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Chapter 1

Atherosclerosis and Current Anti-Oxidant Strategies for Atheroprotection

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Additional information is available at the end of the chapter

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1. Introduction

Cardiovascular diseases (CVDs) remain the leading cause of death in modern societies. The primary cause of dramatic clinical events of CVDs, such as unstable angina, myocardial infarction and stroke, is the atherosclerotic process [1,2,3].

The pathophysiological mechanisms of atherosclerosis are complicated and the integrated picture of the disease process is not yet complete, so currently is largely investigated. It is widely recognized that oxidative stress, lipid deposition, inflammation, Vascular smooth muscle cells (VSMCs) differentiation and endothelial dysfunction play a critical role in the formation, progression and eventually rupture of the atherosclerotic plaque [4]. Multiple risk factors have been associated with the development of atherosclerotic lesions; these include diabetes mellitus, hypertension, obesity and tobacco smoking. The risk factors are influenced by genetic predisposition, but also by environmental factors, particularly diet. Moreover, aging promotes physiological changes, such as oxidative stress, inflammation and endothelial dysfunction strictly associated with the pathophysiology of atherosclerosis [5].

The common belief that signs of atherosclerosis and CVDs are clinically relevant only during adult and elderly age is gradually changing, increasing evidence supports that atherogenesis is initiated in childhood [6].

Low-density lipoproteins (LDL) are crucial to the development of atherosclerotic lesions, whereas high-density lipoproteins (HDL) are inhibitors of the process, primarily through the process of reverse cholesterol transport [4,7]. Dysfunctional lipid homeostasis plays a central role in the initiation and progression of atherosclerotic lesions. Oxidized-LDL (ox-LDL) induces endothelial dysfunction with focal inflammation which causes increased expression of atherogenic signaling molecules that promote the adhesion of monocytes and T

lymphocytes to the arterial endothelium and their penetration into the intima. Early stages of plaque development involve endothelial activation induced by inflammatory cytokines, ox-LDL and/or changes in endothelial shear stress [8,9]. The monocyte-derived macrophages, by taking up ox-LDL, become foam cells, which are typical cellular elements of the fatty streak, the earliest detectable atherosclerotic lesion [10].

After initial injury, different cell types, including endothelial cells, platelets and inflammatory cells release growth factors and cytokines that induce multiple effects: oxidative stress, inflammation, VSMCs differentiation from the contractile state to the active synthetic state and then proliferate and migrate in the subendothelial space [11,12]. Inflammatory cell accumulation, migration and proliferation of VSMCs, as well as the formation of fibrous tissue, lead to the enlargement and restructuring of the lesion, with the formation of an evident fibrous cap and other vascular morphological changes [2,13]. Atherosclerotic plaques result from the progressive accumulation of cholesterol and lipids in oxidized forms, extracellular matrix material and inflammatory cells [14]. In fact, atherosclerosis manifests itself histologically as an arterial lesions known as plaques, which have been extensively characterized: plaques contain a central lipid core that is most often hypocellular and may include crystals of cholesterol that have formed in the foam cells. The lipid core is separated from the arterial lumen by a fibrous cap and myeloproliferative tissue that consists of extracellular matrix and VSMCs. Advanced lesions can grow sufficiently large to block blood flow and so develop an acute occlusion due to the formation of thrombus or blood clot resulting in the important and severe cardiovascular clinical events [2,10].

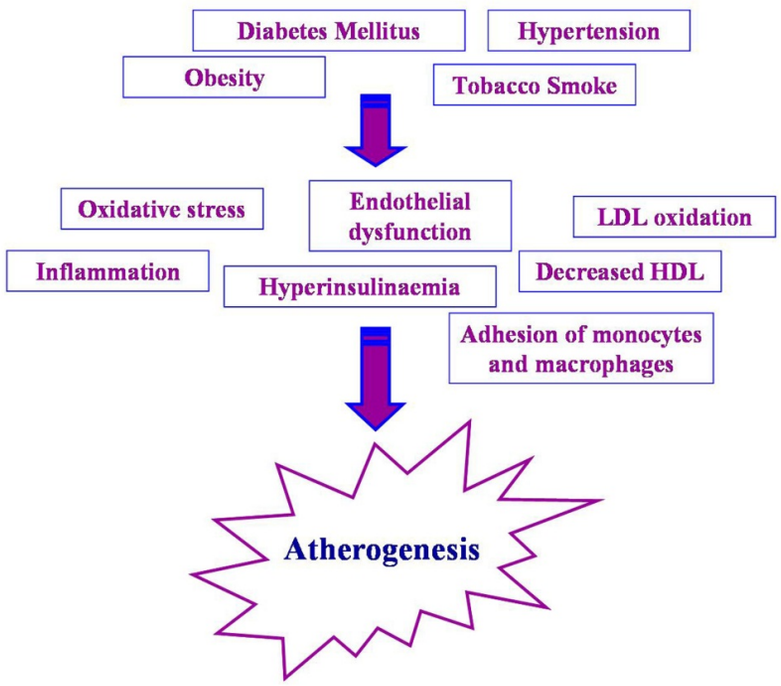

Figure 1. Main vascular alterations observed during atherogenesis. LDL: low density lipoprotein; HDL: high density lipoprotein.

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2. Atherosclerosis and oxidative stress

Oxidative stress is defined as an imbalance between pro-oxidant and anti-oxidant factors in favour of pro-oxidants and is central to the pathophysiology of atherosclerosis. The analysis of plaque composition has revealed products of protein and lipid oxidation, such as chlorinated, nitrated amino acids, lipid hydroperoxides, short-chain aldehydes, oxidized phospholipids, F2α-isoprostanes and oxysterols [15].

Excessive production of reactive oxygen species (ROS) during oxidative stress, out stripping endogenous anti-oxidant defence mechanisms, has been implicated in processes in which they oxidize and damage DNA, protein, carbohydrates and lipids. There are multiple potential enzymatic sources of ROS, including mitochondrial respiratory cycle, heme, arachidonic acid enzyme, xanthine oxidase, nitric oxide synthese and others. However, the predominant ROS-producing enzyme in the VSMCs and in the myocardium is NADPH oxidase, that plays a pivotal role in the atherogenesis [16].

