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Review

Olive oil and the cardiovascular system

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Abstract

Olive oil is the primary source of fat in the Mediterranean diet which is associated with a low mortality for cardiovascular disease. In spite of this, data concerning olive oil consumption and primary end points for cardiovascular disease are scarce. However, a large body of knowledge exists providing evidence of the benefits of olive oil consumption on secondary end points for cardiovascular disease. The benefits of olive oil consumption are beyond a mere reduction of the low density lipoprotein cholesterol. Here, we review the state of the art concerning the knowledge of the most important biological and clinical effects related to the intake of olive oil rich diets on lipoprotein metabolism, oxidative damage, inflammation, endothelial dysfunction, blood pressure, thrombosis, and carbohydrate metabolism. The extent to which we possess evidence of the health benefits of olive oil minor components is also assessed. The wide range of anti-atherogenic effects associated with olive oil consumption could contribute to explain the low rate of cardiovascular mortality found in Southern European Mediterranean countries, in comparison with other western countries, despite a high prevalence of coronary heart disease risk factors.

Keywords: Olive oil; Monounsaturated fatty acids; Phenolic compounds; Cardiovascular disease

Contents

1. Background

Coronary heart disease (CHD) is the main individual cause of death and morbidity in industrialized countries. Myocardial infarction incidence rates, however, present a high regional variability, with rates lower in Mediterranean European countries than those reported in northern European ones, the U.S.A, or Australia [1]. Paradoxically, this low myocardial infarction incidence occurs in spite of a high prevalence of classical cardiovascular risk factors [2]. Protective factors, such as the Mediterranean diet, might contribute to explain this paradox. Although a high degree of adherence to the traditional Mediterranean diet has been associated with a reduction in overall and cancer mortality, the most impressive benefits of this diet are related to cardiovascular morbidity and mortality [3–6]. Olive oil is the primary source of fat in the Mediterranean diet. In spite of this, data concerning olive oil consumption and primary end points for cardiovascular disease are scarce. In a

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hospital-based case–control study in a Spanish population, a high exposure to olive oil consumption was associated with a reduction in the relative risk of having a myocardial infarction [7]. However, in a large cohort study with Greek participants, associations between consumption of individual food groups of the Mediterranean diet, including olive oil, and CHD were generally non significant, although the ratio of monounsaturated lipids to saturated lipids reached an inverse significance [3]. In spite of the lack of data on primary end points, a large body of knowledge exists providing evidence of the benefits of olive oil consumption on secondary end points for cardiovascular disease.

The beneficial effects of olive oil on CHD risk factors are now recognized and often only attributed to its high levels of monounsaturated fatty acids (MUFA). On November 2004, the Federal Drug Administration (FDA) of the U.S.A permitted a claim on olive oil labels concerning: "the benefits on the risk of coronary heart disease of eating about two tablespoons (23 g) of olive oil daily, due to the monounsaturated fat (MUFA) in olive oil" [8]. Olive oil is, however, more than a MUFA fat. Olive oil is a functional food which besides having a high level of MUFA contains other minor components with biological properties [9]. In fact, oleic acid is one of the predominant fatty acids in foods of animal origin which are widely consumed in Western diets, such as poultry and pork [10]. In a Swedish study, oleic acid plasma levels correlated with the meat intake, and were higher in a female population from Malmö than in females from Spain, without differences in polyunsaturated fatty acids (PUFA) levels [11]. Thus, it is plausible that a high oleic acid intake is not the sole primary responsible agent for the healthy properties of olive oil. The minor components of olive oil, which constituted only 1–2% of the total content of a virgin olive oil, are classified into two types: the unsaponificable fraction, defined as the fraction extracted with solvents after the saponification of the oil, and the soluble fraction which includes the phenolic compounds [9]. Components of the unsaponifiable fraction of olive oil by order of their increasing polarity are: hydrocarbons (squalene), tocopherols, fatty alcohols, triterpenic alcohols, 4-methylsterols, sterols, other terpenic compounds, and polar pigments (chlorophylls and pheophytins) [9]. Here, we review the state of the art concerning the knowledge of the most important biological and clinical effects related to the intake of diets rich in olive oil/MUFA, and the extent to which we possess evidence of the health benefits of olive oil and its minor components.

