Dietary antioxidants in preventing atherogenesis

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Abstract

Several naturally occurring constituents have received considerable attention because of their potential antioxidant activity. Consuming a diet rich in natural antioxidants has been associated with prevention from and/or treatment of atherosclerosis. Bioactive components of food, which are of special interest, include the Vitamins E and C, polyphenols, carotenoids—mainly lycopene and β-carotene, and coenzyme Q10, featured by antioxidant properties. Antioxidant therapy is supposed to be effective in the early stages of atherosclerosis by preventing LDL oxidation and the oxidative lesion of endothelium. This review focuses on the effect of dietary antioxidants pertaining to LDL oxidation and to the vascular endothelial dysfunction. Now that the human genome has been completely sequenced, genetic factors involved in oxidation may open new horizons to identify persons at risk for cardiovascular disease, allowing effective dietary intervention strategies to recover normal homeostasis and to prevent diet-related implications. On this basis, current studies on the action of selected antioxidant nutraceuticals on the activity of transcription factors, such as final targets in the signal transduction cascade and gene regulation, may emerge into new treatment concepts.

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Keywords: Atherosclerosis; Cardiovascular; Antioxidants; LDL; Endothelial dysfunction; Gene regulation

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Abbreviations: AP-1, activator protein; CoQ10, coenzyme Q10; COX, cyclooxygenase; CVD, cardiovascular disease; CYP1A1, cytochrome P450 1A1; G-CSF, granulocyte-colony stimulating factor; GSHPx, glutathione peroxidase; ICAM-1, intercellular cell adhesion molecule-1; iNOS, nitric oxide synthase; LDL, low density lipoprotein; M-CSF, macrophage-colony stimulating factor; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor-κB; NO, nitric oxide; NOS, nitric oxide synthase; oxLDL, oxidized low density lipoprotein; PDGF, platelet-derived growth factor; ROS, reactive oxygen species; SOD, superoxide dismutase; TGF-β, transforming growth factor-β; TPA, tissue plasminogen activator; TXA2 (B2), thromboxane B2; VCAM-1, vascular cell adhesion molecule-1

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activate different signaling pathways for cardiac apoptosis. In
pase, proto-oncogene, and p-53 activation. Different ROS
ROS induce cardiac apoptosis include lipid oxidation, cas-
and vascular remodeling processes. The pathways by which
condensation during programmed cell death contributes to
myocardial ischemia/reperfusion. Cytoplasmic and nuclear
portion of cell death linked to myocardial infraction and
in the myocardium, a fact that accounts for a great pro-
oxidative products, have been proven to trigger apoptosis
activation of neutrophils, lipid peroxidation, protein mod-
als cause injury in the myocardium from mitochondrial
electron transport. The generation of large amounts of ROS
ischemic/reperfused experimental animal hearts[6], and in
the area of endogenous antioxidant defenses, excess amount of
ROS leads to the depletion of the protective GSH and
SOD. Because several studies suggest that polymorphic vari-
ations in endogenous antioxidants are linked to increased
risk for atherosclerosis and CVD[3], ROS-induced deple-
tion of antioxidants is fairly a key factor for the initiation of
atherosclerosis and the development of CVD.

Immediate targets of ROS are also long-chain free fatty
acids in the cytosolic compartment and membrane-bound
lipids leading to the formation of lipid peroxides. Chemically
vulnerable to ROS are the polyunsaturated fatty acids in the
lipoproteins. In the case of LDL, which is now recognized to
critically contribute to the pathogenesis and progression of
human atherosclerosis, free radicals attack plasma LDL that
is oxidatively modified (oxLDL) leading to the attraction of
blood monocytes beneath the endothelium. Monocytes dif-
ferentiate into macrophages that are converted to foam cells,
full of cholesterol and oxidized lipids. Macrophage foam cells
form the early atherosclerotic lesions, which are documented
as the pathogenesis of CVD[4]. More than simply cellular
toxicants, ROS seem to modulate cellular gene expression.
The alterations in gene expression are mediated by activation
of transcription activators, such as nuclear factor-
κB (NF-κB) and activation protein-1 (AP-1).

Apart from the mitochondrial production of ROS, other
non-mitochondrial sources of oxidative stress have been rec-
ognized as even more important. Elevated levels of circu-
lating xanthine oxidase can be concentrated in the vascular
tissue and may participate in endothelial dysfunction[5]. It
has been shown that pretreatment with allopurinol, a xan-
thine oxidase inhibitor, improved cardiac function in isolated
ischemic/reperfused experimental animal hearts[6], and in
humans[7].

