, representing 98.5–99.5% of oil, is formed by triglycerides esterifying mainly oleic acid and moderate quantities of other fatty acids (Table 1) [9]. As shown in Table 1 and Fig. 2, the nonsaponifiable fraction, or minor components of VOO, contains a great variety of compounds that represent its hallmark [10]. Hydrocarbons are their main constituents and among these, squalene, followed by carotenes (lutein and β-carotene) in

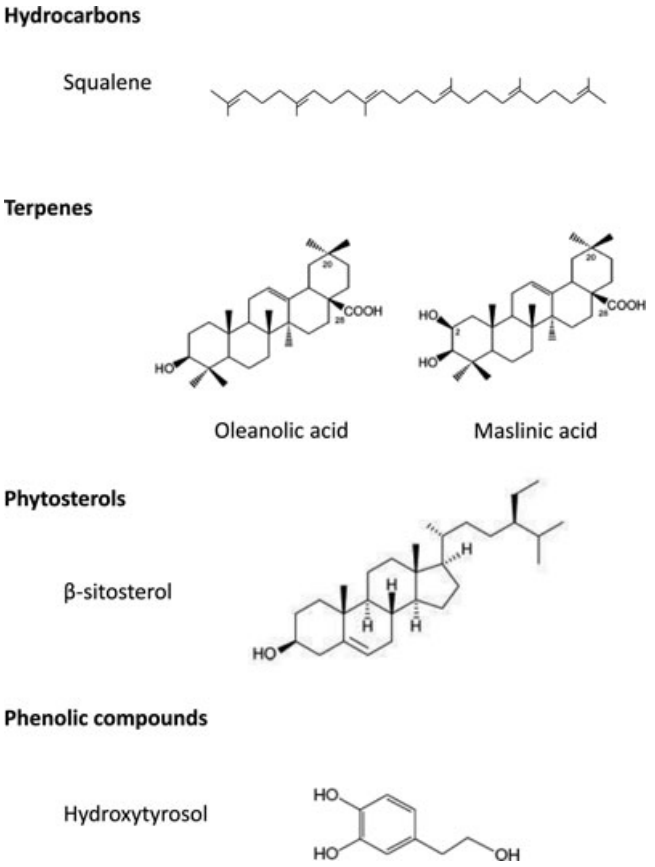

Figure 2. Chemical formulas of representative compounds for each group of minor olive oil components.

smaller amounts [11]. The triterpenes also represent one of the most abundant fractions and may differ according to plant variety. Likewise, two classes of triterpenic compounds are present, dialcohols (uvaol and erythrodiol) and triterpenic acids (oleanolic and maslinic) (Table 1) [12]. Phytosterols such as β-sitosterol, Δ^5-avenasterol, and campesterol are also present [13]. The phenolic compounds, representing the polar fraction, influence the stability and flavor of VOO [14]. Among them, four main groups can be distinguished: (i) simple phenols, either alcohols (tyrosol and hydroxytyrosol) or acids (p-coumaric, vanillic, caffeic, sinapic, protocatechuic, gallic, syringic); (ii) flavonoids (luteolin and apigenin); (iii) secoiridoids (ester derivatives of elenolic acid – glycosylated [oleuropein] or nonglycosylated – with hydroxytyrosol and tyrosol); and (iv) the lignans (+)-pinoresinol and (+)-1-acetoxypinoresinol [15]. Hydroxytyrosol is the most abundant phenolic compound [11]. Part of the nonsaponifiable fraction consists of α-, β-, and γ-tocopherols, present in different quantities depending on the olive grove variety [16]. In view of this complexity, the nature and source of olive oil should be clearly identified.

