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REVIEWS: CURRENT TOPICS

The degree of unsaturation of dietary fatty acids and the development of atherosclerosis (Review)

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Abstract

Atherosclerosis is the principal contributor to the pathogenesis of myocardial and cerebral infarction, gangrene and loss of function in the extremities. It results from an excessive inflammatory-fibroproliferative response to various forms of insult to the endothelium and smooth muscle of the artery wall. Atherosclerotic lesions develop fundamentally in three stages: dysfunction of the vascular endothelium, fatty streak formation and fibrous cap formation. Each stage is regulated by the action of vasoactive molecules, growth factors and cytokines. This multifactorial etiology can be modulated through the diet. The degree of unsaturation of dietary fatty acids affects lipoprotein composition as well as the expression of adhesion molecules and other pro-inflammatory factors, and the thrombogenicity associated with atherosclerosis development. Thus, the preventive effects of a monounsaturated-fatty acid-rich diet on atherosclerosis may be explained by the enhancement of high-density lipoprotein-cholesterol levels and the impairment of low-density lipoprotein-cholesterol levels, the low-density lipoprotein susceptibility to oxidation, cellular oxidative stress, thrombogenicity and atheroma plaque formation. On the other hand, the increase of high-density lipoprotein cholesterol levels and the reduction of thrombogenicity, atheroma plaque formation and vascular smooth muscle cell proliferation may account for the beneficial effects of polyunsaturated fatty acid on the prevention of atherosclerosis. Thus, the advantages of the Mediterranean diet rich in olive oil and fish on atherosclerosis may be due to the modulation of the cellular oxidative stress/antioxidant status, the modification of lipoproteins and the down-regulation of inflammatory mediators. © 2003 Elsevier Inc. All rights reserved.

1. Introduction

Atherosclerosis, the principal cause of heart attack, stroke and gangrene of the extremities, accounts for 50% of mortality in the USA, Europe and Japan. The American Heart Association estimates that cardiovascular diseases affect 57 million Americans, and each year cause 954,000 deaths and cost 259 billion dollars.

Atherosclerosis and inflammation share similar mechanisms in their early phases, when the interactions between the vascular endothelium and circulating leukocytes are increased. Two key initial events within the arterial wall during early atherogenesis are the recruitment and differentiation of circulating monocytes, and the uptake of cholesterol and oxidized low-density lipoproteins (LDL) by tissue macrophages to form lipid-foam cells, involved in atheroma plaque generation. Adhesion molecules participate in leukocyte-endothelial interactions and the extravasation of monocytes. Adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectins are strongly expressed on macrophages within atherosclerotic plaques [1] and the disruption of their expression protects against atherosclerosis [2]. Thus, the initiation and early phases of atherosclerosis like fatty streak and fibrous cap formation depend mainly on the specific conditions of cellular contact and the transient or repeated synthesis of soluble mediators.

Hypolipidemic drugs like cholestyramine, colestipol, statins and fibrates reduce serum triglycerides and LDL-cholesterol to various extents and increase high-density lipopro-

Abbreviations: LDL, low-density lipoproteins; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; HDL, high-density lipoproteins; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; VSMC, vascular smooth muscle cells; AA, arachidonic acid; DHA, docosahexaenoic acid; CETP, cholesterol ester transfer protein; EPA, eicosapentaenoic acid; $^{\bullet}NO$, nitric oxide; $O_2^{\bullet -}$, superoxide anion; IL, interleukin; TNF, tumour necrosis factor; PLA_2, phospholipase A_2; MSR, macrophage scavenger receptor

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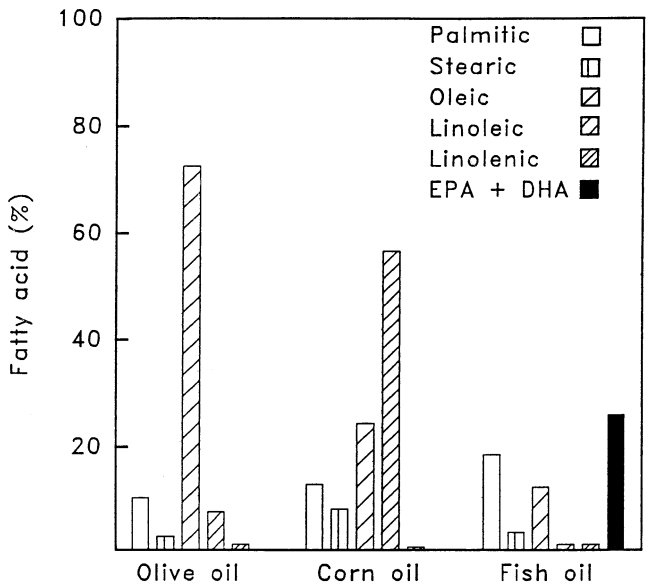

Fig. 1. Comparison of the composition of long-chain fatty acids in olive, corn and fish oils. Oleic acid is the main fatty acid in olive oil, whereas corn oil is rich in PUFA such as linoleic acid, a precursor of AA. Fish oil contains EPA and DHA but not linoleic acid.

tein (HDL)-cholesterol levels. However, these drugs have also numerous and significant side effects. The diet can be improved by modifying the amount and the type of fat ingested, which also conditions the lipid profile, without side effects and a much lower cost than drugs. Data from the seven countries study [3] suggest that the Mediterranean diet, which is rich in monounsaturated fatty acids (MUFA), primarily as olive oil (Fig. 1), is associated with low cardiovascular disease rates. Likewise, epidemiological studies show an inverse correlation between the intake of long-chain n-3 polyunsaturated fatty acids (PUFA) present in fish and fish oil (Fig. 1) and the incidence of cardiovascular diseases such as atherosclerosis [4]. However, there are controversial reports on the cellular and molecular mechanisms involved in these preventive effects. The present review brings together evidence on the effect of the degree of unsaturation of fatty acids in the diet on the mechanisms of development of atherosclerosis, namely cholesterol and LDL levels, LDL oxidation, oxidative stress, the hemostatic system, induction of the atheroma plaque by macrophages, and vascular smooth muscle cell (VSMC) proliferation.