2. Lipoprotein metabolism

2.1. Plasma cardiovascular risk lipid profile

The plasma cholesterol-predictive equations, developed in the mid-1960s by Keys [12] and Hegsted et al. [13] from data of controlled diet studies, showed that consumption of MUFA did not affect total cholesterol levels, but the consumption of saturated fatty acids (SFA) raised them. Consumption of PUFA lowered total cholesterol half as much as SFA raised it. More recent analyses have confirmed these findings, although there were some data that in MUFA consumption the low-density lipoproteins (LDL) cholesterol-lowering effect was less, whereas the high density lipoprotein cholesterol (HDL) was higher, than those observed for PUFA [14–16]. However, the results of a meta-analysis, of 14 studies carried out in the years 1983–1994, showed the replacement of SFA by oils enriched in MUFA versus PUFA had similar effects on total, LDL, and HDL cholesterol, whereas the PUFA-enriched oil had a slight triglyceride-lowering effect [17]. Thus, the hypocholesterolemic effects of replacing SFA by either MUFA or PUFA are comparable. The debate is thus centered on which the ideal substitute for SFA calories is: carbohydrates or unsaturated fatty acids, specifically MUFA when controlling weight conditions. A similar total cholesterol-lowering effect of both a high-fat diet (40% of energy) rich in MUFA and low in SFA, and a low-fat, carbohydrate-rich diet was reported in two different studies. Although both diets lowered total and LDL cholesterol, the high-MUFA diet did not lower HDL cholesterol or increase triglycerides, as the carbohydrate-rich diet did [18,19]. These results were confirmed in further studies [20]. A meta-analysis of 10 studies performed in diabetic patients provided first level evidence of the benefits of MUFA-rich diets in front of carbohydrate-rich diets, not only for healthy, but also for diabetic individuals [21], as is referred to in cardiovascular prevention guidelines [22,23].

Postprandial lipemia has been recognized as a risk factor for atherosclerosis development as it is associated with oxidative changes [24]. Both the amount and the type of the fat ingested influence the postprandial lipemia. Dubois et al. [25] showed that increasing the amount of fat up to 50 g led to stepwise increases in the postprandial rise of the serum triglycerides, while the ingestion of 15 g fat had no effect on postprandial lipemia and lipoproteins in healthy adults. A 31 g fat meal induced considerably less variation in lipemia, chylomicrons, and lipoprotein lipids than a 42 g fat meal [26]. A 25 mL single dose of olive oil does not promote postprandial lipemia [27], whereas 40 mL and 50 mL doses do [28,29] with independence of the phenolic content of the olive oil (Fig. 1). Concerning the influence of the type of fat ingested on the postprandial lipemia, after an oral olive oil fat load the magnitude of postprandial lipemia and remnant lipoprotein has been reported to be lower than after ingestion of butter [30]; similar to that after eating a saturated fat-rich meal [31]; and higher than after the ingestion of safflower oil [32]. In other studies, however, comparisons of the effects of n-6 PUFA-rich oils with olive oil or MUFA-rich meals showed lower [33] or comparable [34,35] postprandial lipemia. Abia et al. [36] have reported that virgin olive oil intake resulted in lower postprandial triacylglyceride-rich-lipoprotein (TRL) levels and a faster TRL-TG disappearance from blood, than after high oleic acid sunflower oil intake. Chylomicrons formed after olive oil [36,37] or n-3 PUFA [38] ingestion seem to enter the circulation more rapidly, and to be cleared at a faster rate, than those formed after intake of fats rich in SFA or PUFA. Although fat intake appears to be the major nutritional determinant of the postprandrial triglyceride response, it is also influenced by other dietary components, including fibre, glucose, starch, and alcohol present in a meal [39].

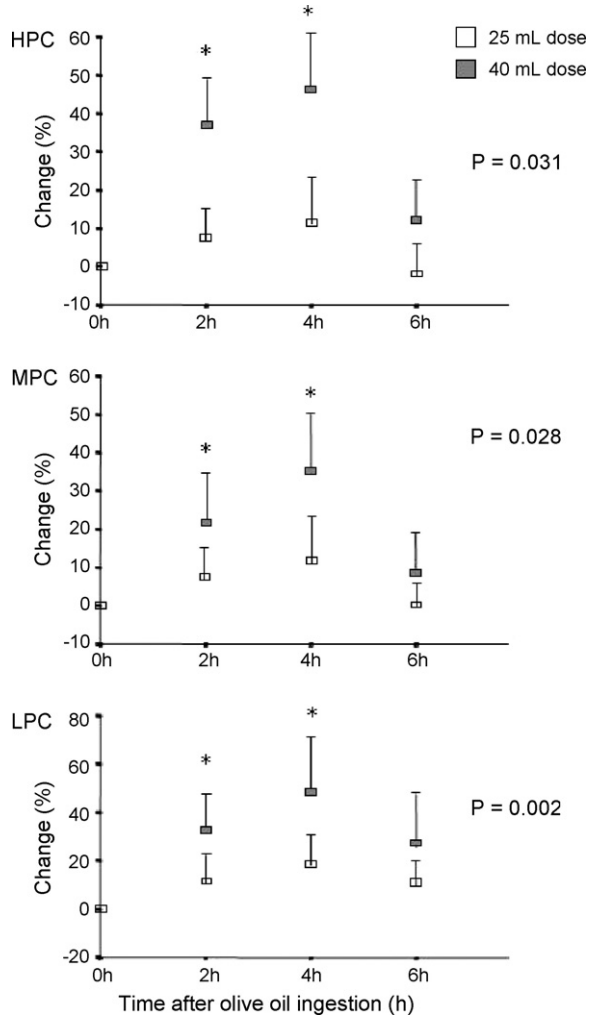

Fig. 1. Postprandial changes from baseline (0 h) after olive oils ingestion. HPC, MPC, and LPC, olive oils with high (>350 mg/kg), medium (120–170 mg/kg), and low (<10 mg/kg) phenolic content. *$P < 0.05$ versus baseline. P for quadratic trend after a 40 mL dose. Adapted from Weinbrenner T. et al. Drugs Exp Clin Med 2004 [27] and Covas M.I. et al. Free Rad Biol Med 2006 [29].