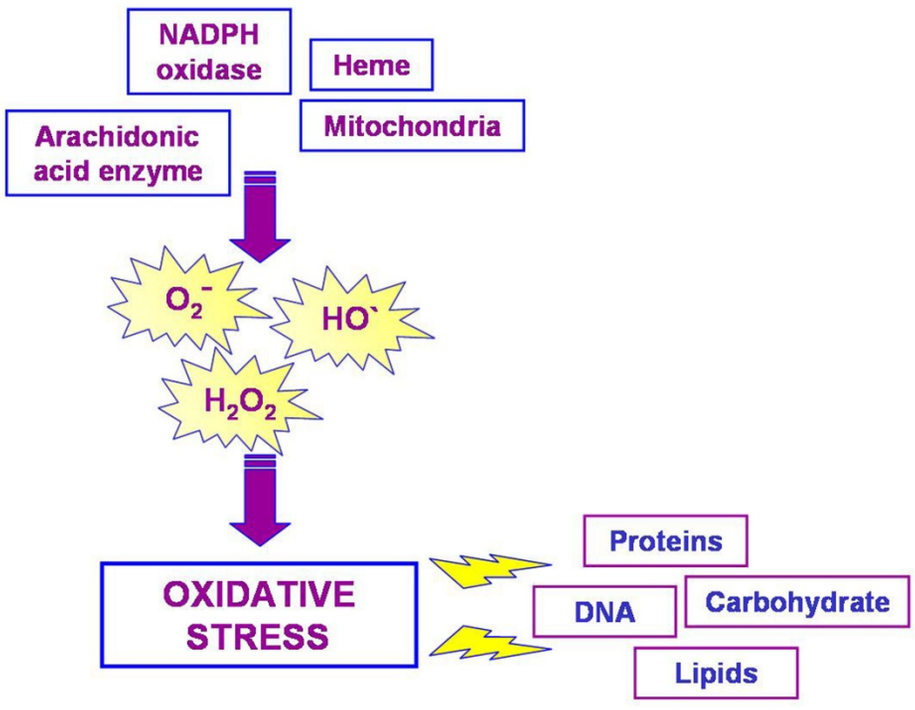

Figure 2. Generation and main damages induced by ROS. Modified from [17]. O_2^-: superoxide; HO`: hydroxyl; H_2O_2: hydrogen peroxide.

ROS may contribute to LDL oxidation, inflammation, local monocyte chemoattractant protein production, upregulation of adhesion molecules and macrophages recruitment, endothelial dysfunction, platelet aggregation, extracellular matrix remodelling through collagen degradation, thus playing a central role in the development and progression of atherosclerosis and eventually in plaque rupture [17,18,19]. Several oxidative systems potentially contribute to LDL oxidation *in vivo*, included NADPH oxidases, xanthine oxidase, myelo-

peroxidase, uncoupled nitric oxide synthase, lipoxygenases and mitochondrial electron transport chain [20,21,22]. Ox-LDL particles exhibit multiple atherogenic properties, which include uptake and accumulation of macrophages, as well as pro-inflammatory, immunogenic, apoptotic and cytotoxic activities, induction of the expression of adhesion molecules on endothelial cells, promotion of monocyte differentiation into macrophages, production and release of pro-inflammatory cytokines and chemokines from macrophages [14].

In particular, at endothelial level, ROS regulates numerous signaling pathways including those regulating growth, proliferation, inflammatory responses of endothelial cells, barrier function and vascular remodeling; while at VSMC level, ROS mediates growth, migration, matrix regulation, inflammation and contraction [23,24,25], all are critical factors in the progression and complication of atherosclerosis.

A vicious cycle between oxidative stress and oxidative stress-induced atherosclerosis leads to the development and progression of atherosclerosis.

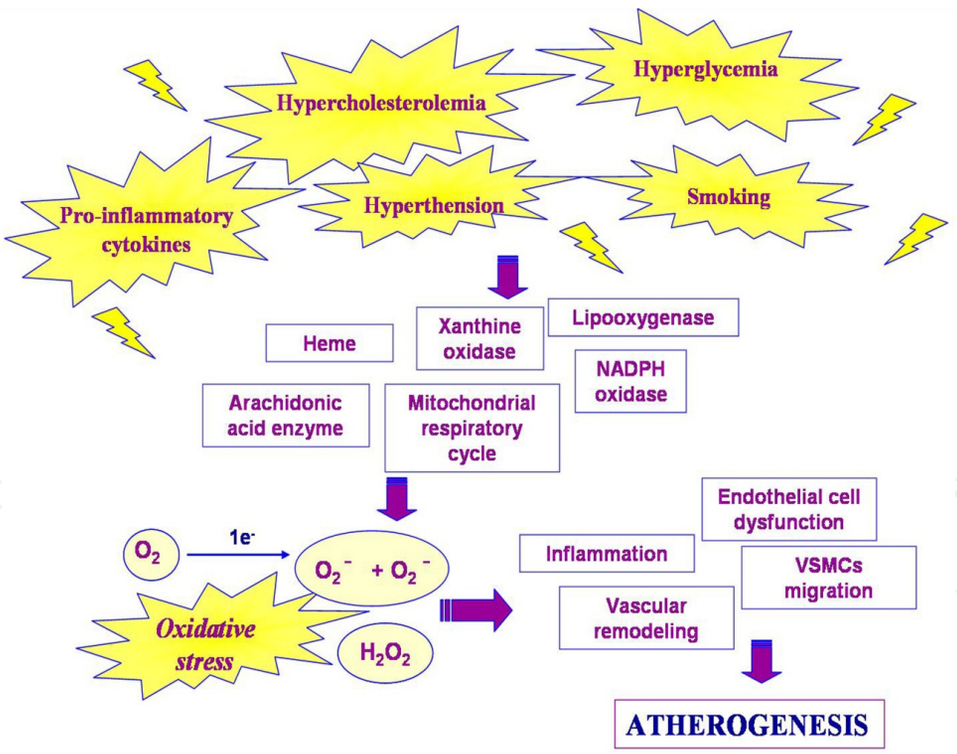

Figure 3. Role of ROS and oxidative stress in the atherosclerosis. Modified from [24]. O_2: oxygen; O_2^{-}: superoxide; H_2O_2: hydrogen peroxide; VSMC: vascular smooth muscle cell.

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3. Atheroprotective strategies

Recently, various pharmacological therapies have been designed to reduce the development and progression of the atherosclerotic plaque and remarkable therapeutic advances in the treatment of CVDs have been made with insulin sensitizers, statins, inhibitors of the renin-angiotensin system and anti-platelet agents [19,26]. However, strictly control of cardiovascular risk factors are often difficult to obtain and the progression of atherosclerosis has not been completely prevented with current pharmacological therapeutic options. Moreover, the modern evolution of Western societies seemingly steers populations towards a profound sedentary lifestyle and incorrect diet is becoming difficult to reverse. Understanding of the mechanisms that explain the fatal effects of physical inactivity and incorrect diet, the beneficial effects of an healthy lifestyle remains largely unexplored [3].

Concerning atherosclerosis prevention by foods, dietary supplements and healthy life style may provide prevention and/or treatment to the onset and development of atherosclerosis. Development of an atheroprotective strategy acting on oxidative stress involved in the pathogenesis of atherosclerosis and with little toxicity or adverse effects may provide an ideal therapeutic treatment for atherosclerosis. Actually, numerous studies have investigated the prevention and treatment of atherosclerosis using naturally-occurring anti-oxidants.

In this review we summarize the many pieces of the puzzle to identified molecular targets for prevention and therapy against atherosclerosis and present that a healthy life style has natural anti-atherogenic activity which has been forgotten by modern societies.