2. Effects of unsaturated fatty acids on lipids and lipoproteins

Post-prandial fats circulate in the blood stream as chylomicrons. They are cleared by the liver, where they are converted to very low-density lipoproteins, LDL and HDL. However, chylomicrons remnants are as atherogenic as LDL cholesterol and stimulate the formation of highly atherogenic small dense LDL. LDL holds cholesteryl esters and triglycerides in the lipophilic core that is surrounded by a monolayer of phospholipids and free cholesterol [5]. LDLs bind many fatty acid molecules and nearly half of them are PUFA. Linoleic acid accounts for 86% of PUFA and is mainly (65%) contained in the cholesteryl esters, whereas arachidonic acid (AA) accounts for 12% and is mostly (68%) found in the phospholipids. Docosahexaenoic acid (DHA) is present in trace amounts, mainly in phospholipids [6]. The type of fatty acids varies greatly from one individual to another, probably owing to differences in dietary habits.

The plasma post-prandial triglyceride concentration has been assessed by Roche et al. [7] in subjects fed either a high MUFA, low saturated fat diet (18% and 12% of energy as fat, respectively) or a low MUFA, high saturated fat diet, (14% and 17% of energy as fat, respectively) for 8 weeks. It returns to near-fasting concentration much earlier in subjects fed on the MUFA-rich diet than in those fed the saturated fat-rich diet. Similar results have been obtained after an extra-virgin olive oil-rich meal [8]. Moreover, numerous intervention studies have shown that the substitution of dietary saturated fatty acids by unsaturated fatty acids also has hypocholesterolemic effects [9,10]. MUFA- and PUFA-rich diets decrease the levels of total plasma cholesterol and LDL-cholesterol and increase HDL-cholesterol in healthy normolipidemic subjects [11,12] and in mouse models of atherosclerosis [13]. The primary source of MUFA that lowers cholesterol levels is olive oil [7,8,14,15], but canola oil [16] and nut- and peanut-derived products [15,17–19] have similar effects. The cholesterol ester transfer protein (CETP) mediates the transfer of cholesteryl esters from HDL to apolipoprotein B-containing lipoproteins. The isoenergetic replacement of a high-saturated fatty acid diet by a high-MUFA or a high-carbohydrate low-fat diet decreases the CETP concentration in young, healthy, normolipidemic subjects [20]. CETP activity may be regulated by the concentration of cholesterol in plasma [21] and it is decreased by an oleic acid-rich diet and enhanced by a palmitic acid-rich diet in hamsters [22]. All these studies shed some light on the beneficial effects of the Mediterranean diet, which may partially explain the lower rate of cardiovascular diseases observed in the Mediterranean area.

Fish and fish oils contain very-long chain and highly unsaturated n-3 PUFA such as eicosapentaenoic acid (EPA, 20:5n-3) and DHA (22:6n-3) (Fig. 1), which are derived from phytoplankton. Fish oils reduce the synthesis of chylomicrons by the intestine and/or increase their removal from circulation, thus decreasing postprandial lipemia [23]. Chylomicron remnants are selectively cleared after the ingestion of n-3 PUFA [24]. In this regard, Lambert et al. [25] reported that labeled [1-^{14}C]oleate and [1,2-^{3}H]cholesterol in chylomicrons remnants derived from fish oil are incorporated into phospholipids more efficiently than those derived from olive, corn or palm oil remnants and that fish oil remnants are metabolized more rapidly than palm oil remnants. We would like to highlight that, the hypolipidemic

effect of fish oil is stronger on hyperlipidemic patients than on normal subjects [26]. Cholesterol concentration in plasma is decreased by fish oil and by n-3 PUFA in patients with type V hyperlipidemia who do not tolerate any other type of dietary fat [26–28]. The slight effect of fish oil on plasma LDL and HDL, as against the decrease in very low-density lipoproteins and triacylglycerol concentrations, is the result of factors such as the smaller very low-density lipoprotein particle produced, which is more likely to be converted to LDL [29], by the direct effect on the synthesis of LDL by the liver and by lowerring the saturated fat intake. These effects depend largely on the dose and type of n-3 PUFA content of fish oil used.

In conclusion, although the ingestion of n-3 PUFA has a lower effect than MUFA on plasma cholesterol and on LDL and HDL cholesterol levels [3,26,30], both diets decrease triacylglycerol levels, increase the HDL/LDL-cholesterol ratio and decrease the total cholesterol/HDL-cholesterol ratio, thus reducing the risk of atherosclerosis and coronary artery disease.