3 Atherothrombosis

Atherosclerosis is the main cause of all manifestations of cardiovascular diseases such as ischemic heart disease, cerebral infarction, gangrene, and loss of function in the extremities. Atherogenesis develops fundamentally in three stages: dysfunction of the vascular endothelium, fatty streak, and fibrous cap formation. The most widely accepted hypothesis for the etiology of atherosclerosis presupposes that the initial lesion occurs in response to injured endothelium due to physical stress, exposure to toxins such as oxidized cholesterol, or infectious agents. This results in endothelial dysfunction, defined as decreased endothelium-dependent vasodilatation due to a reduced availability of nitric oxide (NO). Dysfunctional endothelial cells show increased expression of specific cytokines and adhesion molecules, and recruit monocytes and T-lymphocytes. These migrate through endothelial cells and localize in the subendothelial space, where the monocytes differentiate into macrophages. Uptake of oxidized lipoproteins by macrophages, via scavenger receptors, leads to formation of large foam cells. These, together with T-lymphocytes, release mediators stimulating the proliferation of smooth muscle cells, and all three cell types together form the so-called fatty streak stage. Finally, an excessive inflammatory-fibroproliferative response of macrophages and smooth muscle cells ends up forming an atherosclerotic plaque with a fibrous cap [17]. The rupture of this plaque facilitates the formation of thrombi that result in cardiovascular diseases [18].

4 Effect of VOO on atherosclerosis development in animal models

4.1 ApoE-deficient mice

This model has been widely used to investigate the relationship of atherosclerosis to many genetic and environmental factors [19]. As reflected in Table 2, in the absence of dietary cholesterol, a wide range of VOO doses have been used, those of 6.4 μg/mouse [20] and 300 mg/mouse/day [21] being particularly effective, in contrast to that of 600 mg/mouse/day, which showed no benefit compared with chow diets [22]. These results suggest that a dose–effect relationship may exist. Using the 300-mg/mouse/day dose, females were more susceptible to the action of VOO [21], a finding that might point to sex-specific effects.

An interaction with dietary cholesterol seems to exist since, in presence of 0.1% cholesterol, no difference was observed between chow and VOO groups in terms of atherosclerotic lesions, and the values were higher than in animals receiving diets without cholesterol [22]. In the latter study, aortic lesions were inversely correlated with circulating paraoxonase activity, particularly in males. These results and those

Table 2. Characteristics of studies dealing with the effects of olive oil on atherosclerosis in animal models

Animal model	Duration of intervention (days)	Other dietary components	Study design	Type of olive oil	Participants (*n*)	Daily dose (/animal)	Effect	Ref.
Hamster								
DSN1 Golden Syrian	112	Chow	Progression after 50 days on 3% cholesterol and 15% coconut	EVOO	30♂	1.5 g	Reduced atherosclerosis compared to coconut oil	[46]
Golden Syrian	70	Chow + 0.4% cholesterol	Progression	WOO	60♂	1 g	Reduced atherosclerosis compared to sunflower oil	[47]
Mice								
ApoE-KO	70	Chow	Progression	EVOO	18♀, 22♂	300 mg	Reduced atherosclerosis compared to chow in females	[21]
ApoE-KO	56	Chow	Progression	VOO	24*	6.4 μg	Reduced atherosclerosis compared to chow	[20]
ApoE-KO	70	Chow	Progression	EVOO	14♀, 22♂	600 mg	No change in atherosclerosis compared to chow	[22]
ApoE-KO	70	Chow + 0.15% cholesterol	Progression	EVOO	54♀	600 mg	Reduced atherosclerosis compared to palm diet	[25]
ApoE-KO	77	Chow	Progression	WOO	26♂	300 mg	Reduced atherosclerosis in unsaponifiable-enriched OO	[27]
Rabbits								
NZW rabbit	87	Chow + 0.5% cholesterol	Progression	OO	21♀	14 g	Reduced atherosclerosis compared to coconut oil	[30]
NZW rabbit	180	Chow + 0.1% cholesterol and 5% butter	Progression	OO	50♂	5 g	No change in atherosclerosis compared to coconut or butter	[36]
Dutch belted rabbit	56	Chow + 2% cholesterol	Progression	Heated OO	46♂	6 g	Reduced atherosclerosis compared to heated corn oil	[35]
NZW rabbit	42	Chow + 1% cholesterol	Progression	VOO	40♂	15 g	Reduced atherosclerosis compared to saturated fat	[33]
NZW rabbit	42	Chow	Progression	VOO	24♂	10 g	Reduced atherosclerosis compared to butter or corn oil	[34]