2.2. *Low density lipoprotein oxidation and oxidative damage*

The oxidative modification of LDL plays a key role in atherosclerosis and CHD development. Oxidation of the lipids and lipoproteins present in LDL leads to a change in the lipoprotein conformation by which LDL is better able to enter the monocyte/macrophage system of the arterial wall, and promote the atherosclerotic process [40]. It is currently thought that oxidized LDL is more damaging to the arterial wall than native LDL [41]. Elevated concentrations of circulating oxidized LDL show a positive relationship with the severity of acute coronary events [42,43]; are independently associated with carotid intima-media thickness [44]; and are predictors for CHD both in CHD patients [45] and in the general population [46].

Several studies have been performed comparing the effects of MUFA-rich diets on the susceptibility of LDL to oxidation with those of PUFA- or carbohydrate-rich diets. Oleate-rich LDL have been shown to be less susceptible to oxidation than linoleate rich LDL [47–53]. Compared with the carbohydrate-rich diets, the MUFA ones had a better effect [54–55], or a comparable one [56], on reducing the susceptibility of the LDL to oxidation. In this *in vitro* test the measured parameters are the diene formation, or the time to reach it [57]. PUFA, rich in double bounds, are more prone to form conjugated dienes than MUFA [57]. In many of these studies, instead of natural olive oil, prepared liquid-formula or solid diets highly enriched in MUFA were used. In spite of this, the consistency of the results among the studies leads to the idea that MUFA-rich diets are more protective for LDL in front of oxidation than PUFA-rich diets.

Olive oil minor components have been also involved in the antioxidant activity of olive oil. Although squalene or triterpenes have displayed antioxidant activity in experimental conditions [9], the antioxidant properties of the phenolic compounds have been the most extensively studied. In experimental studies, olive oil phenolic compounds, like other plant-derived polyphenols [58], show powerful antioxidant properties against LDL oxidation [59–61]. In animal models, olive oil phenolics retained their antioxidant properties *in vivo* [62] and delayed the progression of atherosclerosis [63]. The fact that phenolic compounds from olive oil are bioavailable in humans [64], even from doses (25 mL (22 g)/day) [65] lower than those reported as usual in the Mediterranean diet (30–50 g/day) [66], reinforces their possible protective role *in vivo*. Tyrosol (T) and hydroxytyrosol (HT), the major olive oil phenolic compounds [67], are dose-dependently absorbed from olive oil [64,65]. Due to this, they can be used as biomarkers of olive oil consumption, a useful tool for monitoring compliance in clinical studies. Around 98% of T and HT are present in plasma and urine in conjugated forms, mainly glucuronoconjugates, suggesting an extensive first pass intestinal/hepatic metabolism of the ingested primary forms [68,69]. Due to this, the bioactivity of olive oil phenolics it is likely to be mainly derived from their biological metabolites. In fact, it has been reported that the 3-*O*-glucuronide of HT shows stronger activity as a radical scavenger than HT itself [70]. The major metabolites identified in *in vitro* and *in vivo* studies were an O-methylated derivative of HT, glucuronides of HT and T and a novel glutathionyl conjugate of HT [70,71]. The biocatalyzed synthesis of these metabolites has been recently described [72].

The susceptibility of LDL to oxidation depends not only on its fatty content, but also on the LDL antioxidant content (i.e. vitamin E and polyphenols) bound to the LDL [73]. Polyphenols bound to human LDL increase in a dose dependent manner with the phenolic content of the olive oil administered [29]. It has been recently reported that HT and its metabolites are capable of binding human LDL after olive oil ingestion [74]. Phenolic compounds which can bind LDL are likely to perform their peroxyl scavenging activity in the arterial intima, where full LDL oxidation occurs in microdomains sequestered from the richness of antioxidants present in plasma [40].