Elevated levels of cytokines are frequently found in the
plasma of patients with CVD. These include IL-1β, IL-
6, IFN-γ, TNF-α that are considered pro-oxidants together
with several growth factors such as TGF-β1 and insulin like

1. The role of oxidative stress in atherosclerosis and
CVD development

Quite a lot of recent studies have demonstrated that
altered oxygen utilization and/or increased formation of
reactive oxygen species (ROS) contribute to atherogenesis and
CVD progression. For example, in hypertension ROS
induce vascular smooth muscle cell proliferation both in
vitro and in vivo[1,2]. AngII promotes oxidant produc-
tion via NADH/NADPH oxidase and superoxide produc-
tion mediates endothelial dysfunction. In atherosclerosis,
superoxide production mediates endothelial dysfunction and
levels of oxLDL are elevated. In myocardial infarction,
ischernia/reperfusion injury is driven by ROS formation,
while myocarditis necrosis and apoptosis is oxidant resultant.
ROS induce cardiac dysfunction and cardiac apoptosis and/or
necrosis in heart failure.

Several sources of oxygen/nitrogen species do occur
in CVD. ROS are formed intracellularly during mito-
chondrial electron transport chain and are controlled by
antioxidant defense. Numerous experimental hypoxia or
ischemia/reperfusion models have suggested that oxygen rad-
icals cause injury in the myocardium from mitochondrial
electron transport. The generation of large amounts of ROS
can overwhelm the intracellular antioxidant defense, causing
activation of neutrophils, lipid peroxidation, protein mod-
ification, and DNA breaks. ROS per se, as well as their
oxidative products, have been proven to trigger apoptosis
in the myocardium, a fact that accounts for a great pro-
portion of cell death linked to myocardial infarction and
myocardial ischemia/reperfusion. Cytoplasmic and nuclear
condensation during programmed cell death contributes to
the impairment of cardiac performance and to myocardial
and vascular remodeling processes. The pathways by which
ROS induce cardiac apoptosis include lipid oxidation, cas-
pase, proto-oncogene, and p-53 activation. Different ROS
activate different signaling pathways for cardiac apoptosis. In
growth factor-1. The main mechanism, by which cytokines intercede cardiac dysfunction and CVD development, is the elevated production of NO via the induction of NOS. The product coming out from the reaction of NO and superoxide anion, ONOO\(^-\), is a strong oxidant and its biological marker 3-nitrotyrosine is related to the atherosclerotic lesion formation, endothelial cell dysfunction, ischemia/reperfusion injury, myocardial infarction, and heart failure [8]. Also, a pro-apoptotic role of TNF-\(\alpha\) in myocardial cells has been proposed [9]. Increased cytokine activation occurs in acute hypoxia followed by reperfusion and myocardium stunning [10]. Increased plasma levels of pro-inflammatory cytokines and oxidase activities are usually monitored in chronic pathologic cardiovascular conditions, such as heart failure. It is likely that heart itself synthesizes biologically active TNF-\(\alpha\), which in turn may progress heart disease.

2. Antioxidants

2.1. The term 'antioxidant'

Free radicals are electrically charged molecules, which seek out and capture electrons from other substances to finally neutralize themselves. Although the initial attack causes the free radical to become neutralized, another free radical is formed in the process, resulting in a chain reaction. Until subsequent free radicals are deactivated, thousands of free radical reactions may occur within only a few seconds. The term “antioxidant” refers to any molecule capable of stabilizing or deactivating free radicals before they attack cells. These are in particular the primary antioxidants. There are also molecules deserving the “antioxidant” term, because they act as chelating agents binding metal ions (redox activity).

To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly complex antioxidant protection system. It actually involves a variety of components, endogenous and exogenous, that function interactively and synergistically to neutralize free radicals. These include antioxidant enzymes that catalyse free radical quenching reactions, metal binding proteins that sequester free iron and copper ions that can be catalysts of catalyzing oxidative reactions, and diet-derived antioxidants like ascorbic acid, Vitamin E, carotenoids, polyphenols and other low molecular weight compounds such as \(\alpha\)-lipoic acid.