Table 2. Continued

Animal model	Duration of intervention (days)	Other dietary components	Study design	Type of olive oil	Participants (*n*)	Daily dose (/animal)	Effect	Ref.
NZW rabbit	90	Semipurified diet + 0.2% cholesterol	Progression	OO	28♂	13 g	Reduced atherosclerosis compared to coconut	[31]
Dutch belted rabbit	214	Semipurified diet	Progression	OO	30*	14 g	Reduced atherosclerosis compared to peanut oil	[32]
Danish country rabbit	90	Chow + 1.0% or 0.5% cholesterol	Progression with 20 mM plasma cholesterol	OO	36♂	10-15 g	Reduced atherosclerosis compared to control	[38]
Chinchilla rabbit	93	Chow + 2.2% or 1.1% cholesterol	Progression with 20 mM plasma cholesterol	OO	45♂	10 g	No effect compared to margarine and reduced compared to chow	[39]
Danish country rabbit	91	Chow + 2.2% or 1.1% cholesterol	Progression with 20 mM plasma cholesterol	OO	141♂	10 g	No effect compared to margarine, butter, lard or coconut	[40]
NZW rabbit	30	Semipurified diet	Regression after 50 days on 1.3% cholesterol and 3% lard	EVOO	30♂	1.75 g	Reduced atherosclerosis compared to sunflower oil	[41]
Watanabe rabbit	112	Chow	Progression	OO	22 ♀ and ♂	3 g	Increased atherosclerosis compared to fish oil	[37]
NZW rabbit	45	Chow +1% cholesterol	Progression	VOO	40♂	15 g	Antiatherogenic properties of olive oil polar lipids	[43]

Only those models having two studies have been considered. APO, apolipoprotein; EVOO, extra virgin olive oil; NZW, New Zealand White; OO, olive oil; VOO, virgin olive oil; WOO, washed olive oil. Progression is used to denote the estimation of atherosclerosis after a given treatment when the extent of atherosclerosis at the beginning of the experiment was unknown. The term "regression" is used to design studies that first induce atherosclerosis and then quantify its follow-up.
*Sex not indicated.

of Efrat et al. indicate that olive oil consumption increased HDL paraoxonase and macrophage cholesterol efflux [23], and through this action might determine its sex-dependent effect on atherosclerosis. Stimulated cholesterol efflux from peritoneal macrophages has been proposed by Rosenblat et al. [20], and reduced macrophage uptake of oxidized LDL via scavenger receptors was observed in mice consuming VOO [24]. Indeed, reduced macrophage recruitment and atherosclerotic lesions were observed in females consuming extra VOO compared to those receiving palm oil containing diets [25]. Interestingly, in this study, the decrease in atherosclerosis was accompanied by a profound hypercholesterolemia. These results evidence the discrepancy among some secondary markers (cholesterolemia) and the development of atherosclerosis, and the involvement of macrophages in VOO action.

Despite the general acceptance of the term "MUFA-containing oils" to group oils such as those obtained from almond, avocado, high oleic sunflower, olive, and peanut, these oils do not seem to behave similarly in protecting against the development of atherosclerosis in animal models [26]. These discrepancies can be attributed to the presence of the minor components of VOO, and much attention has been paid to the presence of soluble phenolic compounds [15]. The relevance of other components of VOO was addressed by preparing two olive oils using different technological