Postprandial oxidative stress is linked with postprandial lipemia and hiperglicaemia [24]. Contradictory results have been obtained on the *in vivo* antioxidant effect of phenolic compounds from olive oil in postprandial studies. Results are difficult to compare because some studies do not mention whether or not postprandial lipemia and/or hyperglycemia occur after olive oil

ingestion, while in other studies neither hyperlipemia nor hyperglycemia occur at postprandial state after the olive oil ingestion [9]. At olive oil doses at which oxidative stress occur (>40 mL) the phenolic content of the olive oil modulates the degree of lipid and LDL oxidation, the *in vivo* lipid oxidative damage being lower after high- than after low-phenolic content olive oil [29,75]. Concerning sustained olive oil consumption, controversial results were also obtained in the randomized, cross-over, controlled studies, which could potentially provide first level of evidence to give nutritional recommendations to the population [76], on the antioxidant effect of olive oil phenolics in humans. There are extensive differences among the studies in the experimental design, control of diet, sample population, age of the participants, measurement or not of markers of the compliance of the intervention, and in the sensitivity and specificity of the oxidative stress biomarkers evaluated [77–84]. On the basis of the studies referred to above, the Consensus Report made by the Expert Panel in the International Conference of Olive Oil and Health held in Jaen, Spain, October 2004 [9] concluded: (1) data regarding the benefits of olive oil phenolic compounds in humans from real-life daily doses of olive oil are still controversial; (2) the protective effects on lipid oxidation in these trials are better displayed in oxidative stress conditions; (3) in general the best results obtained on lipid oxidation were displayed in those markers directly associated with LDL oxidation; and (4) carefully controlled studies in appropriate populations (individuals with high oxidative status), or with a large sample size (in the case of healthy individuals), are required to definitively establish in which conditions phenolics from olive oil can exert their most beneficial effect controlling oxidative stress.

The recent results of the EUROLIVE study, however, have provided evidence of the antioxidant "*in vivo*" role of phenolic compounds from olive oil in humans and of the fact that olive oil is more than a MUFA fat [85,86]. The EUROLIVE (the effect of olive oil consumption on oxidative damage in European populations) study was a large, crossover, multicentre, clinical trial performed in 200 individuals from five Europeans countries. Participants were randomly assigned for receiving 25 mL/day of three similar olive oils, but with differences in their phenolic content, in intervention periods of 3 weeks preceded by 2-week washout periods. All olive oils increased HDL-cholesterol and the ratio between reduced and oxidized forms of glutathione, and decreased triglycerides, total/HDL cholesterol ratio, and DNA oxidative damage. Consumption of medium- and high-phenolic content olive oil decreased LDL/HDL cholesterol ratio, plasma circulating oxidized LDL, serum uninduced conjugated dienes, and serum hydroxy fatty acids. The greatest effects on increasing HDL cholesterol levels and decreasing lipid oxidative damage were observed after the high phenolic olive oil consumption. Concerning DNA oxidation, protective effects of olive oil phenols on *in vivo* DNA oxidation, measured as 8-oxo-deoxyguanosine in mononuclear cells and in urine, were found in healthy male subjects in a short-term study in which participants were submitted to a very low antioxidant diet [82]. Recently, two new studies on the effect of olive oil phenolic compounds on DNA oxidation have also been reported [87,88]. In one of them, no effects on the etheno-DNA adducts formation, a lipid-peroxidation derived DNA damage, was observed in healthy volunteers [87], whereas a protective effect on DNA oxidation, measured by the comet assay in peripheral blood lymphocytes, was observed in postmenopausal women [88]. Table 1 summarizes the results obtained in the randomized, crossover, controlled studies performed in humans on the antioxidant effects of olive oil phenolic compounds published until October 2006.

LDL particle size is also related with the lipoprotein oxidability. Recent studies suggest that an oxidation reaction is involved in small, dense LDL formation [89]. Small, dense LDL are more prone to oxidation and to enter into the arterial wall more readily than larger buoyant LDL particles, thus, accelerating the development of atherosclerosis [90]. The particle size of the LDL lipoprotein is influenced by the dietary fat. Low-fat diets lead to a decrease in the mean LDL size compared to high-fat diets [91]. High-MUFA diets based on olive oil, however, increase the LDL particle size more than a carbohydrate-rich diets, this effect being influenced by the apoE genotypes [92,93]. In a recent cross-sectional study, however, in 784 individuals with abnormal glucose metabolism and type II diabetes [94], a high PUFA intake was associated to smaller LDL particles, but not with the LDL susceptibility to *in vitro* oxidation.

3. Inflammation and endothelial dysfunction

Atherosclerosis is considered to be an inflammatory disease [95]. Endothelial dysfunction occurs early in the atherosclerosis development. Traditional risk factors for atherosclerosis promote the endothelium activation, and this change induces adhesion and trans-endothelial migration of monocytes [95]. Among the inflammatory mediators released by the endothelium are the eicosanoids derived from the n-6 PUFA arachidonic acid: prostaglandin E_2 (PGE_2), leukotriene B_4, a chemoattractanct and neutrophile activator, and thromboxane a potent vasoconsrtrictor and platelet-aggregating factor [96].