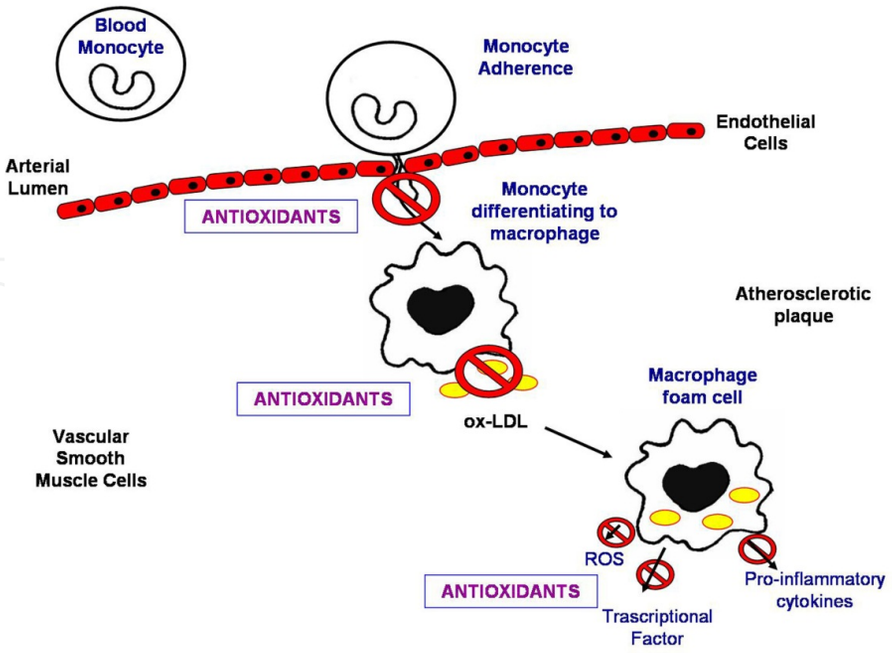

Figure 4. Potential atheroprotective role of anti-oxidants in the atherogenic process. Modified from [27]. ox-LDL: oxidized-low density lipoprotein; ROS: reactive oxygen species.

4. Physical exercise

Physical activity is currently recognized as a potent tool for the prevention of chronic degenerative diseases, including CVDs and common tumors, such as those affecting the colon, breast, prostate and endometrium [28].

There is a body of clinical and experimental evidence showing that voluntary and imposed physical exercise prevents the progression of CVDs and reduces cardiovascular morbidity and mortality. Therefore a physically active state is an appropriate and natural biological condition for human and most animal species [3].

It has been demonstrated that exercise slows the progression of atherosclerosis, promoting its stabilization and preventing plaque rupture in a variety of hypercholesterolaemic animal models, such as apolipoproteinE-deficient mice and LDL receptor-deficient mice, whereas physical inactivity accelerates it [3,29].

Exercise increases blood anti-oxidant capacity through elevating hydrophilic anti-oxidants (uric acid, bilirubin and vitamin C) and decreases lipophilic anti-oxidants (carotenoids and vitamin E) [28]. It is noteworthy that exercise prevents plaque vulnerability and atherosclerosis progression without necessarily correcting classic risk factors, such as hypercholesterolaemia, endothelial dysfunction and high blood pressure, suggesting that exercise can directly affect plaque composition and phenotype, thus preventing the appearance of fatal lesions. Besides the effect of diet and drugs, the protective role of regular exercise against atherosclerosis is well established and its beneficial atheroprotective effects are not limited to one particular cell, but to a variety of cells and tissues involved in the pathogenesis of atherosclerosis and metabolic disorders, such as macrophages and adipose tissue [3].

Regular exercise and a correct diet would be natural atheroprotective approaches which has been forgotten by modern societies.

5. Diet

Several epidemiological studies suggest that a correct diet is significantly associated with reduced risks of CVDs. Phytochemicals including polyphenols like flavonoids, resveratrol and ellagitannins have been shown to be associated with lower risks of CVDs [30,31]. In fact, they are potent anti-oxidants and anti-inflammatory agents, thereby counteracting oxidative damage and inflammation. Actually, dietary anti-oxidants have attracted considerable attention as preventive and therapeutic agents. There is adequate evidence from observational *in vitro*, *ex vivo* and *in vivo* studies that consumption of certain foods results to a reduction in oxidative stress [27]. Evidence linking dietary anti-oxidants to atherosclerosis in humans is still circumstantial and although in some studies the association of anti-oxidant intake and low risk for atherosclerosis is perceptible, in others this

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association cannot be established. The inconsistency of the results reflects the limitations of human studies, the diet differences, the pre-existing total anti-oxidant status, the stage of disease, the interaction between dietary modulation and genetic composition of individuals, the dosage and duration of supplementation, the age and the sex. On the other hand, studies in animal models of atherosclerosis clearly show an atheroprotective effect of dietary anti-oxidants, however, they focus mainly on early atherosclerotic events and not in advanced atherosclerosis as in humans [27].

Cardiovascular prevention and treatment strategies should consider the simple, direct and inexpensive dietary approach as a first-line strategy to the burgeoning burden of CVDs, alone or in combination with pharmalogic treatments [10].

In this review we focus our attention on the main natural anti-oxidants contained in food and on their primary diet source.

6. Polyphenol

Polyphenols are the most abundant anti-oxidants in human diet and are common constituents of foods of plant origin and are widespread constituents of fruits, vegetables, cereals, olive, legumes, chocolate and beverages, such as tea, coffee and wine [32,33].

They are defined according to the nature of their backbone structures: phenolic acids, flavonoids and the less common stilbenes and lignans. Among these, flavonoids are the most abundant polyphenols in the diet [34]. Despite their wide distribution, the health effects of dietary polyphenols have been attentively studied only in recent years [32] and several studies, although not all, have found an inverse association between polyphenol consumption and CVDs motality [35].

Polyphenols exert anti-atherosclerotic effects in the early stages of atherosclerosis development, they decrease LDL oxidation, improve endothelial function, increase vasorelaxation, modulate inflammation and lipid metabolism, improve anti-oxidant status and protect against atherothrombotic events including myocardial ischemia and platelet aggregation [35].

Many polyphenols have direct anti-oxidant properties, acting as reducing agents, and may react with reactive chemical species forming products with much lower reactivity. Polyphenols may also affect indirectly the redox status by increasing the capacity of endogenous anti-oxidants or by inhibiting enzymatic systems involved in ROS formation [36]. The free-radical scavenging activity of many polyphenols has been reported to be much stronger than that of vitamin C, vitamin E or glutathione, the major anti-oxidants present in the body.

In spite of their potent protective effects in the development of atherosclerosis, little is known about aortic distribution of polyphenols [34].

