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Oxidants/Antioxidants balance and Atherosclerosis

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سورة الفاتحة
بسم الله الرحمن الرحيم ١
الحمد لله رب العالمين ٢ الرحمن
الرحيم ٣ مالك يوم الدين ٤
إياك نعبد وإياك نستعين ٥
اهدنا الصراط المستقيم ٦ صراط
الذين أنعمت عليهم غير المغضوب
عليهم ولا الضالين ٧
مد لازم حركات ثريا
مد جائز منفصل ٤ حركات
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رب العالمين : مربيهم ومالكهم ومقدر أمورهم
يوم الدين : يوم الجزاء
الصراط المستقيم : الطريق الذي لا اعوجاج فيه

الإهداء

إلى أمي وأبي

إلى أساتذتي

إلى زملائي وزميلاتي

إلى الشموع التي تحترق لتضئ للآخرين

إلى كل من علمني حرفا

أهدي هذا البحث المتواضع راجياً من المولى

عز وجل أن يجد القبول والنجاح

شكر وتقدير

فإني أشكر الله تعالى على فضله حيث أتاح لي إنجاز هذا العمل بفضله، فله الحمد أولاً وأخرا.

ثم أشكر أولئك الأخيار الذين مدوا لي يدَ المساعدة، خلال هذه الفترة، وفي مقدمتهم أستاذتي المشرفة على البحث

الدكتورة/ زينب نجم.

كما أشكر القائمين على جامعة القادسية وعمادة كلية العلوم.

Contents:

Summary

The oxidation hypothesis suggests that oxidative modification of lipoprotein, and in particular low-density lipoprotein (LDL), increases its atherogenicity by altering receptor- mediated uptake by cells in the intima of blood vessels. Oxidized LDL is taken up by scavenger receptors on monocytes, smooth muscle cells, and macrophages in an uncontrolled process leading to accumulation of lipid and the formation of foam cells, an early feature of atherosclerotic plaque

Balancing oxidant and antioxidant reactions in the body may be important for many diseases, including cancer and cardiovascular disease. Physiologic systems counteract oxidant stress by preventing oxidant formation, trapping oxidants, and repairing oxidant damage. Our inherited capacity to maintain oxidant balance is dependent on the intake of nutritive and nonnutritive dietary antioxidants. In the future, it will become more common for the clinical laboratory to measure not only these antioxidants and free-radical scavenging enzymes but also markers reflecting oxidant damage of target tissue.

1.1 Introduction

Atherosclerosis has been derived from a Greek word,Athero meaning gruel. Marchand introduced the term “atherosclerosis” describing the association of fatty degeneration and vessel stiffening. It is the patchy intramural thickening of the subintima. The earliest lesion is the fatty streak. Fatty streak evolve to fibrous plaque and unstable plaque are responsible for clinical events. patchy intimal plaques. Most common location is lumen of medium sized and large arteries(Figure1). The plaque has cellular component -namely of inflammatory cells, smooth muscle cells, a fibrous component of –connective tissue and a fat component of lipids. Prominent risk factors of consideration are Hypertension, Diabetes, obesity, sedentary life style, Family history, and smoking. Intraplaque rupture, bleeding, thrombosis andenosis cause symptoms. Diagnosis is clinical and definitive diagnosis is made through imaging tests. Management plan includes behavior modifications (Physical activity with low caloric diet, rich in fiber component) and main class of drugs used in treatment are antiplatelet drugs and antiatherogenic drugs[1].