Monocytes and macrophages are critical cells present in all atherosclerotic stages. Besides promoting LDL oxidation through free radical production they secrete proinflammatory cytokines, such as IL-1β and TNFα, which stimulate the expression of adhesion molecules such as intercellular-(ICAM-1), vascular-cell adhesion molecule-1 (VCAM-1), and E-selectin [95]. Circulating monocytes are attracted by these molecules and adhere to the endothelium, from which they transmigrate to the subendothelial space. Once within the endothelium, monocytes differentiate into macrophages, which in turn scavenge oxidized LDL, thus becoming foam cells and leading to plaque formation. The proinflammatory response releases the principal messenger, the cytokine IL6, from macrophages. IL6, after engagement of its receptor on the liver, promotes the secretion of C Reactive Protein (CRP), the prototypic marker of inflammation [97,98]. Serum IL6 and CRP have been shown to be predictors of CHD [97,98]. Serum CRP, IL6, and ICAM-1 concentrations have been associated with atherosclerosis progression, the IL6 measurement being a better predictor of progressive peripheral atherosclerosis [99,100].

Table 1
Randomized, crossover, controlled studies on the sustained effect of phenolic compounds from olive oil on lipids and DNA damage

Subjects (*n* (sex))	Intervention period	Intervention period	Washout	Compliance biomarkers	Oxidative markers	Effects	Reference
10 healthy men	Virgin olive oil vs. oleic acid-rich sunflower oil[a]	3 weeks	1 week with usual diet	No	LDL resistance to oxidation	Decrease of dienes with olive oil phenol content	Nicolaiew et al. [77]
24 (men) Peripheral vascular disease patients	Virgin vs. refined all purposes	3 months	3 months	No	Lipid peroxides in LDL macrophage plasma oxidized LDL uptake	Decrease with olive oil phenol content (all markers)	Ramírez-Tortosa et al. [78]
14 healthy (10 women and 4 men)	Virgin vs. refined olive oil (50 g/day)	4 weeks	4 weeks[b]	No	LDL resistance to oxidation	None	Bonanome et al. [79]
46 healthy (31 women 15 men)	High-phenol vs. low-phenol olive oil (69 g/day) (sauces, baked)	3 weeks	2 weeks without olives and olive oil	No	LDL resistance to oxidation MDA, FRAP, LP, PC	None (all markers)	Vissiers et al. [80]
25 healthy (14 women and 11 men)	High vs. low phenol olive oil (70 g/day, raw)	3 weeks	2 weeks without olives and olive oil	No	LDL resistance to oxidation MDA, FRAP, LP, PC	None (all markers)	Moschandreas et al. [81]
30 healthy men	Virgin vs. common vs. refined olive oil (25 mL/day, raw)	3 weeks with refined olive oil for cooking	2 weeks with refined olive oil for raw and cooking purposes	Yes	LDL resistance to oxidation Plasma oxidized LDL Antibodies against oxidized LDL	Decrease with olive oil phenol content None	Marrugat et al. [65]
12 healthy Patients	High vs. medium vs. low phenol olive oil (25 mL/day, raw)	4 days with refined olive oil for cooking, and very low-antioxidant diet	10 days: refined olive oil for raw and cooking; very-low antioxidant diet	Yes	Plasma, oxidized LDL, MDA in urine, 8-oxo-dG in urine and lymphocytes F_2-isoprostanes GSH-Px	Decrease with olive oil phenol content (all markers) None Increase with olive oil phenol content	Weinbrenner et al. [82]
22 lipemic patients (12 men and 10 women)	Virgin vs. refined (raw) (40 mL/day)	7 weeks usual diet	4 weeks with usual diet	No	Plasma antioxidant capacity F2-Isoprostanes	Increase with olive oil phenol content None	Visioli et al. [83]
Coronary heart disease patients (40 men)	Virgin vs. refined (raw) (50 mL/day)	3 weeks with refined olive oil for cooking	2 weeks with refined olive oil for all purposes	Yes	Plasma Oxidized LDL LP GSH-Px	Decrease with olive oil phenol content Increase with olive oil phenol content	Fitó et al. [84]
10 women post-menopausal	High vs. low phenol virgin olive oil	8 weeks	2 weeks	Yes	Comet assay	Decrease in DNA oxidative damage with olive oil phenol content	Salvini et al. [88]
28 healthy men	Virgin vs. Common vs. refined olive oil	3 weeks	3 weeks without olives and olive oil	Yes	Etheno-DNA adducts in urine	None	Hillestrøm et al. [87]

Table 1 *Continued*.

Subjects (*n* (sex))	Intervention period	Intervention period	Washout	Compliance biomarkers	Oxidative markers	Effects	Reference
200 healthy men	Virgin vs. common vs. refined olive oil	3 weeks	3 weeks without olives and olive oil	Yes	Plasma oxidized LDL	Decrease with the phenol content of the olive oil	Covas et al. [85]
					Uninduced dienes		
					Hydroxy fatty acids		
					Antibodies against oxidized LDL	None	
					F2-isoprostans	None	
					GSH/GSSG	Increase	
					Antioxidant enzymes	None	
					8-oxo-deoxyguanosine	None	Machowetz et al. [86]
					8-oxo-guanine/guanosine		

MDA, malondialdehyde; FRAP, ferric reducing ability of plasma; LP, lipid peroxides; PC, protein carbonyl; 8-oxo-dG,8-oxo-deoxyguanosine ;GSH-Px, glutathione peroxidise; GSH, reduced glutathione; GSSG, oxidized glutathione.