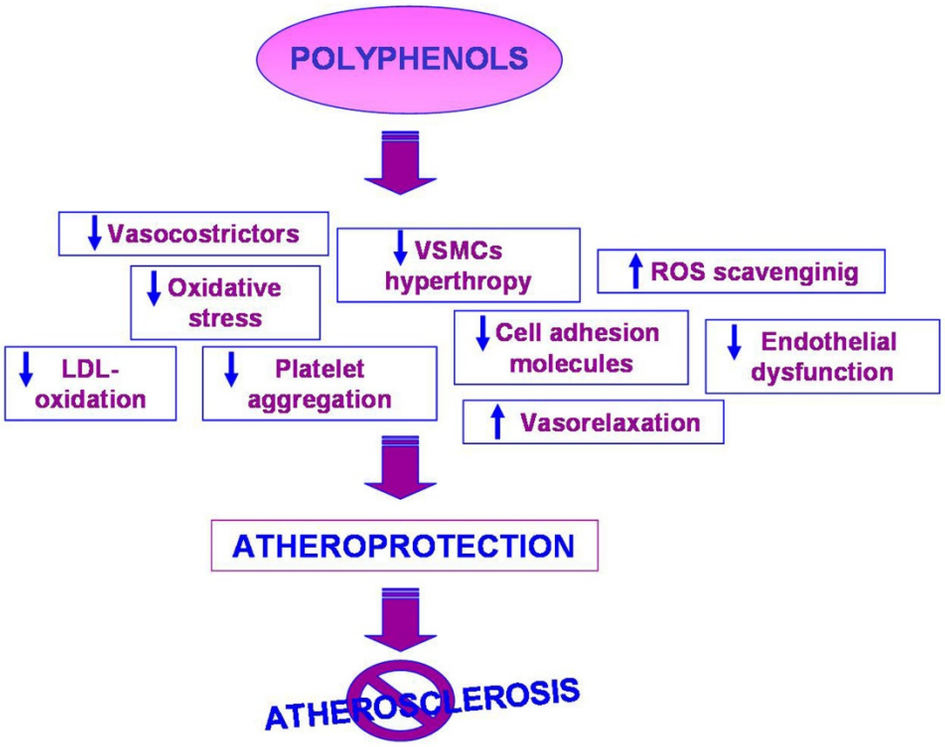

Figure 5. Main atheroprotective mechanisms exert by polyphenols. VSMC: vascular smooth muscle cell; LDL: low density lipoprotein; ROS: reactive oxygen species.

6.1. Resveratrol

Resveratrol naturally occurs as a polyphenol found in grapes and grape products, including wine, as well as other sources, like nuts [37]. In grapes, resveratrol is present in the skin as both free resveratrol and piceid.

Initially characterized as a phytoalexin, a toxic compound produced by higher plants in response to infection or other stresses, such as nutrient deprivation, resveratrol attracted little interest until 1992 when it was postulated to explain some of the cardioprotective effects of red wine [36].

Treatment with resveratrol has been found to reduce oxidative stress and increase the activities of several anti-oxidant enzymes including superoxide dismutase, catalase, glutathione, glutathione reductase, glutathione peroxidase and glutathione-5-transferase [38]. Resveratrol also prevents the oxidation of polyunsaturated fatty acids found in LDL and inhibits the ox-LDL uptake in the vascular wall in a concentration-dependent manner, as well as prevents damage caused to lipids through peroxidation [38]. These effects were found to be stronger respect the well known anti-oxidant vitamin E. Moreover, resveratrol has been proposed to influence and maintain a balance between production of vasodilatators and vasocostrictors respectively [38,39], thereby preventing platelet aggregation and oxidative stress, which leads to reduction in CVD risk [40].

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Resveratrol so has been demonstrated to exert a variety of health benefits including anti-atherogenic, anti-inflammatory and anti-tumor effects. These positive effects are attributed mainly to its anti-oxidant and anti-coagulative properties.

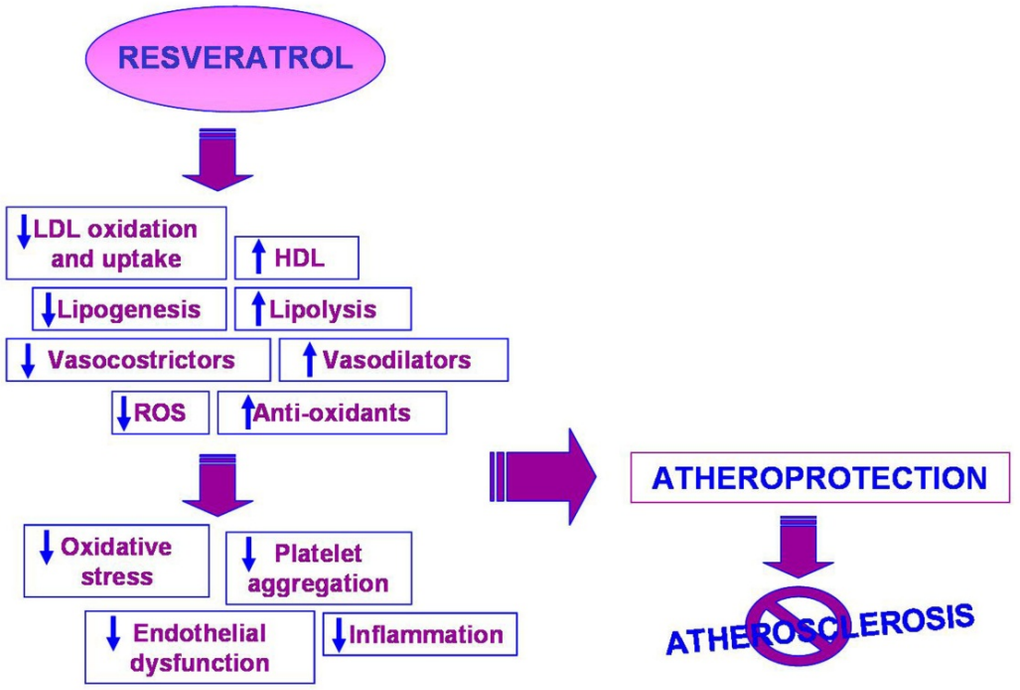

Figure 6. Main atheroprotective mechanisms exert by resveratrol. LDL: low density lipoprotein; HDL: high density lipoprotein; ROS: reactive oxygen species.

Resveratrol reduced not only vascular lipid levels, including LDL and triglycerides, but also the myocardial complications by influencing infarct size, apoptosis and angiogenesis. In addition, resveratrol feeding prevented steatohepatitis induced by atherogenic diets through modulation of expression of genes involved in lipogenesis and lipolysis, reduced total and LDL levels, while increasing HDL levels in plasma.

Several investigations with human and various animal model have demonstrated an absence of toxic effects after supplementation with resveratrol across a wide range of dosages [38].

Promising findings by several groups have demonstrated the potential cardioprotection of resveratrol by reducing atherosclerotic plaque onset and formation.

6.2. Flavinoid

Flavonoids, many of which are polyphenolic compounds, are believed to be beneficial for the prevention and treatment of atherosclerosis and CVDs mainly by decreasing oxidative stress and increasing vasorelaxation [32,40,41]. More than 8.000 different flavonoids have been described and since they are prerogative of the kingdom of plants, they are part of human diet with a daily total intake amounting to 1 g, which is higher than all other classes of phytochemicals and known dietary anti-oxidants. In fact, the daily intake of vitamin C, vitamin E and β-carotene from food is estimated minor of 100 mg. A number of different factors, such as harvesting, environmental factors and storage, may affect the polyphenol content of plants. Additional variability in flavonoid content could be expected in finished food products because its availability is largely dependent on the cultivar type, geographical origin, agricultural practices, post-harvest handling and processing of the flavonoid containing ingredients [32].