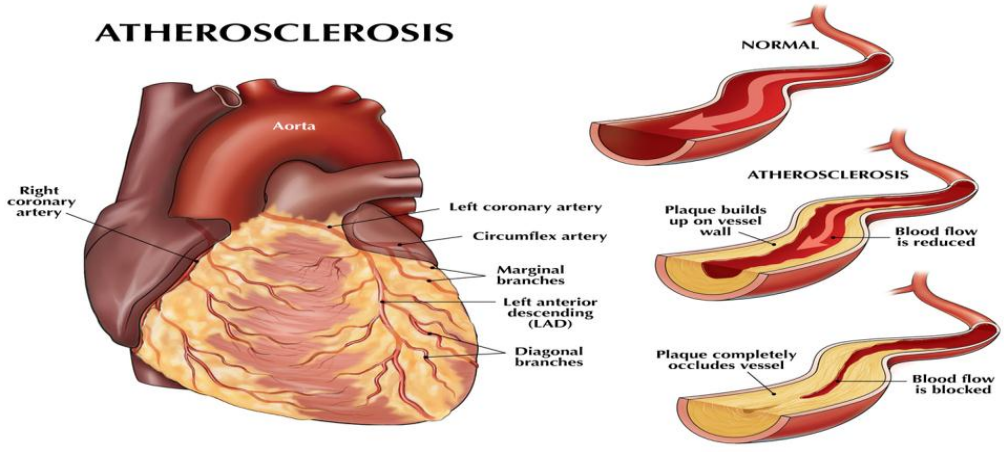

Figure 1: Atherosclerosis

However, there is also evidence that the extent of protection against ex vivo LDLoxidation is not a predictor of an antiatherogenic effect , and thatatherogenesis can be dissociated from aortic lipoprotein lipidper oxidation. Also, the outcomes of interventions with vitamin E in coronary heart disease in

humans continue to be disappointing. These conflicting findings may reflect our limited understanding of the relevance of lipoprotein oxidation to atherogenesis and of how antioxidants protect from disease, and suggest that we need to reassess the strengths and weaknesses of the oxidationtheory. In the present review we focus on oxidants that have been identified to play a role in LDLoxidation in vivo.[2,3]

1.1 Antioxidants and markers of oxidative stress

All aerobes including plants, aerobic bacteria, and humans, suffer damage when exposed to oxygen concentrations higher than normal, signifying that they have no excess of antioxidant defenses.Halliwell and Gutteridge defined free radicals as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbital's. This unpaired electron(s) usually gives aconsiderable degree of reactivity to the free radical. . Reactive oxygen species are produced by various oxidase enzymes, including nicotinamide-adenine dinucleotide phosphate(NADPH) oxidase, xanthine oxidase, cyclooxygenase,glucose oxides, lipooxygenase, and mitochondrial electron transport. An imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage, has been defined "oxidative stress". The term describes a metabolic condition of cells, organs, or the entire organism characterized by an oxidative overload [3]. Indeed, the excess reactive oxygen species can damage cellular lipids, proteins, or DNA impairing their normal function. Because of this, oxidative stress has-been implicated in a number of human diseases as well as in the ageing process. The delicate balance between beneficial and harmful effects of free radicals is a very important aspect of living organisms. Superoxide anion is considered the "primary" reactive oxygen species and can further interact with other molecules to generate "secondary" reactive oxygen species, either directly or prevalently through enzyme- or metal-catalyzed processes . Further, superoxide anion may react with other radicals including NO. The product peroxynitrite is also a very powerful oxidant and belongs to the reactive nitrogen species, *i.e.* derived from NO. The sources of reactive oxygen

species are a variety of cell types, including vascular smooth muscle cells, endothelial cells (ECs) and mononuclear cells. Several lines of evidence demonstrate that oxidative stress plays an important role in the pathogenesis and development of cardiovascular diseases.[3,4]

1.2 Oxidants andAtherosclerosis

1.2.1 Transition metal-mediated LDL oxidation

Free transition metals are strong catalysts for oxidation reactions in the presence of lipid hydro peroxides(LOOH).[5]However, the concentration of free transition metals in vivo is negligible, and there is little convincing evidence that they are important in LDL oxidation in the vessel wall. This indicates that studies that use free transition metals [e.g. Cu(II)] to oxidize LDL in vitro are probably not meaningful biologically [6].These hydroxylated amino acids are increased in humanendarterectomy specimens and in the artery wall of monkeys in early diabetic vascular disease, which is indicative of .OH formation in vivo. However, it is yet tobe established whether less advanced lesions in nondiabetichumans contain elevated levels of these oxidation products. [6,7]