[a] Added to meals, quantity not defined. Only percentage of MUFA (21%) in diet available.

[b] Characteristics of the washout period not defined.

Some findings suggest that both major and minor olive oil components, may modulate inflammation and endothelial activation. In cultured endothelial cell models, oleic acid inhibited the expression of VCAM-1 mRNA levels, the monocyte adhesion, and a key transcription factor: the nuclear factor-kappaB (NFκB) [101,102]. In animal models, a diet rich in olive oil suppressed natural killer cell activity [103] and the expression of receptors for interleukin-2 and transferrin [104]. Several human studies support a beneficial effect of olive oil-rich diets on inflammation. LDL induction of monocyte adhesion to endothelial cells was lower after MUFA consumption than after those of SFA or PUFA in healthy individuals [105]. Isolated human LDL enriched in oleic acid, promoted less monocyte chemotaxis (52% lower) and reduced monocyte adhesion (77%), compared with linoleic-enriched LDL, when exposed to oxidative stress [106]. Yaqoob et al. [107] reported a decrease in the expression of ICAM-1 by peripheral blood mononuclear cells from healthy subjects consuming an oleic acid-rich diet during 2 months. Esposito et al. [108], in a 2-year follow-up of patients with metabolic syndrome, found that, besides an improvement of the cardiovascular risk lipid profile, an intervention with a Mediterranean-style diet improved the endothelial function and levels of vascular inflammatory markers. In the PREDIMED Study [109], a randomized, controlled, intervention study with 772 participants at high risk for CHD, inflammatory markers were reduced after 3 months of a Mediterranean diet versus a low fat diet.

The protective mechanism of oleic acid-rich diets on inflammation has been attributed to a decrease in the LDL linoleic acid content [106]. The low oxidability of oleic acid, and the scavenging capacity of olive oil minor compounds, could decrease the activation of pro-inflammatory transcription factors, such as NFκB, by reducing reactive oxygen spices and peroxyl radicals [110]. In this sense, it has been reported that consumption of an olive oil-enriched meal does not activate NFκB in monocytes as PUFA and SAF-rich meals do [111]. Studies on oleic-acid enriched liposomes, and on vascular endothelium exposed to oleic acid, however, suggest an own protective mechanism of oleic acid on free radical generation, oxidative damage to lipids, and inflammatory activity [112,113]. Recent data support the concept that oleic acid is not the sole responsible for all anti-inflammatory properties of olive oil. In experimental studies, minor components of the unsaponifiable fraction of olive oil, such as α-tocopherol, β-sitosterol, and triterpenes, and phenolic compounds have been shown to have anti-inflammatory and anti-endothelial activation properties [114]. Recently, a phenolic compound from olive oil, typified as oleocanthal, has been described to have similar properties to that of the anti-inflammatory molecule ibuprofene in inhibiting ciclooxigenase (COX)-1 and COX-2 [115]. Several studies have examined the anti-inflamatory and vasculoprotective effect of olive oil phenolic compounds in humans. In these studies, phenolic compounds from olive oil have been shown to be effective in reducing the ecosanoid inflammatory mediators derived from araquidonic acid [83,116–118]. In post-menopausal women, TXB_2 levels in stimulated platelet-rich plasma, but no in urine, were significantly higher after a high phenolic olive oil diet

Table 2
Studies on the anti-inflammatory effect of olive oil phenolic compounds in humans

Subjects	Type of study	Intervention	Biomarkers	Effects	Reference
12 post-menopausal women	2 consecutive periods, no washout	Virgin olive oil vs. oleic acid rich sunflower oil ad libitum	TXB_2 in PRP TXB_2 in urine 6-keto-$PGF_{1\alpha}$	Lower in VOO no differences	Oubiña et al. [116]
Type I diabetic patients	Single intervention	Olive mill waste water (12.5 mg/day of HT) during 4 days	Serum TBX_2	Decrease at day 4	Lèger et al. [117]
Hiperlipemic patients (22) (12 men and 10 women)	Randomized, crossover	Virgin vs. refined olive oil (intervention, 7 weeks; washout period, 4 weeks with usual diet)	Serum TBX_2	Decrease with the phenolic content of the olive oil	Visioli et al. [83]
Healthy Subjects	Randomized, crossover	Virgin vs. refined olive oil postprandial state evaluation	Plasma LTB_4 plasma TBX_2	Decrease with the phenolic content of the olive oil	Bogani P et al. [118]

TXB_2, thromboxane B_2; 6-keto-$PGF_{1\alpha}$, 6-keto-prostaglandin $_{1\alpha}$; LTB_4, leukotriene B_4.

than after a high-oleic acid sunflower oil diet [116]. In diabetic patients, a 46% decrease in serum TXB_2 production was observed after four days of consumption of olive mill waste that provided 12.5 mg/day of hydroxytyrosol [117]. Recently, in two randomized crossover studies, virgin olive oil, rich in polyphenols, was shown to be more effective in lowering LTB_4 and TXB_2 than refined olive oil, with a low phenolic content, both at postprandial state in healthy subjects [118] and after sustained consumption in mildly dyslipidemic patients [83]. Table 2 show a summary of the human studies on the anti-inflamatory effects of olive oil phenolic compounds performed up-to-date. The consistency of the anti-inflamatory effects of olive oil in humans results is promising, and further studies are now required to obtain sustained evidence of the anti-inflammatory activity of olive oil and its minor olive oil components per se in humans.