Flavonoids are widely distributed in the plant and are categorized as flavonol, flavanol, flavanone, flavone, anthocyanidin and isoflavone. Quercetin is one of the most widely distributed flavonoids, which are abundant in red wine, tea and onions. Quercetin intake is therefore suggested to be beneficial for human health and its anti-oxidant activity should yield a variety of biological effects.

The major flavanols in the diet are catechins. They are abundant in green tea (about 150mg/100ml) and lesser extent in black tea (13.9 mg/100 ml) where parent catechins are oxidized into complex polyphenols during fermentation. Red wine (270 mg/L) and chocolate (black chocolate: 53.5 mg/100 g; milk chocolate: 15.9 mg/100 g) are also sources of catechins [34].

Polyphenols and/or flavonoids exhibit a variety of beneficial biological effects, including anti-oxidant, anti-hypertensive, anti-viral, anti-inflammatory and anti-tumor activities; moreover some flavonoids have also been reported to modulate insulin resistance, endothelial function and apoptosis [32,41].

Many studies have shown that flavonoids demonstrate protective effects against the initiation and progression of atherosclerosis. The bioactivity of flavonoids and related polyphenols appears to be mediated through a variety of mechanisms, though particular attention has been focused on their direct and indirect anti-oxidant actions. In particular, it has been shown that the consumption of flavinoids limits the development of atheromatous lesions, inhibiting the oxidation of LDL, which is considered a key mechanism in the endothelial lesions occurring in atherosclerosis.

Mechanisms of anti-oxidant effects include also: suppression of ROS formation either by inhibition of enzymes or chelating trace elements involved in free radical production, scaveng ROS and upregulation or protection of anti-oxidant defences [32]. The phenolic hydroxyl groups of flavonoids, which act as electron donors, are responsible for free radical scavenging activity [27,40].

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Since the evidence of therapeutic effects of dietary flavinoids continues to accumulate, flavinoids could be considered as anti-oxidant nutrients available in everyday life as a protective tool for prevention of atherosclerosis.

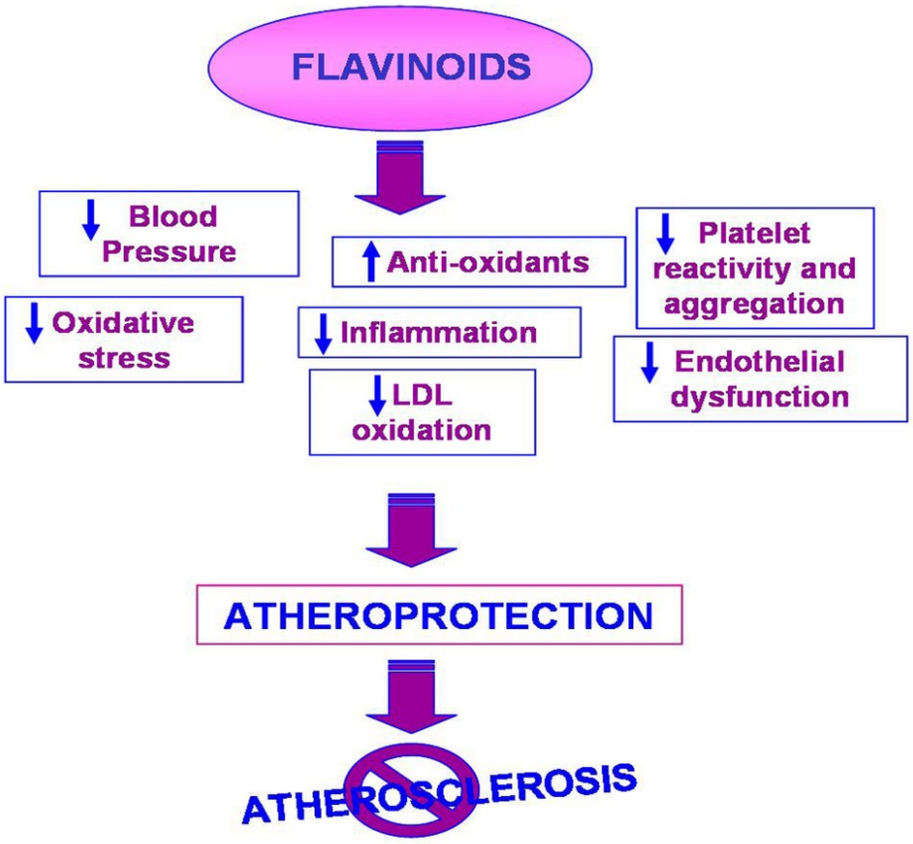

Figure 7. Main atheroprotective mechanisms exert by flavinoids. LDL: low density lipoprotein.

7. Green tea

Tea, a beverage consumed worldwide, is a source of both pleasure and healthful benefits. Originally recommended in traditional Chinese medicine, green tea (*Camellia sinensis*) has gained considerable attention due to its anti-oxidant, anti-inflammatory, anti-hypertensive, anti-diabetic and anti-mutagenic properties [42].

Green tea constitutes 20%-22% of tea production and is principally consumed in China, Japan, Korea and Morocco. Green tea, or non-fermented tea, contains the highest amount of flavonoids, in comparison to its partially fermented (oolong tea) and fermented (black tea) counterparts and, due to its high content of polyphenolic flavonoids, has shown unique cardiovascular health benefits. In green tea, catechins comprise 80% to 90% of total flavonoids, with epigallocatechin gallate, being the most abundant catechin (48–55%), followed by epi-

gallocatechin (9–12%), epicatechin gallate (9–12%) and epicatechin (5–7%) [42]. The catechin content of green tea depends on several factors including how the leaves are processed before drying, preparation of the infusion and decaffeination, as well as the form in which it is distributed in the market (instant preparations, iced and ready-to-drink teas have been shown to contain fewer catechins) [43]. When tea leaves are rolled or broken during industry manufacture, catechins come in contact with polyphenol oxidase, resulting in their oxidation and the formation of flavanol dimers and polymers known as theaflavins and thearubigins [44].

Tea leaves destined to become black tea are rolled and allowed to ferment, resulting in relatively high concentrations of theaflavins and thearubigins and relatively low concentrations of catechins. Consequently, green tea contains relatively high concentrations of catechins and low concentrations of theaflavins and thearubigins. It is important to underline that black tea administration to LDL receptor-deficient mice did not affect aortic fatty streak lesion area, although fatty streak lesion areas in the same animal model supplemented with anti-oxidants, such as vitamin C, vitamin E and β-carotene, were 60% smaller than those of control animals [44,45]. On the other hand, green tea catechins have been shown to inhibit formation of ox-LDL, may decrease linoleic acid and arachidonic acid concentrations [46], elevate serum anti-oxidative activity and prevent or attenuate decreases in anti-oxidant enzyme activities [44]. In addition to having anti-oxidant properties, green tea catechins have also been shown to reduce VSMCs proliferation [42].

In particular, Erba et al. (2005) showed a significant decrease in plasma peroxide levels, DNA oxidative damage and LDL oxidation, as well as a significant increase in total anti-oxidant activity in the plasma of healthy volunteers who consumed two cups of green tea per day in addition to a balanced and controlled diet demonstrating that green tea may act synergistically with a correct diet in affecting the biomarkers of oxidative stress [47]. Much of the evidence supporting anti-oxidant functions of tea polyphenols is derived from assays of their anti-oxidant activity *in vitro*. However, evidence that tea polyphenols are acting directly or indirectly as anti-oxidants *in vivo* is more limited [44].