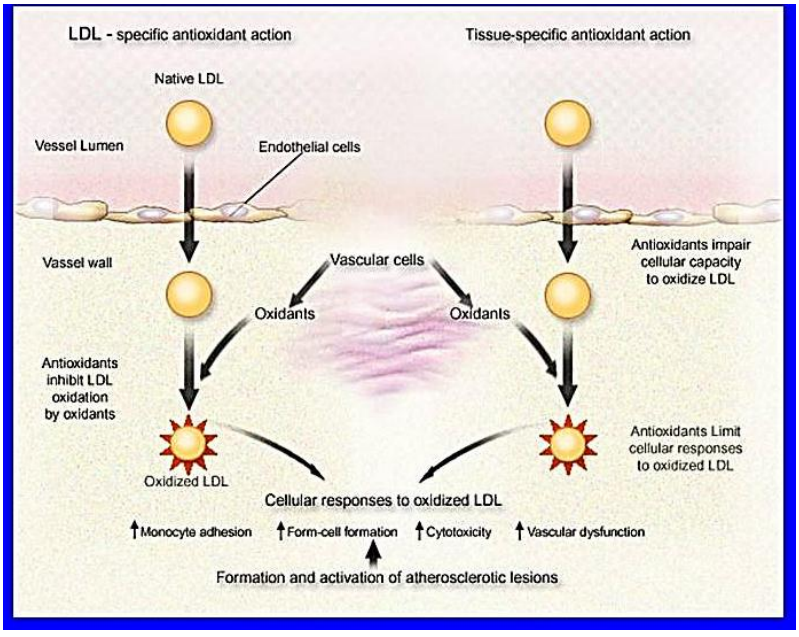

Figure 2: oxidative stress and atherosclerosis

1.2.2 Peroxyl radicals

Lipid peroxyl radicals (LOO.) are regarded as the major chain carrier of free radical-mediated per oxidation, andLOOH are the primary products of polyunsaturated fatty acids, including those that are present in phospholipids andcholesterol esters in LDL. The hydroperoxidesof these lipids [i.e. hydro peroxides of phospholipids (PLOOH) andhydro peroxides of cholesterol esters (CEOOH)] can be analyzed with high sensitivity and specificity by high-performance liquid chromatography withpost column chemiluminescence detection , and their presence has been used as evidence for in vivo lipidperoxidation . However, LOOH are readily metabolized in biological systems, complicating their use as markers of in vivo lipid per oxidation. [8]

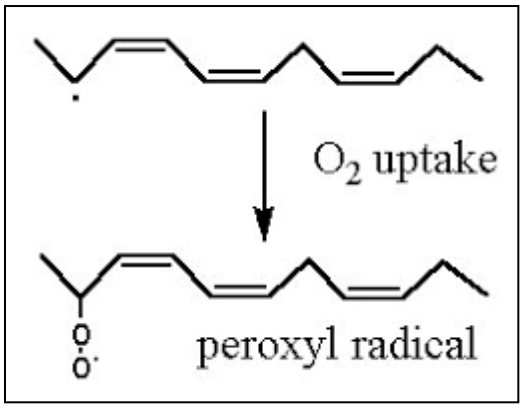

Figure3: Peroxy radical

LOOH are metabolized principally through peroxidasemediated reduction to the corresponding alcohols(LOH). In the extracellular space, where oxidation isbelieved to take place but where suitable peroxidasesare not present, reduction of PL-OOH and methionine residues of apolipoproteins, respectively, can achieve CE-OOH to PL-OH and CE-OH. Therefore, the accumulation of CE-OOH plus CE-OH [i.e.CE-O(O)H] may be a better index of the extent of invivoaortic LDL oxidation than CE-OOH. Indeed,CE-O(O)H are the major oxidized lipids present in advanced atherosclerotic plaque.15-LO and α-tocopherol(α-TOH) can also form LOOHin LDL, so that it is

necessary to discriminate between the different pathways. Peroxyl radical-mediatedperoxidation of linoleate theoretically yields four differentregio-isomers of LOOH that do not accumulate with equal abundance; in pure lipids the thermodynamically favored 9- and 13-trans, trans isomers predominate, whereas a-TOH-containing LDL predominantly yields the 9-trans,cis and 13-cis,trans region-isomers of CE-OOH. This is explained by a-TOH reacting with LOO.before the radical oxidizes other lipids (i.e. before LOO). Can act as a chain carrier). In human plaguethe majority of CE-O(O)H are present as 9-trans,cis and 13-cis,trans region-isomers. This suggests that LOO. is not a majorper oxidation chain-carrying species, and this may have implications with regard to the design of antioxidants that are aimed at preventing no enzymatic lipidperoxidation.[5,9]