3. Effects of unsaturated fatty acids on LDL oxidation

Endothelial cells [31], smooth muscle cells [32] and macrophages [33] oxidize LDL. According to the oxidation theory of atherosclerosis [34], oxidation begins on the phospolipids present on the surface of LDL particles, and propagates to the lipophilic core, rich in cholesteryl esters and triglycerides. The resulting aldehydes, especially hydroxynonenal, bind to the apolipoprotein B moiety of LDL. This modified LDL contains acetyl groups or analogous modifications that are recognized by scavenger receptors. The oxidized LDL bound to the receptors is rapidly taken up by the macrophages present in the subendothelial space. Thus, intracellular cholesterol accumulates and may convert the macrophages into lipid loaded foam cells. The oxidative modifications of LDL are involved in the initiation and progression of atherosclerosis and are mainly due to the depletion of endogenous antioxidants followed by oxidation of PUFA. An excess of oxidized LDL in macrophages can induce necrosis or apoptosis and the subsequent release of proteolytic enzymes and transition-metal ions, such as iron [35] and copper [36], may contribute to the development of advanced atherosclerotic lesions. In this regard, copper ions have been detected in these lesions [37].

Several authors have reported that even in normal subjects, the LDL predisposition to oxidation varies greatly [38,39]. So far this marked variability under the same oxidative stress is not understood. The susceptibility to oxidation is modulated by intrinsic and extrinsic factors to LDL particles [40] and the production of reactive oxygen species in the vicinity of LDL.

3.1. Intrinsic factors

3.1.1. Concentrations of LDL and HDL

As the oxidative modifications of LDL are crucial for the initiation of atherosclerosis, a reduction in plasma LDL levels may be involved in the prevention mechanisms. Moreover, it seems that high levels of HDL also prevents the generation of an oxidative modified LDL. Several mechanisms have been involved in the beneficial effect of HDL, e.g. reverse cholesterol transport [41] and, transfer of peroxidized lipids from LDL to HDL [42], followed by their degradation by enzymes associated with HDL, like paraoxonase, which prevent LDL oxidation [43]. However, the exact mechanisms by which HDL provides protection against coronary heart disease are still a matter of debate. Thus, the reduction of LDL levels and the increase in HDL levels by a MUFA-rich diet are correlated with the beneficial effects against atherosclerosis even stronger than those of PUFA supplementation.

3.1.2. Degree of unsaturation of fatty acids in LDL

There is considerable disparity in the indices of LDL susceptibility to oxidation measured *ex vivo*. The most currently used techniques to asses LDL oxidation is the conjugated dienes or the thiobarbituric reactive substances formation mediated by metal ion-dependent (copper) and -independent methods (2,2′-azobis(2-aminopropane) dihydrochloride). The copper-mediated oxidation involves a site-specific mechanism which may be more relevant *in vivo* [44]. Copper binds to the surface of LDL particles and breakdown performed lipid peroxides to chain-propagating radicals. Copper-mediated oxidation also allows the measurement of the phosphatidylcholine hydroperoxides and cholesteryl ester hydroperoxides generated during the oxidation process from the LDL surface and the lipophilic core, respectively. In addition it is possible to identify the cholesteryl ester hydroperoxides derived from specific fatty acids. The 2,2′-azobis(2-aminopropane) dihydrochloride generate peroxyl radicals in an aqueous phase [45] and produces random attack of free radicals in LDL. It does not require the binding to LDL and can act in the absence of lipid peroxides.

The amount of MUFA and PUFA in LDL may provide great insight to the LDL susceptibility to oxidation. Ramirez-Tortosa et al. [46] studied the effect of two tablespoons/day of either extra-virgin oil to men with peripheral vascular diseases for 3 months. Extra-virgin oil was significantly more effective in reducing the slope of the line reflecting thiobarbituric acid reactive substances formation after 24 hr of oxidation *ex vivo* in the presence of copper. The antioxidants present in extra-virgin oil (not thermally or chemically treated) may contribute to this effect. However, the performance of olive oils has not been compared with that of other types of vegetable oils. On the other hand, other sources of dietary MUFA have distinct effects on LDL oxidation. Thus, supplementation with peanut oil to humans

increases the oxidation rate when compared with olive oil, without modifying the lag time or the amount of conjugated dienes [47]. This may be due to the lower ratio of saturation/unsaturation [48] or 18:1/18:2 ratios [49–53]. Thus, a safflower oil-enriched diet (high in n-6 PUFA) containing cholate was compared with a Paigen diet (saturated fat diet containing cholate) in mouse and results showed a shorter lag time in the former group [13]. The administration of olive oil (rich in MUFA) or grape seed oil (high in diunsaturated fatty acids) to humans in a crossover study design revealed that the rate of LDL oxidation *ex vivo* was higher after administering the diunsaturated fatty acid diet [49]. In this regard, numerous authors have reported that LDL particles from subjects fed olive oil-rich diets are less susceptible to oxidation than LDL particles from subjects fed a baseline diet [54], a PUFA-rich diet [49–51,55,56], or even a carbohydrate diet [57].

Despite the favorable effects of diets high in unsaturated fat on lipid profiles [58,59], there is concern that PUFA-rich diets may increase the oxidative susceptibility of LDL. Studies evaluating this issue have revealed conflicting results after the administration of fish oil-rich diets or the long-chain PUFA, e.g. the EPA and DHA contained in fish and fish oils. In some studies, LDL showed enhanced oxidizability [60–65], whereas other studies revealed no increase in LDL oxidation [66–73]. Among the most plausible factors involved in these controversial results are the dose and time of supplementation with n-3 PUFA and the method and the criteria chosen to evaluate LDL oxidation. Higdon et al. [72] studied several parameters related to LDL oxidation in postmenopausal women fed moderate doses of fish oil (15 g/day) for five weeks. The results indicated a more rapid loss of α-tocopherol and a shorter lag time in phosphatidylcholine hydroperoxide and cholesteryl linoleate hydroperoxide formation. However, the maximal rates of both hydroperoxides were lower and the loss of total PUFA was not greater in LDL from subjects supplemented with fish oil than in those supplemented with oils rich in oleic and linoleic acids. In conclusion, these observations leave open the possibility that overall, the total oxidation of the LDL particle *ex vivo* is not increased by FO supplementation.