procedures so that, without phenolic compounds, they had similar levels of MUFA and squalene, but they differed in their content of linoleic acid, phytosterols, tocopherols, triterpenes, and waxes. ApoE-deficient mice consuming oil enriched in those components as 10% (w/w) of their diet showed reduced atherosclerotic lesions compared to those not receiving enriched olive oil [27]. Therefore, the antiatherosclerotic effect of VOO may also be due to compounds other than phenolics, and needs to be explored. The possibility of using extra VOO as vehicle to transfer other components and to test their effect on atherosclerosis has also been studied. In this regard, enrichment with green tea polyphenols [20] or seal oil [28] significantly reduced atherosclerotic lesion formation through antithrombotic, antihypertriglyceridemic, and antioxidant actions. To summarize, the use of this animal model has confirmed the antiatherosclerotic properties of VOO, the existence of a therapeutic range, and the influence of sex (Table 2). Likewise, an interaction with dietary cholesterol exists since the VOO effect was lost when both were administered. The combined antiatherosclerotic action of minor components, such as phytosterols, tocopherols, triterpenes, and waxes, indicates that these may be relevant in olive oil properties.

4.2 Rabbits

The rabbit represents a classical model in atherosclerosis research, with a tradition that dates back to the beginning of the 20th century [29]. With a regimen of 14% of dietary fat and 0.2% cholesterol, olive oil-fed rabbits developed less severe hyperlipidemia and atherosclerosis than those receiving the coconut oil containing diet [30]. With similar percentages of fat, olive, and avocado oils were less atherogenic than coconut oil [31], and olive oil was associated with significantly lower levels of aortic atherosclerosis than the two peanut oil fed groups, despite the higher levels of serum lipids [32]. With a slightly higher amount of VOO (15%, w/w), De la Cruz et al. observed less-severe vascular lesions compared with those produced by a saturated fatty acid-enriched diet [33]. Using 10% (w/w) olive oil supplementation, Bayindir et al. reported vascular improvement [34]. With a lower amount of fat (6%, w/w) in diets including heated corn or olive oil, in presence of 2% cholesterol, Kritchevsky and Tepper reported a decrease in atherosclerosis in the olive oil group [35]. However, when 5% was used, the olive oil effect was lost compared to coconut oil [36]. All these results indicate that administration of olive oil is more favorable than saturated fat and that not all MUFA-containing oils (avocado, olive, or peanut) behave in similar ways. According to these data (Table 2), a threshold of 6 g/animal would be the dose required to observe any positive effect. Not surprisingly, when Watanabe heritable hyperlipidemic rabbits received a lower dose, an increased development of aortic atherosclerosis was observed [37].

In radical experimental designs with established atherosclerosis, using rabbits maintained at plasma cholesterol levels of about 20 mM over a period of 12 weeks and then separated into groups receiving 10–15% fat or control diet, the aortic cholesterol concentrations in the olive oil group were significantly lower than the level in the control group [38]. With a similar experimental design, no differences were found between 10% olive oil and 10% margarine [39], nor were any differences detected when olive oil was compared with butter, lard, and coconut oil at such extreme levels of plasma cholesterol [40]. However, in male New Zealand rabbits, replacement of a high cholesterol-saturated (1.3%) fat diet (3% lard) by another enriched with 1.75% extra VOO stopped the progression [41], a consequence that was found to be associated with an enhancement of the hepatic antioxidant defense system [42, 80]. These results indicate the marked influence of the experimental design on the outcome and, in this setting, there were no clear differences between olive oil and other fats or oils.

When olive oil [43] and pomace [44] polar lipid extracts were administered to rabbits to investigate the influence of VOO components, the results were decreased platelet aggregation and reduced vascular lesions compared to animals receiving the neutral lipid extract. These polar extracts were particularly enriched in a glycerylether-sn-2-acetyl glycolipid that could be responsible for the reported action [45], an interesting observation that requires confirmation in other models. In conclusion, the antiatherosclerotic effects of olive oil in this model (Table 2) show a dose-dependent response and are sensitive to differences in the experimental approach, such as source of fat, strains of rabbits, preparation of olive oils, type of olive oil (virgin or not), etc.