1.2.3 Tocopheroxyl radicals

α-Tocopheroxyl radical participates in lipid peroxidationof LDL exposed to one-electron (i.e. radical) oxidants inviter. Preferential accumulation of 9-trans,cis and13-cis,trans region-isomers of CE-O(O)H in human lesions suggests that α-tocopheroxyl radical participates in the generation of oxidized LDL in vivo. Support for this comes from animal interventions with inhibitors ofα-tocopherol -mediated per oxidation that effectively attenuate lipoprotein lipid per oxidation in the vessel wall, suggesting that such inhibitors are potential antiatherogenic compounds.[10]

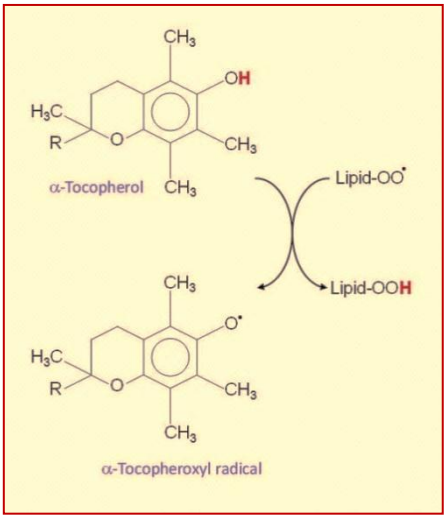

Figure 4: Tocopheroxylradical

1.2.4 Lipoxygenase

Several lines of evidence support a role for 15-LO in atherosclerosis, the strongest of which is that disruption of the 12/15-lipoxygenase gene diminishes disease inapolipoprotein E (ApoE) gene knockout (ApoE7/7)mice [11]. Also, 15-LO represents a potential anti-atherosclerotictarget for pharmaceutical intervention, as supported by a study in hypercholesterolemiarabbits. What is less clear is how precisely 15-LOcontributes to atherogenesis. Human 15-LO oxidizes unsaturated free fatty acids to LOOH, with a regio- andstereo-specific product distribution. 15-LO also oxidizesLDL in vitro [6,9], in part by initiating α-tocopherol mediatedperoxidation . However, in lesions 15-LOis associated with macrophages, raising the question of how the cytosolic oxygenize oxidizes extracellular LDL.[8,11]

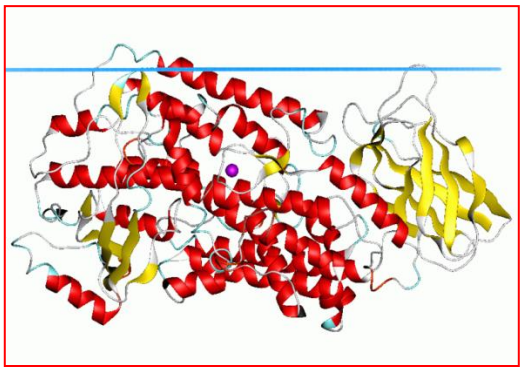

Figure 5: Structure of rabbit reticulocyte 15S-lipoxygenase

The idea that 15-LO `seeds' LDL with LOOH,rendering it more susceptible to subsequent oxidation, received support from LDL oxidation studies with cells over-expressing 15-LO. However, those studies did not determine whether the oxidized lipids detected in LDLwere enzymatic or no enzymatic products; also, they did not rule out involvement of contaminating transition metals in the cellular LDL oxidation .[12]

Therefore, it remains to be shown whether, and if show, cellular 15-LO may oxidize extracellular LDL.