Short lag time and slow oxidation rates have been extensively reported in humans after feeding a fish oil-rich diet [63,70,72,74,75]. The slow oxidation rate appears paradoxical and supports the new hypothesis that LDL with highly-unsaturated fatty acids are not necessarily oxidized more rapidly than those with fewer double bonds. Several explanations have been postulated. Brude et al. [71] reported that a reduced oxidation rate following n-3 PUFA supplementation results from the tight packing of EPA and DHA, making double bonds less available for free radical interactions. Moreover, the amount of conjugated dienes or thiobarbituric acid reactive substances generated during LDL oxidation depends on the lipid composition. It has been suggested that after the administration of a fish oil-rich diet, n-3 PUFAs form more polar radicals [76], which may be located on the surface of LDL particles, resulting in slower propagation rates because the more polar radicals reach the lipophilic core more slowly, thus decreasing the rate of termination.

At present it is difficult to ascertain the proatherogenic role of n-3 PUFA by increasing the oxidative susceptibility of LDL to oxidation. Further studies are needed to reach a conclusion, and other factors may be involved in the cardiovascular protective effects of fish and fish oil.

3.1.3. Antioxidants in LDL

The rate of lipid hydroperoxides formation during LDL oxidation is inversely correlated with the level of α-tocopherol [5]. Moreover, as the LDL particle must first be depleted of its antioxidants before oxidation can proceed, lag time may be a more relevant indicator of the oxidation status *in vivo* than the rate of oxidation. A higher degree of unsaturation of fatty acids incorporated into LDL particles may be indicative of lipid radical generation and so reduced protection against oxidation by the consumption of antioxidants in LDL.

Two studies have compared the supplementation of a normal diet with a mixture of high amounts of β-carotene, α-tocopherol and ascorbic acid (equal or higher than 60 mg/day, 800 mg/day and 1 g/day, respectively) for three months with supplementation of α-tocopherol alone at the same dose used in the mixture [77,78]. The combined antioxidants and α-tocopherol alone increased the lag time and decreased the oxidation rate to the same extent. On the other hand, β-carotene alone did not protect LDL against oxidation [79–81]. It can be concluded that α-tocopherol may be the most effective antioxidant in protecting LDL against oxidation. Moreover, lower amounts of combined antioxidants were also effective [71,82]. In this regard, Brude et al. [71] administered a capsule containing a combination of 15 mg of β-carotene, 75 mg of vitamin E and 150 mg of vitamin C plus another capsule containing 30 mg of coenzyme Q_{10} for 6 weeks to hyperlipidemic male smokers. They observed increases of 28% and 35% in the lag time after 3 and 6 months of supplementation, respectively, but no changes in the level of malondialdehyde generated. However, we must consider that α-tocopherol can also be pro-oxidant rather than protective for lipids in isolated LDL [83], through the generation of α-tocopheroxyl radicals. Thus, the beneficial effect of supplementing with vitamin E alone may be explained by the simultaneous uptake of other antioxidants present in the diet [83].

LDL isolated from mild hypercholesterolemic subjects receiving a linoleic acid-rich diet and an oleic acid-rich diet showed the same levels of vitamin E and coenzyme Q_{10} [84]. However, α-tocoferol levels in LDL decrease when administering fish oil [72,73], although according to Turini et al. [64] the LDL antioxidant status is not affected by supplementing 25 g/day of fish oil (provided 7,5 g of n-3 PUFA) for 30 days.

The propagation phase of phosphatidylcholine hydroperoxides generation begin prior to the depletion of α-tocopherol and is slowed once the LDL particle is depleted of α-tocopherol in subjects fed a fish oil-rich diet [72]. This effect was even stronger when subjects were fed with a fish oil-rich diet than when fed with a safflower- or sunflower-rich diets [72]. Practitioners are advised to supplement an n-3 PUFA-rich diet with antioxidants to protect fatty acids against oxidation. Brude et al. [71] also supplemented subjects with capsules containing n-3 PUFA (5 g of EPA and DHA) and with the same combination of antioxidants as described above. The results obtained indicated no changes in the oxidizability parameters of control group or the group fed only with the n-3 PUFA-rich capsules, but protection was lower than when only the antioxidant was supplemented.

The various conditions of the diet and the complexity of LDL particles and the *ex vivo* methods used to evaluate LDL oxidation hinder the extrapolation of the *ex vivo* results to an *in vivo* situation. The measurement of antioxidants in the vessel wall, the basal levels of conjugated dienes or thiobarbituric acid reactive substances in LDL, the relative electrophoretic mobility of LDL or the presence of oxidized LDL antibodies in plasma were expected to give a more realistic indication of the *in vivo* oxidation status of LDL. However, the correlation between oxidized LDL and circulating oxidizied LDL autoantibodies is not clear [85,86].

3.2. Extrinsic factors

Extrinsic factors such as the level of antioxidants and the free radical generation in the vicinity of LDL particles can also modulate LDL oxidation. Moreover, dietary fats are known to interact with these extrinsic factors. The beneficial effects of an olive oil-rich diet on LDL oxidation may be due to its high levels of oleic acid and the associated reduction of PUFA and to the presence of minor components such as flavonoids and phenolic compounds [87–89]. De la Cruz et al. [90] reported that olive oil supplements in the diet reduce lipid peroxidation in heart, aorta and platelets. Visioli et al. [91] found that the polyphenolic compounds of olive oil, which are regarded as natural antioxidants, inhibit the formation of cytotoxic products such as LDL lipid peroxides and thus delay the onset of atherosclerotic damage. Thus, the intake of extra-virgin olive oil, containing 32-35 mg/day of total phenols and other unsaponifiable compounds, may limit LDL oxidation [46].