4.3 Other animal models

In hamsters fed 15% coconut oil and 3% cholesterol for 3 months to induce initial atherosclerotic lesions and then switched to olive oil or coconut oil diets for 16 weeks, the former group showed decreased lesion size [46]. In the absence of added cholesterol, Golden Syrian hamsters fed on olive oil (15% wt/wt) also showed reduced atherosclerotic lesions compared to animals fed on coconut oil. Again, in hamsters fed 10% olive oil or sunflower oil plus 0.4% cholesterol (wt/wt) for 10 weeks (Table 2), aortic cholesterol ester was reduced in the olive oil groups relative to the sunflower oil, an effect associated with reduced oxidative stress [47]. The antiatherosclerotic effect of olive oil was also found in atherosclerosis-prone Japanese quail fed 2% olive or fish oils for 9 months [48]. In male Wistar rats, administration of 10% v/w VOO reduced aortic atherosclerotic lesions compared with the sunflower or fish oil treated groups [49]. A favorable pattern of thrombosis prevention characterized by increased plasma 6-ketoprostaglandin $F_{1\alpha}$ and decreased serum thromboxane B_2 was observed in rats fed olive oil compared to those fed sunflower oil [50]. A threshold of 10% w/w olive oil in diets was required to elicit an increase in APOA1 in this animal [51]. In Wistar-Kyoto and spontaneously hypertensive rats, only the chronic feeding of VOO diet was capable of

modulating the vascular response of rat aorta compared with high oleic acid sunflower diet; this model also reinforces the role of minor components in the vascular protective effect [52]. The above results substantiate the positive antiatherosclerotic effects of olive oil reported in these three animal models.

5 Effect of VOO on atherosclerosis development in humans

5.1 Epidemiological studies

Case–control studies have demonstrated an inverse association between olive oil and myocardial infarction [53]. In a Greek population, exclusive use of olive oil was also associated with a 47% lower likelihood of having acute coronary syndrome compared to nonuse, after adjusting for several physical and lifestyle characteristics and different diseases [54]. In subjects at high cardiovascular risk, an inverse association was observed between olive oil consumption and the carotid intima-media thickness, a fact that could suggest a protective role of olive oil against the development of carotid atherosclerosis [55] and could be due to changes in plasma phospholipids [56]. Thus, end points and carotid atherosclerosis are associated with olive oil intake.

5.2 Clinical trials

In asymptomatic high cardiovascular risk subjects, those with carotid intima-media thickness greater than 0.9 mm showed a significant decrease when they consumed a diet enriched with VOO for one year [57]. However, in a small number of patients with angiographically documented coronary heart disease and normal plasma lipid levels, a clinical trial using either 6 g of fish oil or olive oil capsules for 28 months revealed that coronary atherosclerosis did not improve with either oil [58]. Also, in a clinical trial involving patients with peripheral vascular disease (Fontaine stage II), a 3-month intervention with VOO was not found to produce clinical benefit, despite the reduced susceptibility of LDL to oxidation [59]. When refined olive oil was administered, no reduction in LDL oxidation was noted in the same type of patients, suggesting that minor compounds were involved in the action [60]. These results indicate that the different sites of atherosclerosis might display different susceptibilities to VOO administration, and that no surrogate marker exists to easily monitor progress. Other issues raised in animal studies such as doses and period of administration are open questions.

5.3 Ex vivo studies

More prolific have been the studies addressing the effect of olive oil on intermediate parameters related to atherosclerosis. In this regard, in human umbilical endothelial cells incubated with sera of subjects consuming different diets, a diet enriched with VOO induced lower intracellular reactive oxidative species production, cellular apoptosis, and percentage of cells with telomere shortening, compared with the saturated or carbohydrate diets [61]. The results indicate that this diet protects the cells from oxidative stress, prevents cellular senescence, and reduces cellular apoptosis. Interestingly, this diet has been shown to induce plasma brain-derived neurotrophic factor in depressed patients [62]. Olive oil was found to reduce susceptibility of lipoproteins to oxidation [63], expression of adhesion molecules [64], and other inflammatory factors [65]. As a consequence, it may inhibit monocyte recruitment and transformation into foam cells [66], and control blood platelet function, blood coagulability, and fibrinolytic activity [67] in normal subjects [68] and in patients with different diseases [69]. Collectively, and in agreement with other authors [70], the knowledge of the effects of VOO and its components on human atherosclerosis is very limited at this time and further studies considering these inconclusive reports are required.