4. Blood pressure

Several intervention studies in humans showed that the replacement of SFA by MUFA in the diet led to a decrease in blood pressure, both in men and women [119–121]. Moreover, an inverse relationship between arterial blood pressure and both the Mediterranean diet and olive oil consumption *per se* has been observed in population studies [122–124]. In hypertensive patients, olive oil was more effective in reducing systolic (SBP) and diastolic blood pressure [125,126], and the antihypertensive treatment [126], than PUFA-rich diets. Ruíz-Gutiérrez et al. [127] compared the effect of two similar MUFA-rich diets (olive oil and high-oleic sunflower oil) in hypertensive women. These authors [127] reported that only the olive oil rich-diet induced a significant reduction of blood pressure, suggesting a role for the minor olive oil components on blood pressure levels. Supporting this hypothesis, Fitó et al. [84] reported a decrease in the SBP after high-phenolic olive oil consumption, in comparison with low-phenolic olive oil, in hypertensive stable CHD patients. This fact was particularly marked in those who were SBP $\geq$140 mmHg at the beginning of the study. In Fito's study [84] a concomitant decrease in circulating oxidized LDL and lipid peroxides was also observed related with the phenolic content of the olive oil. The potential vasodilator activities of olive oil triterpenoids, such as oleanolic acid or erythrodiol, are currently a subject of interest. Although their presence in virgin olive oil is low, they are in high concentrations, up to 120 mg/kg, in pomace olive oil, a mixture of the refined product of the drupe after virgin olive oil extraction and virgin olive oil [128]. Both oleanolic acid and erythrodiol evoked an endothelium-dependent vasorelaxation in rat aorta, associated with the nitric oxide (NO) endothelial production [129].

In essential hypertension, a major cause for endothelial dysfunction is a decreased availability of NO. Oxidative stress, through superoxide anion production, decreases NO availability [130], and an inhibition of the NO synthase expression by oxidized LDL has been reported [131]. The antioxidant effect of olive oil, and that of its minor components, could account for the protective effect of virgin olive oil on blood pressure levels. In this sense, antihypertensive effects have been reported for other dietary polyphenols [132]. Polyphenols from red wine have been shown to be able to enhance the expression of nitric oxide synthase, with the subsequent NO release, in endothelial cultured cells [133]. The endothelium plays a key role in the regulation of vascular tone through the release of vasodilator and vasoconstrictor substances [134]. An olive oil rich-diet was shown to be able to attenuate the vascular reactivity response of the aorta ring, in spontaneously hypertensive rats [135]. Olive oil rich diets have been observed to improve the flow-mediated (endothelium-dependent) dilatation in hypercholesterolemic males [136] and metabolic syndrome patients [108]. Ryan et al. [137] showed that an olive oil diet attenuated the endothelial dysfunction present during the consumption of a baseline diet high in PUFA. The role of phenolic compounds from olive oil controlling endothelial-dependent vasodilation has been recently depicted. Ruano et al. [75] reported that a meal containing high-phenolic virgin olive oil improved the endothelial-dependent vasodilatation during postprandial state more than when the meal was taken with a similar olive oil, but with low-phenolic content. In Ruano's study [75], besides an improvement in ischemic reactive hyperaemia, a concomitant decrease in oxidative stress and NO metabolites was observed, thus suggesting a link between the phenomena. Thus, the benefits of olive oil and its phenolic compounds on

blood pressure could be mediated through their protective effect on the vascular endothelial function.

5. Carbohydrate metabolism

An individualized approach, taking in account patient's preferences, based on the nutritional assessment and desired outcomes for each patient is a goal recommended from the American Diabetes Association [23]. To achieve these nutritional goals, in the control of hyperglycemia and dyslipemia, either low-saturated-fat, high-carbohydrate diets or high-MUFA diets can be advised. In some studies [119,138,139] performed in diabetic patients, MUFA-enriched diets reduced the insulin requirements in diabetic patients versus a low-fat/high-carbohydrate diet. A meta-analysis of various studies, comparing these two approaches to diet therapy in patients with type 2 diabetes, revealed that high-MUFA diets improve lipoprotein profiles as well as glycemic control, while having no effect on fasting insulin and glycated haemoglobin concentration [21]. Posterior studies have not found differences between high-carbohydrate and high-MUFA diets on glycemic control in diabetic patients [20]. Fasting glucose was lower and insulin resistance decreased in metabolic syndrome patients [108] as well as in non-diabetic participants of the PREDIMED Study [109] after 2 years and 3 months, respectively, of a Mediterranean rich-olive oil diet versus a recommended low-fat diet. Furthermore, there is no evidence that high-MUFA diets induce weight gain in patients with diabetes mellitus provided that energy intake is controlled. Therefore, a diet rich in olive oil can be advantageous for both patients with type 1 or type 2 diabetes who are trying to control the body weight.