N-3 PUFA affects endogenous antioxidant systems. Vitamin E requirements are higher in a fish oil-rich diet than in a soybean oil-rich diet [92]. However, fish oil supplementation raises platelet and plasma β-carotene levels [93,94]. We found low levels of α-tocopherol and catalase activity in erythrocyte membranes after administration of a fish oil-rich diet to rats for four months [95]. Such adaptive effects may explain why the enhanced oxidizability of LDL is more frequent after short-term [60–63] than after long-term supplementation of n-3 PUFA [66–70,73]. Further studies are needed to explain the wide range of antioxidant levels found in various compartments of the body following the ingestion of n-3 PUFA-rich diets.

Another extrinsic factor involved in the pathogenesis of many cardiovascular diseases, including atherosclerosis, is oxidative stress. The free radical species generated by monocytes/macrophages and endothelial cells from the artery wall may oxidize LDL. Moreover, the fatty acids present in the diet can modulate LDL production and their susceptibility to oxidation.

4. Effects of unsaturated fatty acids on cellular oxidative stress

Endothelium dysfunction may be due to the enhancement of reactive oxygen species and to the increased rate of inactivation of nitric oxide ($^{\bullet}NO$) [96]. Cherny et al. [97] have described the activation of NADPH oxidase and the subsequent superoxide ($O_2^{\bullet -}$) production by free AA. MUFA- and n-3 PUFA-rich diets, by replacing AA by oleic acid and by EPA and DHA in phospholipid membranes, respectively, impair the release of AA from membranes [98] and thus modify the release of free radicals. Compared with a diet rich in linoleic acid, dietary olive oil has no effect on $O_2^{\bullet -}$ release by human monocytes [99] or mouse [100] and rat [101] macrophages *ex vivo* (Table 1). Results obtained with n-3 PUFA vary according to the animal species and experimental model tested. Thus, monocytes/macrophages from humans fed with n-3 PUFA show a lower production of $O_2^{\bullet -}$ [102]. However, $O_2^{\bullet -}$ production in rat and mouse macrophages is higher than in animals fed a diet rich in oleic acid [100] or linoleic acid [100,101]. Robinson et al. [103] reported that PUFA such as AA, EPA and DHA induced $O_2^{\bullet -}$ production *in vitro* by human neutrophils through phospholipase A_2 (PLA_2) activation. These data may explain the lower $O_2^{\bullet -}$ production in cells of rats fed an olive oil-rich diet.

As mentioned in the previous section, virgin olive oil contains numerous minor components with antioxidant activity. Visioli et al. [104] reported that the administration of phenol-rich olive oil was dose-dependently associated with the decreased urinary excretion of 8-iso-$PGF_{2\alpha}$, a biomarker of oxidative stress. Leger et al. [105] observed that polyphenol-rich olive oil wastewater fractions decreased $O_2^{\bullet -}$ production in cultured human promonocyte cells and scavenged $O_2^{\bullet -}$. Given the role of $O_2^{\bullet -}$ in LDL oxidation and oxidized LDL in atheroma plaques, these results point to the anti-atherogenic role of minor components of olive oil such as polyphenols.

No studies have been carried out on the direct effect of MUFA on $^{\bullet}NO$ generation. However, it has been shown that an olive oil-rich diet markedly lowers the daily anti-hypertension dosage required, possibly through enhanced $^{\bullet}NO$ levels [106]. The effects of n-3 PUFA-rich diets on $^{\bullet}NO$

Table 1
Effect of the degree of unsaturated fatty acids on $O_2^{\bullet -}$ and $^{\bullet}NO$ generation and on vascular response

Cells or tissues	Species	Administration	$O_2^{\bullet -}$	$^{\bullet}NO$	Response	Ref.
Macrophages						
Olive oil	Human	Diet	NC	—		99
Olive oil	Rat	Diet	NC	↑		101
Olive oil	Mice	Diet	NC	↑		100
Fish oil	Human	Diet	↓	NC		102
Fish oil	Rat	Diet	↑	↑		101
Fish oil	Rat	Diet	—	↑ cGMP		107
Fish oil	Rat	Diet	—	↑		108
Fish oil	Mice	Diet	↑	↑		100
n-3 PUFA	Human	Diet	↓	—		99
Endothelial cells						
EPA		In vitro		↑ EDRF		109
Smooth muscle cells						
EPA		In vitro		↑		109
Arteries						
Olive oil	Human	Diet			↓ Art. pressure	106
Fish oil	Human	Diet			Vasodilation	113
Fish oil	Rat	Diet			Vasodilation	111
n-3 PUFA	Human	Diet			↓ Art. pressure	115
n-3 PUFA	Pig	Diet			Relaxation	117

EDRF: endothelial derived relaxing factor.
-: not measured.
NC: not change.
↓: decrease, ↑: increase.

generation by rat and mouse macrophages through the activation of inducible NO synthase are uniform when a linoleic acid-rich diet is supplemented, and enhance $^{\bullet}NO$ generation [100,101,107,108]. Endothelial cells contain endothelial NO synthase, a constitutive isoform that also generates $^{\bullet}NO$. The Vanhoutte group described the release of relaxing factors by n-3 PUFA both *in vitro* [109] and *in vivo* [110]. We observed that the administration of a Menhaden oil-rich diet increases the resting level of $^{\bullet}NO$ generation by aortic vessels (unpublished data) and acetylcholine-induced vasorelaxation in phenylephrine pre-contracted aortic rings [111]. This relaxation is mediated by $^{\bullet}NO$ without modification of $O_2^{\bullet -}$ release by the vessel wall or relaxation due to the cyclooxygenase pathway [111].