Dietary compounds that do not neutralize free radicals, however, enhance endogenous antioxidant activity also belong to antioxidants. Enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione-S-transferase within the cell can convert oxygen free radicals to oxygen and water. Glutathione is initially reduced in cells to provide a ready source of electrons for the conversion of hydrogen ions and oxygen radicals to water by these enzymes. Peroxidases are also present in the cell, able to remove peroxy radicals, hydrogen peroxide, or alkyl hydroperoxides by reaction with hydrogen ions or water. Various enzymes such as oxidases, dehydrogenases, deaminases, and oxidase activities are usually monitored in chronic pathologic cardiovascular conditions, such as heart failure. It is likely that heart itself synthesizes biologically active TNF-\(\alpha\), which in turn may progress heart disease.

2.2. Dietary antioxidants

Based on the ‘oxidation theory’ for atherosclerosis, dietary antioxidants have attracted considerable attention as preventive and therapeutic agents. There is adequate evidence from observational, in vitro, ex vivo, controlled intervention and animal model studies that consumption of certain foods results in a reduction in oxidative stress and myocardial infarction biomarkers. Extracts obtained from several natural sources are of intense scientific interest to further include either the whole extract or the drastic compound to complementary medicine supplements. In their majority, extracts contain Vitamins C and E, \(\beta\)-carotene, and polyphenols.

Vitamin C is water-soluble 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 (\(\alpha\)-, \(\beta\)-, \(\gamma\)- and \(\delta\)-tocopherol, and \(\alpha\)-, \(\beta\)-, \(\gamma\)- and \(\delta\)-tocotrienol) synthesized only by plants. The natural sources are vegetable oils. Olive oil contains Vitamin E and many of its beneficial effects are attributed to this constituent. \(\beta\)-Carotene is also a lipid soluble vitamin, which is carried with Vitamin E in the fatty core of lipid particles. It is chiefly found in high concentrations in carrots and in dark green leafy vegetables. Amongst the carotenoids, lycopene activity has attracted substantial interest more recently. Lycopene is a red color pigment present mainly in tomatoes and tomato products.

Generally, phenolic compounds or polyphenols occur widely in plants. Interest in polyphenols as antioxidants has attracted substantial interest more recently. Lycopene is a red color pigment present mainly in tomatoes and tomato products. Generally, phenolic compounds or polyphenols occur widely in plants. Vitamin C and Vitamin E are antioxidants that are naturally found in many natural foods. However, when under exposure to alcohol, medications, trauma, cold, infections, poor diet, toxins, radiation or strenuous physical activity, the endogenous antioxidant defense is not adequate to counteract the oxidative stress, protection against it is dependent upon the adequacy of antioxidants that are derived from the diet.

### Table 1

Main dietary sources of antioxidants

<table>
<thead>
<tr>
<th>Antioxidants</th>
<th>Main dietary sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin E</td>
<td>Wheat germ oil, almonds, sunflower oil, safflower oil, olive oil, hazelnuts</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Fruits (mainly citrus fruits), vegetables, tomatoes</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Potatoes, tomatoes, lettuce, onions, apples, wheat bran, dark chocolate, red wine, coffee, black tea</td>
</tr>
<tr>
<td>Lycopene</td>
<td>Tomatoes and tomato products, apricots, pink grapefruits, guava, skin of red grapes, papaya and watermelon</td>
</tr>
<tr>
<td>(\beta)-Carotene</td>
<td>Carrots, pumpkins, papayas, peaches, sweet potatoes, apricots, cabbage, green beans, broccoli, brussel sprouts, lettuce, peas, spinach, tomatoes, pink grapefruits, oranges</td>
</tr>
<tr>
<td>CoQ10</td>
<td>Fish and meat</td>
</tr>
</tbody>
</table>
3. Observational studies

There is a large body of observational (epidemiological, case-control, or prospective and retrospective cohort) studies on the dietary antioxidant intake link to prevention of CVD progression. Amongst the most established are: (i) the CHAOS study where an inverse correlation between death for myocardial infarction and Vitamin E was observed [11], (ii) the ARIC study that reported an inverse correlation between Vitamin C and carotid wall thickness [12], (iii) the Kuopio Atherosclerosis study showing that Vitamin C deficiency may be associated with an increased risk of myocardial infarction [13], (iv) the Zutphen Elderly study, in which intake of flavonoids (30 mg/day) was correlated with 50% reduction in CVD mortality compared to 19 mg/day flavonoid intake [14], (v) the John Hopkins University study that reported an inverse correlation between carotenoids and myocardial infarction [15], and (vi) the EURAMIC study that clearly showed a relationship between adipose tissue lycopene and the risk for myocardial infarction [16].