It is very important to underline also that while green tea beverage consumption is considered part of a healthy lifestyle, green tea extracts supplements should be used with caution. Very high doses of green tea extracts (6 g–240 g) have been associated with hepatotoxicity in patients who used them for a duration of 5 to 120 days, changing in blood biochemical parameters included an elevation of serum levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin and albumin levels. Although, it was observed a reversal of symptoms when subjects stopped taking the green tea supplement [42].

In addition, in a number of countries, tea is commonly consumed with milk. Interactions between tea polyphenols and proteins found in milk have been found to diminish total anti-oxidant capacity *in vitro*, but it is presently unclear whether consuming tea with milk substantially alters the biological activities of tea flavonoids *in vivo*. The addition of milk to tea did not significantly alter areas under the curve for plasma catechins or flavonols in human volunteers, suggesting that adding milk to tea does not substantially affect the bioavail-

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ability of tea catechins or flavonols. Two studies in humans found that the addition of milk decreased or eliminated increase in plasma anti-oxidant capacity induced by tea consumption, whereas another found no effect [44].

Nevertheless, a diet rich in foods containing anti-oxidant polyphenols, like green tea beverages, combined with physical activity and a correct diet may offer primary prevention against CVDs. While future clinical trials could further elucidate the cardioprotective benefits of green tea beverages, on the basis of existing reports, freshly prepared green tea appears to be a healthy dietary choice to consider as an atheroprotective strategy.

8. Herbal

Studies of the herbal medicines for the prevention and treatment of atherosclerosis have received much attention in recent years. Single compounds isolated from some herbal materials have been shown to reduce the production or remove the build up of cholesterol *in vitro* or *in vivo* studies. Glabrol from Glycyrrhiza glabra has been found to be an acyl-coenzyme A: a cholesterol acyltransferase inhibitor that blocks the esterification and intestinal absorption of free cholesterol. Curcumin from Curcuma longa inhibited cholesterol accumulation. Puerarin from Pueraria lobata can promote cholesterol excretion into bile by upregulating the rate-limiting enzyme in the synthesis of bile acid from cholesterol. Moreover, these extracts have anti-oxidative effects and may reduce the levels of ox-LDL and increased the levels of HDL [48].

9. Pomegranate juice

Pomegranate juice consumption slowed atherosclerosis progression through the potent anti-oxidant properties of pomegranate polyphenols [35].

Pomegranate fruit (*Punica granatum L.*) has been rated to contain the highest anti-oxidant capacity in its juice, when compared to other commonly consumed polyphenol rich beverages. The anti-oxidant capacity of pomegranate juice was shown to be three times higher than that of red wine and green tea, based on the evaluation of the free-radical scavenging and iron reducing capacity [30]. It was also shown to have significantly higher levels of anti-oxidants in comparison to commonly consumed fruit juices, such as grape, cranberry, grapefruit or orange juice. The principal anti-oxidant polyphenols in pomegranate juice are ellagitannins and anthocyanins. Ellagitannins account for 92% of the anti-oxidant activity of pomegranate juice and are concentrated in peel, membranes and piths of the fruit. The bioavailability of pomegranate polyphenols is affected by several factors, including: interindividual variability, differential processing of pomegranate juice, as well as the use of analytical techniques sensitive enough to detect low postprandial concentrations of these metabolites [30].

One pomegranate fruit contains about 40% of an adult's recommended daily requirement of vitamin C and is high in polyphenol compounds. The pomegranate plant contains alkaloids,

mannite, ellagic acid and gallic acid and the bark and rind contain various tannins. The polyphenols in pomegranate are believed to provide the anti-oxidant activity and protect LDL against cell-mediated oxidation directly by interaction with the LDL [49]. In fact, the supplementation of pomegranate juice revealed a significant reduction in the atherosclerotic lesion area compared to the water-treated group reporting significant anti-oxidant capacities of all pomegranate extracts.

The principal mechanisms of action of pomegranate juice may include: increased serum anti-oxidant capacity, decreased plasma lipids and lipid peroxidation, decreased ox-LDL uptake by macrophages, decreased intima-media thickness, decreased atherosclerotic lesion areas, decreased inflammation and decreased systolic blood pressure, thereby reducing/inhibiting the progression of atherosclerosis and the subsequent potential development of CVDs [30,50].

On the basis of limited safety data, high doses of pomegranate polyphenol extracts may have some deleterious effects: gastric irritation, allergic reactions, including pruritus, urticaria, angioedema, rhinorrhea, bronchospasm, dyspnea and red itchy eyes. Moreover, dried pomegranate peel may contain aflatoxin, a potent hepatocarcinogen; thus, it should be used cautiously by patients who have hepatic dysfunction or who are taking other hepatotoxic agents. Pomegranate may also increase the risk for rhabdomyolysis during statin therapy, as a result of intestinal CYP3A4 inhibition and increased absorption of active drugs [49].

10. Wine

The last two decades have seen renewed interest in the health benefits of wine, as documented by increasing research and several epidemiologic observations showing that moderate wine drinkers have lower cardiovascular mortality rates than heavy drinkers or teetotalers. Most of the beneficial effects of wine against CVDs have been attributed to the presence in red wine of resveratrol and other polyphenols. Wines contain polyphenolic compounds that can be roughly classified in flavonoid and non flavonoid compounds; both classes of compounds have been implicated in the protective effects of wine on the cardiovascular system. Resveratrol is one of the most biologically active polyphenols contained in wine.

Moderate wine intake reduces cardiovascular risk [51]. In addition, it is known that alcohol favourably modifies the lipid pattern by decreasing total plasma cholesterol, in particular LDL, and by increasing HDL. Cardiovascular risk reduction seems to be linked largely to the effect of non-alcoholic components, mainly resveratrol and other polyphenols, on the vascular wall and blood cells and a great part of the beneficial effects of resveratrol on vascular function are due to its anti-oxidant effects.

The effect of resveratrol and other wine polyphenols on oxidative stress has been scarcely explored in humans and only a few studies have analyzed the effects of wine supplementation on indexes of oxidation *in vivo* [36].

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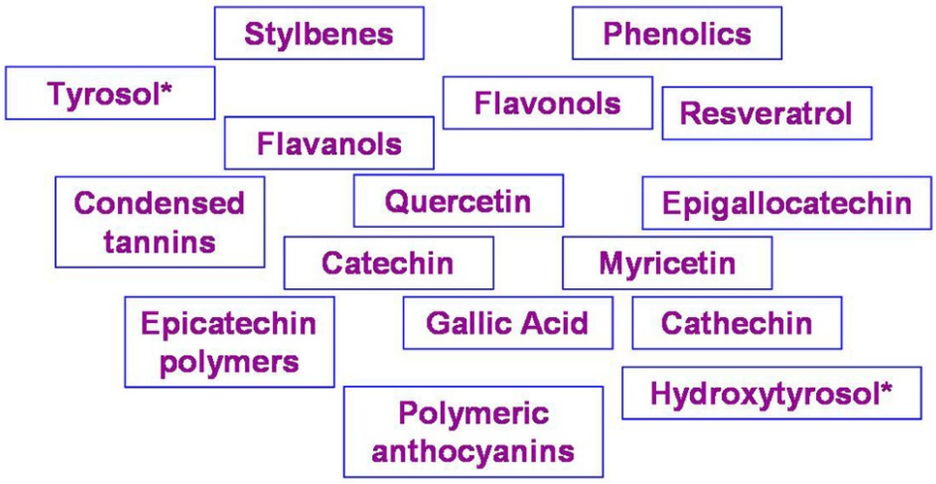

Figure 8. Main polyphenols in wine. * Polyphenols contained only in white wine. Modified from [36].