The anti-hypertension effects of the increase in $^{\bullet}NO$ by MUFA rich- and n-3 PUFA rich-diets when $O_2^{\bullet -}$ generation is not enhanced may partly explain the beneficial properties of both types of fatty acids. However, the cardiovascular disease may be prevented through the vasorelaxation induced by $^{\bullet}NO$ or, by the powerful antioxidant activity of $^{\bullet}NO$ on LDL [112]. The first hypothesis is supported by the increase in coronary artery vasodilation in response to acetylcholine infusion in heart transplant patients [113] and by the reduction of blood pressure in healthy volunteers [114], in patients with mild hypertension [115] and in rats genetically predisposed to hypertension [116] after their diet is supplemented with fish oil or DHA (Table 1).

At the vascular level, $O_2^{\bullet -}$ reacts with $^{\bullet}NO$ to generate peroxynitrite and lipid-derived products such as LONO and LOONO [117]. The stimulation of $O_2^{\bullet -}$ production reduces $^{\bullet}NO$ levels unless NO synthase is induced simultaneously with NADPH oxidase. Thus, the $^{\bullet}NO/O_2^{\bullet -}$ ratio in cells from rats fed an olive oil-rich diet is higher than in cells from animals fed fish oil- or corn oil-rich diets [101].

5. Effects of unsaturated fatty acids on the hemostatic system

The endothelium has a major function in both thrombotic and coagulant activities. Heparan sulfate, $^{\bullet}NO$ and prostaglandin I_2 released by the endothelium are all antithrombotic agents. The endothelium also binds factors that prevent coagulation. Moreover, it can balance interactions between the coagulation and fibrinolytic systems. In atherosclerotic lesions, this balance is broken in favor of a prothrombotic state, which can be particularly deleterious in the later stages of the disease [118]. Furthermore, the maintenance of the vascular tone depends on the release of vasodilators ($^{\bullet}NO$ and prostaglandin I_2) and vasoconstrictors ($O_2^{\bullet -}$ and endothelin) [119]. De la Cruz et al. [120] and Oubina et al. [121] noted that olive oil reduced thrombogenicity and platelet activation by modulating lipoprotein peroxidation and the subsequent eicosanoid production. Moreover, a MUFA-rich diet decreases the plasma levels of both the von Willebrand factor, a tissue factor pathway inhibitor, and the plasminogen activator inhibitor type 1, the main inhibitor of fibrinolysis [122]. The beneficial effects of a MUFA-rich diet on atherosclerosis may also be partly due

to the changes that protect against thrombogenesis. Olive oil also decreases the blood coagulation factors VII, a key factor in thrombogenesis [123], XIIa and α2-antiplasmin [124].

Chen et al. [125] reported that a fish oil-rich diet delays the formation of arterial thrombus, probably by reducing platelet aggregation, and the $O_2^{\bullet -}$ release associated which arterial injury. Thus, EPA and DHA decreased collagen-induced platelet aggregation and thromboxane production [126]. The loss of endothelium-derived $^{\bullet}$NO may have a key function in early atherosclerosis through the enhancement of platelet aggregation, monocyte adherence and chemotaxis, and the loss of vasorelaxation [127]. Likewise, we observed that a fish oil-rich diet raises the endothelial production of $^{\bullet}$NO induced by acetylcholine in intact aortic rings [111]. Thus, fish oil may counterbalance the loss of $^{\bullet}$NO in atherosclerosis and to reduce platelet aggregation, monocyte adherence and chemotaxis.

6. Effects of unsaturated fatty acids on macrophages and atheroma plaque

Oxidized LDL may directly damage the endothelium and contribute to atheroma plaque formation through increased adherence and migration of monocytes and T lymphocytes into the subendothelial space. Oxidized LDL particles induce cell adhesion by the formation of adhesive cell-surface glycoproteins, e.g. VCAM-1, by the endothelium. Once monocytes and lymphocytes enter the intima of the artery, oxidized LDL from the endothelium and other substances associated with atherogenesis trigger the differentiation of monocytes into macrophages [128]. The uptake of oxidized LDL by the macrophages, through scavenger receptors leads to foam cell formation [128] and may alter the gene expression of many growth-regulatory molecules and cytokines.

Thus, monocytes/macrophages are present at all stages of atherogenesis [129,130]. Macrophages do not only behave as antigen-presenting cells to T lymphocytes, but also as scavenger cells that remove noxious materials and as indicators of the fibroproliferative process by their capacity to form numerous growth factors, namely the platelet-derived growth factor [131], interleukin-1 (IL-1) [132] and tumor necrosis factor (TNFα) [132]. They are thus the principal inflammatory mediator of cells in the atheromatous plaque microenvironment.