The extent of atherosclerosis at early subclinical stages of the disease is measured by high-resolution ultrasound measurements of the arterial wall intima-media thickness (IMT). In the EVA study in 1384 subjects, Vitamin E, but not carotenoid, plasma levels was associated with less thickening of the arterial wall, after adjustment for normal CVD factors [17]. In men, after adjustment for age and cardiovascular disease risk factors, higher plasma Vitamin C or β-carotene concentration was associated with a smaller IMT [18]. Inverse relationship of plasma lycopene with IMTmax were hypothesized to be compatible with a protective role of this natural dietary antioxidant in atherosclerosis [19]. The results of the study by Aviram and co-workers [20] suggest that pomegranate juice – which contains potent tannins and anthocyanins – consumption by atherosclerotic patients with carotid artery stenosis for one year decreases carotid IMT.

Despite the interesting results, because observational studies are based upon measurements of micronutrient intakes through the use of dietary recall questionnaires, they may be imprecise. The correlations provide strong hypotheses, but do not clarify the mechanisms underlying the effect. Therefore, observations need to be further verified by studies in vitro, controlled intervention, ex vivo and animal model studies (Table 2).

4. LDL oxidation: the effect of dietary antioxidants

4.1. The role of oxidized LDL in atherogenesis

The evidence supporting the hypothesis that LDL is the major atherogenic lipoprotein comes from epidemiological studies, clinical trials, studies in laboratory animals, heritable hypercholesterolemia, pathologic investigations, and studies in model systems. Several theories exist on the mechanisms by which LDL induces atherosclerosis. The concentration, the size and the chemical modification of LDL are important for atherogenesis.

Modified LDLs are produced during chemical modification that LDLs undergo after synthesis. Modifications take place in either plasma or in the inner layer of the artery and pertain to either the lipid or the protein fraction, induced by hydrolytic or proteolytic enzymes. O2 or •OH radicals or other non-enzymatic mechanisms. Additionally, modifications concern the production of lipoprotein–proteoglycan complexes or lipoprotein–autoantibody complexes [21]. Chemically modified LDLs are taken up by receptors or scavenger receptors in monocytes/macrophages. Kupffer cells and endothelial cells. oxLDLs represent the most important chemically modified LDLs, and glycosylated LDLs are more susceptible to oxidation than native LDLs [22].

oxLDLs are associated with the pathogenesis of atherosclerosis, a key early stage of CVD. Oxidation takes place when naturally occurring antioxidant agents such as Vitamin E and β-carotenes that normally inhibit LDL oxidation do not occur. The level of LDL oxidation varies. Thus, oxLDLs consist of a heterogeneous numeral of modified lipid and protein molecules. Polysaturated fatty acid oxidation is induced by several products of the metabolism in the artery wall, endothelial cells, smooth muscle cells, monocytes/macrophages, lymphocytes, platelets, O radicals or 15-lipoxygenase. Tyrosine radicals and hypochlorous acid that are regularly formed by the influence of myeloperoxidase secreted by phagocytes, copper or iron ions, either in free or as metal proteins, induce non-enzymatic oxidation. Oxidation results to LDL quite different compared to native LDL as to physicochemical and biological properties, lipid, fatty acid, and antioxidant composition [23]. In vitro research points out acceleration in oxidation by vascular cells.

Nevertheless, there are controversies among researchers as to the biochemical pathways via which LDL is modified to its oxidized form and this is due to the differences in the experimental conditions. The role of oxLDLs in atherosclerosis, and consequently in CVD, is manifold. oxLDL induces cell adhesion molecule expression in aortic endothelial cells [24]. Enhanced endothelial cell expression of chemotactic and adhesion molecules (i.e. E-selectin, ICAM-1 (CD54), and VCAM-1 (CD106), within the artery wall result in monocyte binding to endothelial cells, their entry into the vascular system, their differentiation into macrophages, and final conversion into foam cells. oxLDLs also stimulate T-cells through the major histocompatibility complex (MHC) and CD4+ helper T-cell receptor [25]. Stimulated T-cells secrete: (i) IL-1 that increases smooth muscle cell proliferation, (ii) IL-2 that activates monocytes and increases T-cell proliferation, and (iii) IFN-γ that induces MHC expression in endothelial and smooth muscle cells. Activated macrophages also secrete a number of cytokines (TNF-α, TGF-β, M-CSF, G-CSF, PDGF) that influence the expression of mediators of endothelial activation, such as NO. Besides, the aldehydic products of LDL oxidation exert a direct toxic effect on the endothelium and platelets.
### Table 2

The effects of dietary antioxidants assessed in vitro, ex vivo and in vivo.