1.2.5 Isoprostanes

The isoprostanes are prostaglandin-like compounds formed in vivo from the free radical-catalyzed peroxidation of essential fatty acids (primarily arachidonic acid) without the direct action of cyclooxygenase (COX) enzymes. The compounds were discovered in 1990 by L. Jackson Roberts and Jason D. Morrow in the Division of Clinical Pharmacology at Vanderbilt University . These nonclassical eicosanoids possess potent biological activity as inflammatory mediators that augment the perception of pain. These compounds are accurate markers of lipid peroxidation in both animal and human models of oxidative stress. [5,13]

Elevated levels of isoprostanes are suspected of contributing to increased risk of heart attack in patients taking Coxibs. Isoprostanes and their metabolites have also been shown to be elevated in the urine of cigarette smokers, and have been suggested as biomarkers of oxidative stress in smokers.[4]

1.2.6 Reactive nitrogen species

Nitric oxide (*NO) is a potent vasodilator generated within endothelial cells that can react with metal ions andmetalloproteinase to form reactive nitrogen species (RNS). Among the latter, peroxynitrite is formed by the diffusion-controlled reaction of *NO with superoxide anionradical. Chemical systems that simultaneously generate*NO and superoxide readily oxidize and convert LDL into a high-uptake form. Nitrogen dioxide, formed from peroxynitrite and carbonate, readily nitrates protein tyrosine Using a sensitive and specific method involving gas chromatography and mass spectrometry, Leeuwenburgh reported that LDL from aortic atherosclerotic intima contains 90-fold higher levels of protein-standardized 3-nitrotyrosine than does plasma LDL. These studies implicate RNS in aortic oxidation of LDL, and therefore potentially in atherosclerosis.[13]

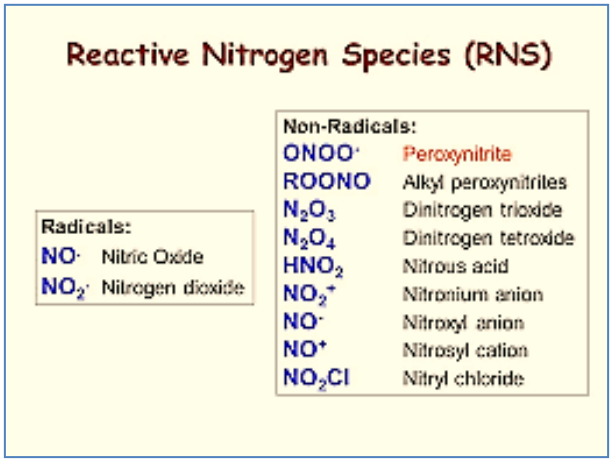

Figure 6: Nitrogen species

In support of this, treatment with an inhibitor of inducible *NO synthase (NOS) marginally decreased the progression of coronary atherosclerosis in hypercholesterolaemicrabbits , although in apoE7/7 mice a genetic defect in inducible NOS had no effect on atherosclerosis [3] .

1.2.7 -derived oxidantsMyeloperoxidase

Myeloperoxidase is a home-containing protein that produces two-(hypochlorous acid) and one-electron oxidants (tyrosyl radical), as well as Reactive species such as chlorine gas and p-hydroxyphenylacetaldehyde(pHA), which are capable of oxidizing LDL [14].There is now good evidence that myeloperoxidasederivedoxidants participate in LDL oxidation in human atherosclerosis. As this has been reviewed recently [10],we discuss only selected aspects.

pHA reacts with LDL amino phospholipids, and the levels of pHA-ethanolamine associated with LDLincrease with the extent of disease. A potential significance of this pathway is that it represents a lipidperoxidation-independent path for the in vivo formation of modified phospholipids. Modified phospholipids detected by immunohistochemistry are formedearly in atherogenesis . and are generally assumed to represent modifications caused by secondary lipidperoxidation products, such as malondialdehyde-

and 4-hydroxynonenal. A potential problem with this view is that malondialdehyde- and 4-hydroxynonenalare formed only after depletion of endogenous a-TOH, but the vitamin is not generally depleted inhuman lesions. The novel pathway identified by Heinecke's group. may resolve this apparent conundrum. It will be interesting to determine the relationship between pHA-ethanolamine and epitopesrecognized by the antibodies used by Witztum and others.[15]

1.2 Antioxidants and atherosclerosis

The antioxidant content of LDL is critical for its protection. In theory, if sufficient lipophilic antioxidants were present, LDL would be protected from even profound oxidant challenge. The balance between the prooxidant challenge and the presence of antioxidants determines the extent of arterial wall modification of LDL. Antioxidants such as probucol, N,N'-diphenyl phenylenediamine, and BHT have been shown to decrease the degree of oxidation and the extent of atheromatous lesions in animal models of atherosclerosis , but have side effects. Thus, dietary antioxidants such as α-tocopherol, a-carotene, and ascorbic acid become attractive alternatives.[16]

1.3.1 Antioxidants

The role of antioxidants as potential antiatherogeniccompounds has been the subject of numerous reviews.Thus, the following discussion is limited to summarizingmore recent results.