6 Role of minor VOO components in atherosclerosis development

6.1 Effect of squalene

As mentioned, squalene represents the main minor component of VOO and, therefore, its properties warrant an explanation [71]. The early observations that small amounts of this compound were present in normal aorta and in atherosclerotic plaques of humans and rabbits [72–76] raised an open discussion regarding its role at these sites. Recent works have even fueled the debate after finding squalene accumulation in normal and stenotic human aortic valves [77] and higher concentrations of serum squalene in patients with familial hypercholesterolemia [78] or coronary artery disease [79]. These authors also found elevated ratios of serum squalene to cholesterol, leading them to propose that reduced cholesterol synthesis may be related to coronary atherosclerosis [80]. On the other hand, experimental administration of a 3% squalene-containing diet for 7 wk to rabbits showed the absence of changes in atheroma development, despite an increase in cholesterol-rich lipoproteins [81]. Squalene administration also reversed endothelial activation and lower cellularity in gingival mucosa of atherosclerotic rabbits [82]. Likewise, 1 g/kg/day squalene feeding for 10 weeks reduced atherosclerotic lesion size in male ApoE-deficient mice. In this case, the changes were independent of plasma lipids and associated with decreased hepatic fat content. In contrast, squalene intake did not decrease lesion area in females. These data suggest that squalene administration modulates lesion development in a sex-specific manner and that it could be used as a safe alternative to correct hepatic steatosis and atherosclerosis, particularly in males [83]. Moreover, a cardioprotective

action has been reported in male albino rats in which prior administration of a 2% squalene-containing diet for 45 days effectively prevented isoproterenol-induced myocardial infarctions, mainly by blocking lipid peroxidation and through a hypolipidemic action [84, 85]. Similarly, squalene acted as a cytoprotectant capable of attenuating cyclophosphamide-induced alterations in rat myocardium [86]. In this animal, squalene feeding significantly decreased blood pressure and body weight gain as well [87]. Based on these findings, it could be suggested that squalene is a promising agent in cardiovascular prevention.

Equally controversial has been the effect of squalene on plasma lipids in humans and animals. While some authors did not observe any increase in plasma cholesterol [87–89], others did [90, 91]. A less-explored hypothesis is that squalene may have an antiinflammatory effect since it inhibited the myeloperoxidase activity induced in auricular edema of mice exposed to either arachidonic acid or 12-*O*-tetradecanoylphorbol acetate [92]. Overall, these studies indicate that squalene administration may play a cardioprotective role that needs to be confirmed in different experimental approaches, species, and sexes before being considered an important contributor to the action of VOO.

6.2 Effect of triterpenes: erythrodiol, uvaol, oleanolic, and maslinic acids

A great number of experiments have been carried out to elucidate their effect on atherothrombotic risk factors such as lipid profile, oxidative stress, hyperglycemia, endothelial dysfunction, hypertension, and inflammation.

Antihyperlipidemic effects of OA have been demonstrated in Wistar [93] and in Dahl salt-sensitive rats [94]. In this regard, OA has been found to decrease hepatic expression levels of lipogenic genes such as acetyl-CoA carboxylase and glycerol-3-phosphate acyltransferase [95]. OA administration also increased the expression of genes related to cell proliferation and suppressed the expression of several cytochrome P450 genes, possibly to switch cellular metabolic energy to an acute-phase response [96]. A change in expression of cytochrome P450 genes has been observed in response to treatment with MA as well [97]. These results indicate that dietary triterpenes are highly active in controlling hepatic gene expression.