11. Olive oil

A high intake of some unsaturated fatty acid and/or anti-oxidant compounds can both reduce pro-atherogenic risk factors and the susceptibility of the vascular wall to pro-inflammatory and pro-atherogenic triggers.

Many Authors started to recognize olive oil as one of the key elements in the cardioprotection and longevity of inhabitants of Mediterranean regions. The healthful properties of olive oil have been often attributed to its high content of monounsaturated fatty acids, namely oleic acid [7]. However, it should be underlined that olive oil, unlike other vegetable oils, contains high amounts of several micronutrient constituents, including polyphenolic compounds (100– 1000 mg/kg) [10].

The major phenolic compounds in olive oil are: simple phenols (*i.e.*, hydroxytyrosol, tyrosol); polyphenols (oleuropein glucoside); secoiridoids, dialdehydic form of oleuropein and ligstroside lacking a carboxymethyl group and the aglycone form of oleuropein glucoside and ligstroside and lignans. Around 80% or more of the olive oil phenolic compounds are lost in the refination process, thus, their content is higher in virgin olive oil (around 230 mg/kg) than in other olive oils.

Olive oil supplementation (50 mg/day) to the diet enriched LDL with oleic acid and significantly reduced human LDL susceptibility to *in vitro* oxidation, thus making them significantly less atherogenic. In part, this reflects the lesser susceptibility of monounsaturated fatty acids to lipid peroxidation compared with that of polyunsaturated fatty acids, which are particularly prone to peroxidation due to the greater number of double bonds [10,52].

Olive oil consumption could reduce oxidative damage, on one hand, due to its richness in oleic acid and, on the other hand, due to its minor components of the olive oil particularly

the phenolic compounds. The phenolic content in virgin olive oil could reduce the lipid oxidation and inhibit platelet-induced aggregation [53].

Moreover, olive oil minor components have also been involved in the anti-oxidant activity of olive oil. Some components of the unsaponifiable fraction, such as squalene, β-sitosterol or triterpenes, have been shown to display anti-oxidant and chemopreventive activities and capacity to improve endothelial function decreasing the expression of cell adhesion molecules and increasing vasorelaxation [54].

Olive oil phenolic compounds are able to bind the LDL lipoprotein and to protect other phenolic compounds bound to LDL from oxidation. The role of phenolic compounds from olive oil on DNA oxidative damage remains controversial and perhaps more sensitive methods would be required to detect differences among the types of olive oil consumed. Further studies are required to establish the potential benefits of olive oil and those of its minor components on DNA oxidative damage.

One of the most well known and important characteristic of the Mediterranean diet is the presence of virgin olive oil as the principal source of energy from fat. In contrast to other edible oils with a similar fatty composition, like sunflower, soybean and rapeseed canola oils, virgin olive oil is a natural juice, while the seed oils must be refined before consumption, thus changing its original composition during this process. Virgin olive oils are those obtained from the olives solely by mechanical or other physical means under conditions that do not lead alteration in the oil. The olives have not undergone any treatment other than washing, decantation, centrifugation or filtration [53].

Virgin olive oil is a source of healthy unsaturated fatty acids and hundreds of micronutrients, especially anti-oxidants, as phenol compounds, vitamin E and carotenes.

Results of the randomized cross-over clinical trials performed in humans on the anti-oxidant effects of olive oil phenolic compounds are controversial. The protective effects on lipid oxidation in these trials have been better displayed in oxidative stress conditions, i.e. males, submitted to a very strict anti-oxidant diet, hyperlipidaemic or peripheral vascular disease patients. Carefully controlled studies in appropriate populations, or with a large sample size, are urgently required to definitively establish the *in vivo* anti-oxidant properties of the active components of virgin olive oil [55].

12. Oligoelements in water

Epidemiological studies have revealed both a higher incidence of CVDs and cerebrovascular mortality in soft water areas and a negative correlation between water hardness and cardiovascular mortality [56,57]. Actually, there is not enough evidence to determine whether hard water contains protective substances not present in soft water or if there are detrimental substances in soft water.

Water contains oligominerals, such as calcium, magnesium, cobalt, lithium, vanadium, silicon, copper, iron, zinc and manganese, that are some important factors in reducing the risk

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of CVD onset. On the other hand, elements like cadmium, lead, silver, mercury and thallium are considered to be harmful [58].

Magnesium deficiency is considered to be a risk factor of CVDs, in fact its supplementation delays the onset of atherosclerosis or hinders its development. On the other hand, silicon is a major trace element in animal diets and humans ingest between 20-50 mg/day of silicon with the Western diet [59]. Main dietary sources are whole grain cereals and their products (including beer), rice, some fruits and vegetables and drinking water, especially bottled mineral waters with geothermal and volcanic origin [60]. Numerous studies showed that silicon has a role in maintaining the integrity, the stability and the elastic properties of arterial walls [61,62] and postulated silicon as a protective factor against the development of age-linked vascular diseases, such as atherosclerosis and hypertension [62,63].

In addition, vanadium is considered to have anti-atherosclerotic properties; lithium can also inhibit the synthesis of cholesterol, but has an atherogenous activity that can be inhibited by supplementation with appropriate quantities of calcium. A copper-deficient diet can induce hypercholesterolemia and hypertriglyceridemia that is, in turn, intensified by high levels of dietary zinc [58,64].

On the basis of these limited data, intakes of silicon, magnesium and vanadium in water and avoiding exposure to cadmium and lead are important elements of the prophylaxis of CVDs, so hard water has positive health effects and should not be replaced by drinking water with insufficient amounts of beneficial elements [58]. It is important to remember also that water has small contribution of mineral trace respect to total dietary intake (7% from liquid vs 93% from solid food) [58,65].

13. Melatonin supplementation

Melatonin, an endogenously produced indoleamine, is a remarkably functionally pleiotropic molecule [66] which functions as a highly effective anti-oxidant and free radical scavenger [67,68]. Endogenously produced and exogenously administered melatonin has beneficial actions on the cardiovascular system [69,70,71].

Exogenously administered melatonin is quickly distributed throughout the organism; it may cross all morphophysiological barriers and it enters cardiac and vascular cells easily. Highest intracellular concentration of melatonin seem to be in the mitochondria; this is especially important as the mitochondria are a major site of free radicals and oxidative stress generation. Moreover, melatonin administration in a broad range of concentration, both by the oral and intravenous routes, has proven to be safe for human studies [72,73].