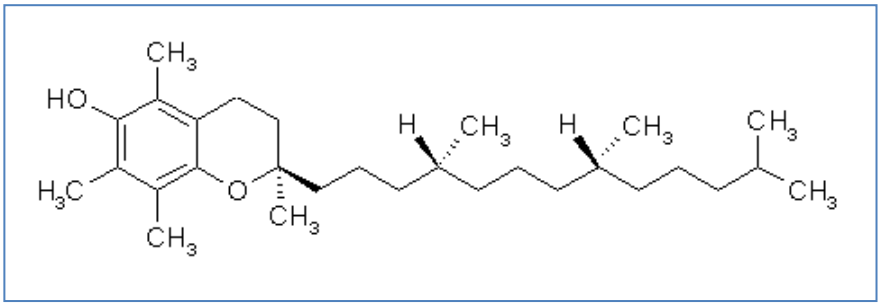

Figure 7: Vitamin E

Although α-TOH is the most abundant lipid-soluble antioxidant in LDL, animal interventions and controlled prospective clinical trials using vitamin E supplements have yielded conflicting results. Overall, a-TOH does not consistently prevent atherosclerosis in animals or cardiovascular disease in humans. Despite one recent finding of a decrease in myocardial infarction in patients with end-stage renal disease,the conclusion from recent studies is that a-TOH is ineffective in decreasing coronary artery disease.[17]

The reasons for these disappointing results are probably complex. A common criticism is that vitamin E may be more effective in primary rather than secondary interventions. However, this argument itself may be flawed, because the only large-scale studies with `positive ‘results [6,11] themselves employed individuals with established disease.

1.3.2 Probucol

Probucol is a phenolic antioxidant that is structurallyunrelated to vitamin E. Its pharmacological propertiesand atheroprotective activities were reviewed previously. The latter arguably provide the strongest evidencefor a protective activity of an antioxidant in cardiovascular disease. However, a problem with probucol is thatit lowers HDL and prolongs the QT interval withproarrhythmic risk . Also, the benefit of probucol on establishedlesions in mature WHHL

rabbits and nonhuman primates has been questioned, as has the effective dose Paradoxically, probucolpromotes atherosclerosis in the aortic root of LDLreceptor-deficient and apoE7/7 mice [8], raising the question of its molecular action in vivo.

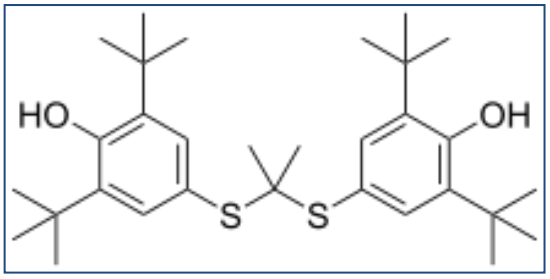

Figure 8:Probucol

In the cardiovascular literature, probucol is often referred to as a `potent' antioxidant, perhaps because it prolongs the apparent `lag phase' of LDL oxidation induced byCu(II) more effectively than does a-TOH. As mentioned above, though, the biological relevance of this in vitroactivity is questionable. However, the antioxidant potency of probucol, as assessed by chemical parameters, is clearly less than that of a-TOH . For example,probucol is not able to reduce α-tocopheroxyl radical inLDL . It is also known that probucol has several potential antiatherogenic properties in addition to inhibition of LDL oxidation.[17]

Important evidence for a cardio protective effect ofprobucol independent of its antioxidant activity comes from studies in restenosis. Thus, the landmark Multi-Vitamins and Probucol Trial showed that probucolreduces restenosis after balloon angioplasty, whereas an antioxidant cocktail comprising of a-TOH, ascorbate andb-carotene not only failed to be effective, but alsoprevented the beneficial action of probucol. A follow-up study of the MultiVitamins and Probucol Trialsuggested positive vascular remodeling as the underlyingmechanism of probucol's anti-restenotic action. We observed that the marked site-specific proatherogenicand antiatherogenic effects of probucol in the aortas ofapoE7/7 mice are not associated with parallel changes inaortic CE-O(O)H [8], supporting the view that probucoldid not affect

lipoprotein lipid peroxidation in the vesselwall. In support of such alternate function, preliminaryresults employing balloon-injured rabbits as a model ofrestenosis indicate that probucol promotes reendothelialization. [20]