MA has shown a potent dose-dependent antioxidant effect on LDL peroxidation [98]. It has exhibited antioxidant properties against lipid peroxidation in vitro, and also reduced the generation of hydrogen peroxide by stimulated macrophages in a dose-dependent manner [99]. OA has also shown a remarkable protection against in vitro lipid peroxidation in isolated rat liver microsomes [100, 101]. Similar results have been obtained for uvaol and erythrodiol [98, 102]. The potential antioxidant effect could be explained by the increases in glutathione peroxidase and superoxide dismutase activities [103], by the membrane-stabilizing action [93] [104], and by the improvement of mitochondrial function [105]. In the case of OA, these antioxidant actions could be executed by nuclear factor erythroid 2 p45-related factor 2 [106].

MA showed hypoglycemic effects by reducing insulin resistance in a mouse model of genetic type-2 diabetes [107]. Similar results were obtained for OA in Dahl salt-sensitive rats [94, 103] and in obese mice [108]. OA may promote insulin signal transduction and inhibit oxidative stress-induced hepatic insulin resistance and gluconeogenesis [109].

Consumption of high triterpene content olive oil improved endothelial function in spontaneous hypertensive rats [110] by improving the agonist-mediated NO response [111]. OA, MA, uvaol, and erythrodiol showed vasodilatory properties, also mediated by the endothelial production of NO [112–116] or increased release of prostaglandin I_2 [117]. In addition, these compounds exert diuretic and antidysrhythmic effects, so they may control hypertension and cardiac ischemia [94, 103, 118]. OA protected against rat myocardial ischemic injury, and the protective effect could be attributed to its antiarrhythmic properties, as well as to its membrane-stabilizing action [93] and a mitochondrial increase in reduced glutathione [119]. Allouche et al. have shown that uvaol and erythrodiol significantly decreased thrombin formation [98], and they also inhibit cell proliferation in a dose- and time-dependent manner [102]. Finally, OA has been found to reduce hydrogen peroxide-induced cell apoptotic death of vascular smooth muscle cells by enhancing HO-1 expression and activity in a concentration- and time-dependent manner [120]. The above results indicate that these compounds have interesting therapeutic potential as cardiovascular drugs.

Pentacyclic triterpenes exhibited pro- and antiinflammatory properties depending on the chemical structure and dose [121]. Interestingly, MA significantly inhibited the enhanced production of NO induced by lipopolysaccharides. This inhibitory effect was exerted by modulating inducible NO synthase gene expression [99]. In the human monocyte cell line, THP-1, incubated with triacylglycerol-rich lipoproteins of subjects with high OA intake, there was a decrease in the secretion of monocyte chemoattractant protein-1, which was associated with a decrease in cyclooxygenase-2 gene expression [122]. Likewise, analogues of OA were powerful inhibitors of cellular inflammatory processes such as the induction of NO synthase and cyclooxygenase 2 by IFN-γ [123] or release of phospholipase A_2 [124]. Overall, these facts support an antiatherogenic potential of dietary triterpenes through modulation of different proatherogenic risk factors. In fact, a high dose of OA (100 mg/kg) has been shown to reduce atherosclerotic lesions in ApoE-deficient mice by decreasing inducible NO synthase [125].

6.3 Effect of phytosterols

These compounds have been widely studied [126]. Animal studies suggest that they reduce atherosclerosis [127], but this association has not been addressed in humans [128].

Reviews of their effects on cholesterol metabolism [129] and cardiovascular risk factors [130] have also been published.