6. Thrombosis

Two processes play a key role in thrombus formation: coagulation and fibrinolysis. MUFA rich-diets reduce platelet aggregation, a key step in the blood-clotting process, in front of SFA diets [140,141]. Platelet activating factor (PAF) causes platelets to aggregate and is a strong inflammatory lipid mediator essential for the activation of leukocytes and their binding in the endothelial cells [142]. PAF antagonists have been shown to exert a protective action against platelet aggregation and atherosclerotic development [143]. Olive oil, particularly its polar lipid fraction, is rich in PAF antagonists in comparison with seed oils [144]. In a recent study, in rabbits fed with olive oil or olive oil polar lipid extract, blood platelet activating factor acetyl-hydrolase increased, platelet aggregation was attenuated, less oxidation occurred in plasma, lesion thickness was reduced, and vessel walls retained elasticity [145]. Olive oil isochromans, derivatives of the phenolic compound hydroxytyrosol, have been shown to inhibit human platelet reactivity in experimental studies [146]. Thromboxane A2 (TXA_2), produced by activated platelets increases the platelet aggregation. The effect of olive oil phenolic compounds on TXB_2, the TXA_2 metabolite, generation in human studies has been referred to previously (Table 2) [84,116–118]. The effect of triglyceride-rich lipoproteins on PGE_2 and TXB_2 generation in cultured endothelial cells was lower after the ingestion of a virgin olive oil enriched with its unsaponifiable fraction than after the non-enriched virgin olive oil or high-oleic sunflower [114]. Rats fed with an olive oil-rich diet showed: (1) a significant delay in the aortic thrombotic occlusion, a lower incidence of venous thrombosis, and a prolonged bleeding time in comparison with the control group fed the usual diet [147]; and (2) a decreased platelet hyperactivity and subendothelial trombogenicity when compared with a SAF fed group [148].

A coagulation component associated with the formation of platelet thrombus and endothelial injury marker is the von Willebrand factor (vWF). A high-MUFA rich diet has been shown to reduce plasma levels of vWF both in diabetic patients [149] and in healthy individuals [150]. Factor VII (FVII), a key protein in thrombosis and a risk factor for CHD, has been shown to be decreased after rich-oleic acid diets in front of lauric and palmitic-rich diets [151]. No differences were observed among oleic-rich and linoleic-rich diets [152]. As a general pattern, meals rich in MUFA seem to have postprandial FVII responses lower than that after SFA-rich meals and similar to that of PUFA- or rapeseed oil-rich meals [20]. The background diet seems to influence the FVII postprandial activation, olive oil sustained dietary patterns promoting lower postprandial FVII peaks after high fat meals than SFA-rich dietary patterns [153]. In the stabilization and progress of thrombus, fibrinolysis plays an important role as a mechanism regulated by the equilibrium between the tissue plasminogen activator (t-PA) and its strongest natural inhibitor, PAI-1. MUFA-rich diets have been shown to decrease PAI-plasma levels in comparison with SFA diets, and to not differ from PUFA-rich or low-fat diets [20].

7. Comments

On the basis of the information discussed above, diets in which olive oil is the main source of fat could be an useful tool against risk factors for cardiovascular disease. The benefits of olive oil consumption are beyond a mere reduction of the LDL cholesterol. Olive oil rich diets reduce the insulin requirements and decrease plasma concentration of glucose and insulin in type 2 diabetic patients, compared with the effect of high-SFA and low-fat, high-carbohydrate diets. Oleic acid-enriched LDL is more resistant to oxidative modifications. Moreover, oxidative damage is also related in a dose-dependent manner with the phenolic content of the olive oil. Directly, or through a reduction in the oxidative status, dietary olive oil influences the endothelium functions. These include endothelium-dependent vasodilatation and a reduced capacity of oleic-enriched LDL to promote the adhesion and chemiotaxis of monocytes. Olive oil, and particularly a virgin olive oil-rich diet, decreases prothrombotic environment, modifying platelet adhesion, coagulation and fibrinolysis. Olive oil is the main fat in the Mediterranean diet. The wide range of antiatherogenic effects associated with olive oil consumption could contribute to explain the low rate of cardiovascular mortality found in Southern European Mediterranean countries, in comparison with other western countries, despite a high prevalence of CHD factors. The mechanisms by which olive oil exerts its beneficial effects merit further investigation, and

further studies are required to obtain evidence of the benefits of olive oil consumption on primary end points for cardiovascular disease.

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