1.3.3 Carotenoids

Research on the antioxidant properties of carotenoidshas focused on the reaction of b-carotene and lycopenewith singlet oxygen. However, there is no evidence that singlet oxygen plays a significant role in cardiovascular disease. Carotenoids can also scavenge other oxidants, although this appears to be of minor importance in humans when compared with the action of other natural antioxidants. Consistent with this, there is no evidence from controlled studies that carotenoid supplement reduces the risk of CVD.[5,9]

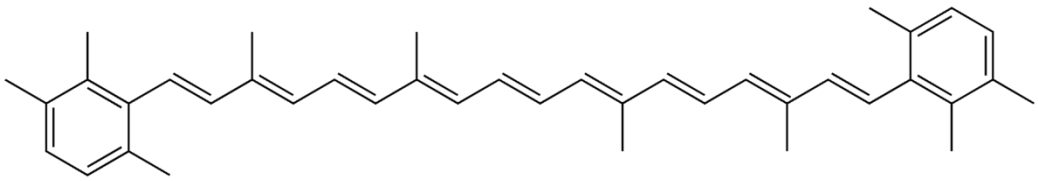

Figure9: Carotenoid structure

1.3.4 Vitamin C

Vitamin C (ascorbate) is a water-soluble antioxidant that inhibits LDL lipid per oxidation in vitroand lipidper oxidation in vivo even under iron-overload condition, by directly scavenging aqueous radicals or by reducing the chain-carrying α-tocopheroxylradical. Assessing changes in plasma vitamin C may provide a sensitive marker for oxidative stress, such as that associated with cigarette smoking , and low plasma ascorbate concentrations have been reported to be an independent predictor for an unstable coronary syndrome[15].

Vitamin C also shows a variety of other potentially important biological functions. Foremost in human endothelial cells, ascorbate increases [103] and stabilizes tetrahydrobiopterin, a cofactor that is involved in the synthesis of .NO by NOS. Tetrahydrobiopterin may stabilize NOS protein through allosteric and antioxidant mechanisms. This may enhance .NO production, which itself may inhibit restenosis . Also, in cardiac transplant recipients, plasma vitamin C levels correlate with nitroglycerin and acetylcholine response ,suggesting that endogenous vitamin C may preserve *NO bioavailability through the above-proposed mechanisms.[17]

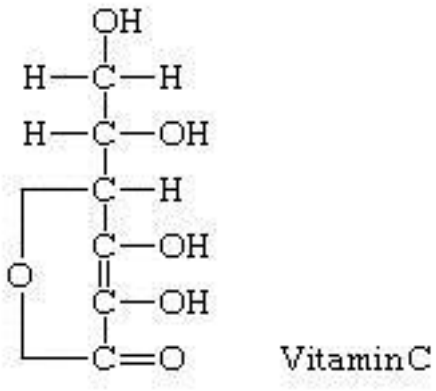

Figure10: The structure of Vitamin C

1.3.5 Flavonoids as antioxidants

Oxidative stress has been considered a mechanism involved in the pathogenesis of ischemic heart disease and atherogenesis, in cancer and other chronic diseases, and it also plays a major role in the aging process . Oxidative damage by free radicals has been well investigated within the context of oxidant/antioxidant balance. Indeed, oxidative stress describes various deleterious processes resulting from an imbalance between the excessive formation of reactive oxygen and/or nitrogen species and limited antioxidant defences. In this regard, cardiovascular risk factors significantly