6.1. Effects on macrophage adhesion

Yaqoob et al. [133] showed that a diet rich in MUFA (rich in olive oil) significantly reduced ICAM-1 (CD54) expression in human peripheral blood mononuclear cells. Moreover, this MUFA diet also tends to decrease the expression of the macrophage-associated adhesion molecule (CD11b), whereas it does not affect the expression of CD2, CD3, CD4, CD7, CD8, CD21 or CD64. Taken together, these data point to a direct link between the expression of adhesion molecules and both the intake and composition of dietary fat. The n-3 fatty acids, EPA and DHA, hinder the adhesion and migration of monocytes and the processes involving leukocyte-endothelial cell interactions such as atherosclerosis and the inflammation associated with increased endothelial expression of leukocyte adhesion molecules or endothelial activation [134]. Collie-Duguid and Wahle [135] noted that EPA and DHA do not affect the expression of ICAM-1, VCAM-1 or E-selectin in resting human umbilical vascular endothelial cells. However, they attenuate the induction of these adhesion molecules in IL-1β-activated endothelial cells, which is expected to decrease the adherence and migration of leukocytes into the vascular intima and other tissues, and thus decrease plaque formation. De Caterina et al. [136] have recently reported that DHA reduces the endothelial expression of VCAM-1, E-selectin, ICAM-1, IL-6 and IL-8 in response to IL-1, IL-4, TNF and bacterial endotoxin. They also noted that the magnitude of these effects parallels the incorporation of DHA into cellular phospholipids. In addition, DHA also decreases the adhesion of human monocytes and monocytic U937 cells to cytokine-stimulated endothelial cells and VCAM-1 levels. Likewise, a fish oil-rich diet reduces basal ICAM-1 expression in murine peritoneal macrophages [137].

Stockton and Jacobson [138] have suggested the involvement of AA in adhesion-signaling pathways. Thus, lipoxygenase oxidation generates leukotriene metabolites, which regulate the spreading stage of cell adhesion, whereas ERK1/2-induced cyclooxygenase synthesis generates prostaglandins, which regulate the later migration stage. Furthermore, Barnett et al. [139] reported that cytosolic PLA_2, which mobilizes AA, is involved in the ICAM-1 expression of endothelial cells. As cell spreading, adhesion and migration are regulated by AA metabolites and olive oil- and fish oil-rich diets reduce the AA levels of membranes, AA release and proinflammatory eicosanoid synthesis [101], the impairment of eicosanoid (prostaglandins/leukotrienes) production may be involved in the decrease of adhesion molecule expression, as suggested elsewhere [140].

Soluble forms of ICAM-1, VCAM-1 and E-selectins are found in the plasma [141], probably as a result of shedding from the surface of activated endothelial cells [142]. There is a positive correlation between the extent of atherosclerosis and the plasma concentrations of soluble adhesion molecules [143–146]. Thus, soluble adhesion molecules levels represent a molecular marker of atherosclerosis and predict future myocardial infarction [147]. Fish oil diets modulate soluble adhesion molecule levels [148,149]. However, the functional significance of these effects is not fully understood.

6.2. Effects on receptor scavengers

Tissue macrophages within the aortic intima take up cholesterol through the binding and uptake of oxidized LDL to become the lipid-laden foam cells characteristic of early atherosclerotic lesions [150,151]. Oxidized LDL uptake is mediated via macrophage scavenger receptors (MSR) [152]. The role of MSR type A in plaque formation has been determined by studies revealing that MSR-A knockout mice fed a high-cholesterol diet show a significantly lower development of atherosclerotic plaques [153].

An olive oil-rich diet impairs the mRNA levels of MSR-A type I and type II [154]. The presence of lesions in the double knockout mice suggested that other LDL scavenger receptors, such as CD36, are involved in atherogenesis [153]. An olive oil-rich diet decreases the mRNA levels of CD36 in murine macrophages [154]. These data suggest that part of the protective effect of olive oil against atherosclerosis is exerted via the reduction of the macrophage uptake of oxidized LDL and subsequent foam cell formation. However, no study has revealed whether this is due to the down regulation of gene transcription directly by unsaturated fatty acids or by other components of olive oil. On the other hand, Miles et al. [137] reported that a fish oil-rich diet can decrease the expression of MSR-A, thus altering macrophage phenotype and function and enhancing macrophage-induced plaque formation. MSR-A also appears to have a role in macrophage adhesion. Therefore, blocking MSR-A with antibodies prevents macrophage binding to tissue-culture-treated plates [155]. Incubation of murine macrophages with n-3 PUFA also decreases macrophage adhesion to tissue cultured-plastic surfaces [156]. In summary, the MUFA and n-3 PUFA from the diet may reduce macrophage extravasation and persistence and, so fatty streak formation by macrophages within the arterial intima. These effects may be related to the modulation of the expression of adhesion molecules and scavenger receptors.

6.3. Effects on macrophage secretion

Macrophages are effective scavenger cells, and they are also involved in the hemostatic and fibroproliferative process thanks to their capacity to secrete cytokines, eicosanoids, $^{\bullet}$NO and numerous growth factors. During the process of atherogenesis, these autacoids may act in cell recruitment and migration, cell proliferation and the control of lipid and protein synthesis as well as in vascular events such as vasodilation, vasoconstriction and coagulation.