Melatonin itself appears to have an atheroprotective activity during LDL oxidation and also melatonin's precursors and breakdown products inhibit LDL oxidation, comparable to vitamin E. Because of its lipophilic and nonionized nature, melatonin should enter the lipid phase of the LDL particles and prevent lipid peroxidation [9] and may also augments endogenous cholesterol clearance.

Melatonin also counteracts the cell oxidative burden indirectly by stimulating the production of cell ROS detoxifying enzymes, specially glutathione peroxidase, glutathione reductase and superoxide desmutase. Melatonin besides being a more effective anti-oxidant than resveratrol can reverse the pro-oxidant DNA damage induced in low concentration of resveratrol, when added in combination [74].

Moreover, 6-hydroxymelatonin, the main *in vivo* metabolite of melatonin, and its precursor, *N*-acetyl-5-hydroxytryptamine, were potent in reducing *in vitro* LDL peroxidation. The ability of the parent molecule melatonin as well as its metabolites to function in radical detoxification greatly increases its ability to limit oxidative abuse at many levels within cells [9]. Therefore it can be suggested that although melatonin *per se* would have physiologically or pharmacologically effects to inhibit *in vivo* LDL oxidation, its action sinergically with its main catabolite would be more active [75]. Melatonin may exert protective and benefical effects against CVDs reducing the risk of atherosclerosis and hypertension [9].

It is important to underline that the recent discovery of melatonin in grapes [74] opens new pespectives in the field of natural anti-oxidative atheroprotective strategies.

14. Vitamins

Vitamin C is a water-soluble vitamin and is believed to regenerate vitamin E from its oxidized state back to its activated state. The principal sources of vitamin C are citrus fruits, tomatoes and potatoes. Natural vitamin E is a mixture of tocopherols and tocotrienols synthesized only by plants and the natural sources are vegetal oils. In fact, olive oil contains vitamin E and many of its beneficial effects are attributed to this constituent.

Vitamin E acts as a chain-breaking anti-oxidant for LDL lipids [27]. *In vitro* enrichment of LDL in vitamin E drastically increases their resistance to oxidative stress and it has also been reported to inhibit the cytotoxicity of ox-LDL toward cultured endothelial cells. Vitamin E has been reported to retard atherosclerosis progression in certain arteries of primates fed an atherosclerosis diet. In humans, both women and men, exhibited reduced vascular disease parameters [75], beneficial effects in the reduction of risk of onset and progression of atherosclerosis, due to its inhibition of LDL oxidation and association with molecular modulation of the interaction of immune and endothelial cells. A long term supplementation with vitamin E in hypercholesterolemic patients and/or chronic smokers increase levels of autoantibodies against ox-LDL. There is also a quite convincing evidence from *in vitro* studies that vitamin C strongly inhibits LDL oxidation [27].

It is important to underline that there are no definite recommendations on the dose and duration of supplementation with vitamins in human. Although, high dietary intake of fruit and vegetables is associated with a reduction in the incidence of atherosclerosis, stroke and cardiovascular mortality in general [27]. Moreover, epidemiologic studies have reported that high dietary intake of foods rich in vitamin E, vitamin C and β-carotene have been inversely associated with the incidence of CVDs [35].

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Actually, it is difficult to conclude that a clinical benefit of anti-oxidants in CVD is established. Thus, it is necessary to clarify why anti-oxidants showed their beneficial effects *in vitro*, whereas less satisfactory results were observed in some, although not all, clinical conditions [40].

15. HDL-based diet

It is well known that LDL are crucial to the development of atherosclerotic lesions, whereas HDL are inhibitors of the process, so the primary focus of pharmaceutical lipid modulation is reduced LDL; this strategy has reduced cardiovascular morbidity and mortality by up to 25% [76].

Recent studies also suggest that HDL inhibits oxidation, prevents the expression of inflammatory mediators and the expansion of pro-atherogenic myeloid cells and reduces the expression of pro-coagulant enzymes, each of which may contribute in smaller ways to atheroprotective effects [77].

The synthesis and release of HDL into the peripheral vasculature is the first step in reverse cholesterol transport that is proposed to be a major mechanism by which HDL mediates its atheroprotective effects [78]. However, HDL possesses multiple anti-atherosclerotic properties in addition to reverse cholesterol transport. HDL acts as a transporter of a variety of fat-soluble vitamins, including vitamin E, and also as a natural anti-oxidant protecting for LDL in a multifactorial manner. Moreover, HDL are associated with enzymes with anti-oxidant capacity, like paraoxonase that is a major contributor to the anti-oxidant activity of HDL [78]. Paraoxonase is synthesized in the liver and released into the circulation, where it becomes closely associated with HDL.

HDL has also been demonstrated to improve endothelial function, maintain the integrity of vascular endothelium and may induce the production of vasodilators, such as prostacyclin, by the endothelium. HDL has also been demonstrated to exhibit anti-thrombotic and anti-inflammatory activities.

The combination of a low saturated fat diet and increased exercise raises HDL levels by 5–14% and lowers triglyceride, LDL and total cholesterol levels by 4–18%, 7–15% and 7-18%, respectively. Thus, simple lifestyle measures including a correct diet and increased activity represent a cost-effective and low-risk intervention that is associated with a range of health benefits [76].

There is considerable interest at present in the possible therapeutic effects of elevating HDL levels to capitalize on their vasculoprotective effects. Although, clinical evidence to date has provided inconsistent results and suggests that raising HDL levels may not be the straightforward answer to atheroprotection [79,80]; HDL-based therapies, also combined with other atheroprotective strategies, may be a valide future atheroprotective approach.

16. Conclusion

As a result of increased understanding of the characteristics and production of ROS and oxidative stress and demonstrated a link either directly or indirectly to atherosclerosis, the reduction of ROS or decreasing their rate of production may delay the onset and progression of atherosclerosis. Aging promotes physiological changes, such as oxidative stress, inflammation and endothelial dysfunction strictly associated with the pathophysiology of atherosclerosis. Actually, compelling evidence indicates that increased consumption of correct diet containing nutritive and non-nutritive compounds with anti-oxidant properties may contribute to the improvement of the quality of life by delaying onset and reducing the risk of CVDs and, in particular, the development of an atheroprotective strategies acting on oxidative stress involved in the pathogenesis of atherosclerosis and with little toxicity or adverse effects may provide an ideal simil-therapeutic treatment against atherosclerosis. Actually, cardiovascular prevention and treatment strategies should consider the simple, direct and inexpensive dietary approach as a first line approach to the burgeoning burden of CVDs, alone or in combination with pharmacological treatments. In this context, wine, tea, fruit and olive oil received much attention, because they are particularly rich in natural anti-oxidants.

However, a better understanding of the oxidative stress-dependent signal transduction mechanisms, their localization, and the integration of both ROS-dependent transcriptional and signaling pathways in vascular pathophysiology is anyway a prerequisite for effective pharmacological and non pharmacological interventions for cardiovascular protection from oxidative stress.

In conclusion, the proposal that anti-oxidants may retard the progression of atherosclerosis is very interesting and promising, but further studies are needed to better understand the mechanisms that underline the biological effect of healthy life style.

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