cause oxidative stress, which contributes to a disruption in the balance between nitric oxide (NO) and reactive oxygen species, with a resulting relative decrease in NO bioavailability. The resulting endothelial dysfunction has been supposed to be the first step of atherosclerosis. Further, the majority of cardiovascular diseases follow from complications of atherosclerosis. In addition, an important initiating event for atherosclerosis may well be the transport of oxidized low-density lipoprotein across the endothelium into the artery wall. Diet and nutrition play a fundamental role in cardiovascular prevention and in maintaining physiological homeostasis. Recent literature emphasizes the potential therapeutic effects of micronutrients found in natural products, indicating positive applications for controlling the pathogenesis of chronic cardiovascular disease driven by cardiovascular risk factors and oxidative stress [18]. Nutritional compounds that display anti-inflammatory and antioxidant effects have specific applications in preventing oxidative stress-related injury, which characterizes the pathogenesis of cardiovascular disease. Polyphenolic compounds, mainly flavonoids, are ubiquitous dietary components. Dietary flavonoids represent a diverse range of polyphenolic compounds that occur naturally in plant foods. Flavonoids from food have been reported to be potentially involved in cardiovascular prevention mainly by decreasing oxidative stress and increasing NO bioavailability.

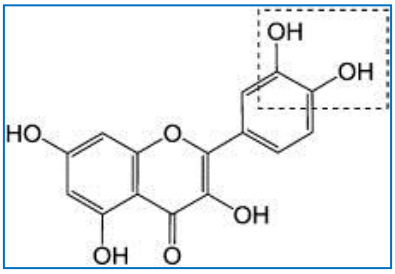

Figure 11: The structure of Flavonoids

They are able to modulate genes associated with metabolism, stress defence, drug metabolizing enzymes, detoxification and transporter proteins . Their overall effect is protective in overcoming damaging effects of cardiovascular risk factors and in delaying the onset of atherosclerosis [1,7]. Thus, they have naturally been associated with the hypothesis that their redo activities may confer them with specific health benefits. Their prevalence in plant-derived foods has supported this point of view and inspired new research for human intervention trials with flavonoidrichfood items in order to investigate their ability to protect from cardiovascular risk. In recent years, there has been a remarkable interest in scientific studies focusing on oxidative stress. The reasons seem to be the knowledge about reactive oxygen and nitrogen species metabolism, the definition and clinical role of markers for oxidative damage, the evidence linking cardiovascular diseases and oxidative stress, and the identification of flavonoids and other dietary polyphenolantioxidants able to act as bioactive molecules with health benefits deriving from flavonoid-rich diet.[10]

1.4 Dietary Strategies against Oxidative Stress

Based on evidence of the importance of oxidative stress in cardiovascular damage, there has been great interest in developing strategies that target reactive oxygen species in the treatment of cardiovascular diseases. Therapeutic approaches that have been considered include mechanisms to increase antioxidant bioavailability or to reduce reactive oxygen species generation. The mechanisms involved in radical scavenging activity are complex, determined by the structure of the compound,redox status of the environment and interactions with other agents. In this regard, it is of interest that purified micronutrients isolated from natural products may be less effective than a combination observed in the natural product due to synergistic effects of interacting agents.[2]

Relatively scant data still characterize the in vivo implications of these findings. Nevertheless, there have been studies suggesting that the regular or occasional consumption of polyphenols andorflavonoid-rich foods exerts

beneficial effects on blood pressure, insulin resistance, endothelial function, and oxidative stress[6]. Dietary antioxidants constitute a large group of compounds that differ in mechanism of action, bioavailability and side effects. A systematic analysis of the role of the various antioxidants in chronic diseases is retarded by the difficulty of employing death or clinical events as ends in intervention studies. Therefore, valid markers for oxidative stress, which show dose response and are sensitive to changes in dietary supply of antioxidants, are potentially of great value when trying to establish healthy dietary patterns, or when one component, like cocoa, tea or redwine, is studied. Epidemiologic studies indicate that diets rich in fruit and vegetables are associated with a decreased incidence of adverse cardiovascular events, such as coronary artery disease and stroke. This effect was ascribed, at least in part, to the high content of antioxidants, in particular polyphenolic compounds, such as flavonoids, in plant-based foods. In this context, cocoa, some chocolates, red wine, and tea received much attention, because they are particularly rich in flavonoids, photochemical with strong antioxidant properties in vitro [19].

Several lines of evidence suggest that flavonoids, a major class of polyphenols, are important bioactive constituents of the above mentioned foods and that there may be a causal relationship betweenflavonoid consumption and improvements in cardiovascular function. [5, 20]

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