6.4 Effect of phenolic compounds

Much of the research focused on explaining the reduced cardiovascular mortality and morbidity experienced by people consuming the Mediterranean diet has been concentrated on olive oil phenolic compounds [131]. These compounds have been shown to possess strong antioxidant and free-radical scavenging activity in vitro. In addition, they are absorbed by a pathway independent of chylomicron formation [132, 133]. A great deal of experimental work has also evidenced potential roles for these compounds in protecting the cardiovascular system: (i) by direct antioxidant defense, especially against lipid peroxidation and DNA oxidative damage [134]; (ii) by preventing endothelial dysfunction, including increased NO production and inducible NO synthesis, as well as reduced endothelial adhesion molecule expressions, and quenching vascular endothelium intracellular free radicals [135]; (iii) by inhibiting platelet-induced aggregation and thrombi generation [136]; (iv) by enhancing the mRNA transcription of the enzymes involved in antioxidant defense [137]; (v) by inducing apoptosis and antiproliferative effects [15]; and (vi) by modulating immune system function [138]; they are also capable of modifying homeostasis [139], exerting beneficial effects on the control of blood pressure [140].

Among these compounds, hydroxytyrosol and oleuropein have deserved special attention [141]. Oleuropein has antioxidant, antiinflammatory, antiatherogenic, anticancer, antimicrobial, and antiviral activities, and hypolipidemic and hypoglycemic effects [142]. The antiinflammatory action of oleuropein aglycone in prevention of tumor necrosis factor (TNF)-α stimulation of metalloproteinase-9 was reported to be due to the impairment of NF-kappaB (where NF is nuclear factor) signaling [143]. The latter cascade in mononuclear cells has been targeted in subjects consuming VOO [144] to decrease expression of the p65 subunit of NF-kappaB [145] and proinflammatory cytokines, interleukin-6, and TNF-α [146]. The modulation of metalloproteinases could be reflected in plaque stabilization.

6.5 Hydroxytyrosol and atherosclerosis

Hydroxytyrosol, the most abundant phenolic compound in VOO, has a powerful antioxidant activity both in vivo and in vitro [147] and is a better antioxidant and radical scavenger than oleuropein or tyrosol [148]. In porcine pulmonary artery endothelial cells, it has been shown to positively regulate the antioxidant defense system involving catalase [149].

So far, it is the only phenolic compound to be tested in this field. To verify the effect of hydroxytyrosol on the development of atherosclerosis, Acin et al. administered this compound, at a dose of 10 mg/kg/day, to male ApoE-deficient mice on a standard chow diet of low cholesterol content. They reported increased atherosclerotic lesion size, mainly associated with the degree of monocyte activation. These results indicate that phenolic-enriched products, outside the original matrix, could not only fail to be useful, but be harmful, and their formulation as possible functional foods should approximate as closely as possible the natural environment in which active molecules are found [150]. In rabbits, administration of hydroxytyrosol at 4 mg/kg body weight in presence of saturated fat and cholesterol reduced the size of atherosclerotic lesions when compared with animals receiving this diet without hydroxytyrosol [151]. Collectively, these works suggest that both the dose of hydroxytyrosol administered and the experimental model could be important. Potential interaction with dietary cholesterol or with other phenolic compounds should be further considered, as should the response in different animal models with special variants of lipid metabolism.

Randomized, controlled clinical trials in humans are required to provide evidence that olive oil phenolic compounds contribute significantly to health benefits to enable the establishment of recommendations for the general population [152].

7 Conclusions and outlook

In several animal models, VOO-enriched diets prevent the development of atherosclerosis as efficiently as a chow diet enriched in carbohydrates. There are sex- and dose-related effects. Moreover, dietary cholesterol interferes with the antiatherogenic effects of VOO, but even in presence of cholesterol, the latter is more effective than diets enriched in saturated fat. Minor components present in VOO play a determinant role in the antiatherogenic properties, either alone or in synergy with oleic acid containing triglycerides. Some triterpenes and phenolic compounds may be promising antiatherosclerotic agents, considering their effects on risk factors related to this disease. In view of these conclusions, new experiments are required to compare the efficacy of VOO versus polyunsaturated fatty acid containing diets, considering the influence of cholesterol and sex on the outcome. More work will be necessary to identify the minor components involved in their actions, their mechanisms of action and their interactions. Finally, the profusion of experimental approaches should be narrowed, with the determination of the most effective dose and the careful chemical characterization of VOOs employed.

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