Macrophages from rats fed olive oil or fish oil diets reduced the levels of AA in membranes, their release and their subsequent metabolism to biosynthesize proinflammatory (proatherogenic) eicosanoids such as prostaglandins (series 2) and leukotrienes (series 4) [98,101]. These data are in agreement with Yaqoob and Calder [100] and Wallace et al. [157] who observed that macrophages isolated from mice fed with fish oil diet produced less PGE_2 and LTB_4 than cells from animals fed with a low fat diet or a high fat diet containing coconut oil. Macrophages from mice fed a Menhaden oil-rich diet showed suppression of basal TNFα mRNA and TNFα and IL-1β induced by lipopolysaccharides [157,158]. This way, the human gene expression of platelet-derived growth factor and monocyte chemoattractant protein-1 can be reduced by dietary n-3 PUFA in unstimulated and adherence-activated monocytes [159]. Likewise, dietary supplementation with n-3 PUFA ethyl esters decreases procoagulant activity and IL-6 production by human mononuclear cells [160]. The production of lipoprotein lipase also decreased in these conditions, while the release of $^{\bullet}$NO in response to TNF increased [156]. Phorbol-stimulated cells from animals fed an olive oil- or fish oil-rich diet produced higher levels of $^{\bullet}$NO than rats fed a corn oil-rich diet [101]. There is a growing body of evidence that alterations of both the synthesis and activities of $^{\bullet}$NO can promote atherosclerosis-related diseases and thrombosis-mediated vascular injury [127], as mentioned above.

7. Effects of unsaturated fatty acids on vascular smooth muscle cell proliferation

The proliferation of VSMC is a key characteristic of atherosclerosis progression [119] and a strategic target for preventing the development of arterial lesions. Furthermore, it is a major limitation to the success of interventional revascularization procedures. The correlation between lipoproteins and VSMC growth has been extensively studied. LDL may enhance growth in mitogen-stimulated cells [161]. Elinder et al. [162] reported an increase in PLA_2 in atherosclerotic lesions, mainly in macrophages and VSMC. Furthermore, oxidized phospholipids of LDL are more susceptible to hydrolysis by PLA_2 [163], with the subsequent AA release. Anderson et al. [164] provided direct evidence that PLA_2 is involved in the control of VSMC proliferation and indicated that cytosolic PLA_2-mediated AA release is critical for this event.

Terano et al. [165] observed that the PUFA of both n-6 and n-3 series inhibit VSMC proliferation. EPA and DHA hinder DNA synthesis and cyclin-dependent kinases and stopped the progression from G_1 to the S phase of the cell cycle. These effects are related to the amount of lipid peroxides formed in the cells [166]. These results are supported by Nakayama et al. [167] who showed that EPA significantly inhibits the expression of tumor growth factor β mRNA and Cdk2 activity in VSMC. Likewise, Mata et al. [168] cultured VSMC in the presence of sera from volunteers fed with olive oil, sunflower oil or fish oil diets. [^{3}H]thymidine incorporation into DNA was significantly reduced by MUFA- and n-3 PUFA-rich diets. These data may be linked to the impairment of eicosanoid synthesis in these dietary conditions [98,101] and to a critical effect on

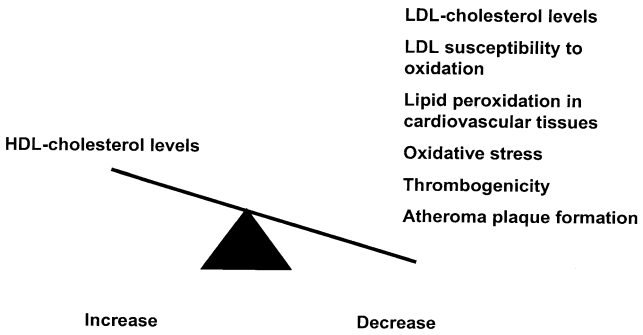

Fig. 2. Effects of olive oil on the main mechanisms of the development of atherosclerosis. Olive oil consumption increases HDL-cholesterol levels, whereas it decreases LDL-cholesterol levels, LDL susceptibility to oxidation and lipid peroxidation. The reduction of cellular oxidative stress, thrombogenicity and the formation of atheroma plaque can explain the preventive effects of olive oil on atherosclerosis development.

cell cycle control through modulation of the levels of the proteins of the cell cycle machinery [169].

8. Conclusions

The effects of MUFA and n-3 PUFA on LDL levels, LDL oxidation, prooxidant species production, cellular resistance to oxidative stress, $^{\bullet}$NO and eicosanoid production, cell adhesion molecules, receptor scavenger expression and VSMC proliferation show that both dietary fats are crucial in modulating pivotal elements of the development of atherosclerosis. The preventive effects of MUFA on atherosclerosis development may be due to the enhancement of HDL-cholesterol levels and the impairment of LDL-cholesterol levels, LDL susceptibility to oxidation, cellular oxidative stress, thrombogenicity and the formation of atheroma plaque (Fig. 2), whereas n-3 PUFA consumption increases HDL-cholesterol levels. This and the reduction of thrombogenicity, atheroma plaque formation and VSMC proliferation may account for the beneficial effects of PUFA on the prevention of atherosclerosis (Fig. 3). These findings point to the beneficial effects of certain components of the Mediterranean diet, which is rich in olive oil and fish. However, we cannot confirm whether this is merely due to changes in the fatty acid composition of the diet or to other oil components.

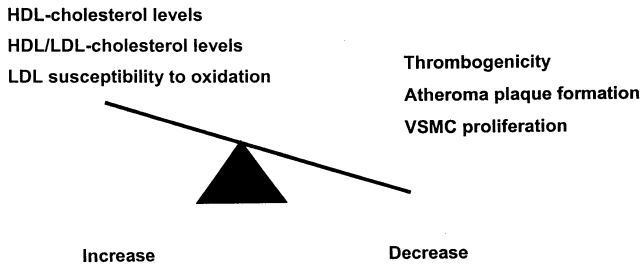

Fig. 3. Effects of fish oil on the main mechanisms of the development of atherosclerosis. Fish oil consumption increases HDL-cholesterol levels. This effect, along with the reduction of thrombogenicity, the formation of atheroma plaque and VSMC proliferation, can explain the preventive effects of fish oil on atherosclerosis development.

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