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>In Vitro</th>
<th>Ex vivo</th>
<th>In Vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vitamin E</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibition of LDL oxidation [26]</td>
<td>Increase in LDL oxidative resistance [27-32]</td>
<td>Decrease in agonist-induced platelet aggregation [61,63,65]</td>
<td>Lower risk for MI, the CHAOS study [11]</td>
</tr>
<tr>
<td>Downregulation of PKC activity [98]</td>
<td></td>
<td></td>
<td>Increase in autoantibodies against oxid LDL [28]</td>
</tr>
<tr>
<td>Downregulation of SRA and CD16 expression [10-112]</td>
<td></td>
<td></td>
<td>Decrease in plasma MDA levels [33,64]</td>
</tr>
<tr>
<td>Upregulation of LDL-r gene expression [113]</td>
<td></td>
<td></td>
<td>Decrease in plasma β-thromboglobulin concentration [63]</td>
</tr>
<tr>
<td>Downregulation of collagen a1(1) and glycoprotein IB mRNA expression [114]</td>
<td>Inhibition of PKC mediated endothelial dysfunction [98]</td>
<td>Downregulation of ICAM-1 and VCAM-1 expression in the aorta [100,123]</td>
<td>Decrease in COX activity and PGE2 production [125]</td>
</tr>
<tr>
<td>Downregulation of IL-4 gene expression [115]</td>
<td>Decrease in agonist-induced platelet aggregation [67-69]</td>
<td>Decrease in macrophage accumulation and severity of the lesion in the aorta [100,101,123]</td>
<td>Decrease in carotid wall thickness, the ARIC study [12,18]</td>
</tr>
<tr>
<td>Downregulation of IL-1β gene expression [116]</td>
<td></td>
<td></td>
<td>Downregulation of TGF-β gene expression [115]</td>
</tr>
<tr>
<td>Downregulation of CD95 ligand expression [117]</td>
<td></td>
<td></td>
<td>Decrease in arterial stiffness [67]</td>
</tr>
<tr>
<td>Downregulation of VCAM-1 gene expression [119,120]</td>
<td></td>
<td></td>
<td>Normalization of gene expression of antioxidant enzymes [127]</td>
</tr>
<tr>
<td>Downregulation of CD11b/CD18 gene expression [120]</td>
<td></td>
<td></td>
<td>Downregulation of ICAM-1 mRNA expression [130]</td>
</tr>
<tr>
<td><strong>Vitamin C</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibition of LDL oxidation [34,35]</td>
<td>Increase in LDL oxidative resistance [36]</td>
<td>Decrease in carotid wall thickness, the ARIC study [12,18]</td>
<td>Decrease in arterial stiffness [67]</td>
</tr>
<tr>
<td>Decrease in collagen I mRNA stability [129]</td>
<td>Decrease in agonist-induced platelet aggregation [67-69]</td>
<td>Decrease in macrophage accumulation and severity of the lesion in the aorta [100,101,123]</td>
<td>Decrease in arterial stiffness [67]</td>
</tr>
<tr>
<td>Downregulation of transcription of elastin [129]</td>
<td></td>
<td></td>
<td>Normalization of gene expression of antioxidant enzymes [127]</td>
</tr>
<tr>
<td>Downregulation of ICAM-1 expression [132]</td>
<td></td>
<td></td>
<td>Downregulation of ICAM-1 mRNA expression [130]</td>
</tr>
<tr>
<td>Upregulation of Bcl-2 gene expression [133]</td>
<td></td>
<td></td>
<td>Downregulation of NOS expression [131]</td>
</tr>
<tr>
<td>Downregulation of VEGF expression [134]</td>
<td></td>
<td></td>
<td>Upregulation of HOOG1 and IdMTH1 gene expression [135]</td>
</tr>
</tbody>
</table>

| **Flavonoids** | | | |
| Inhibition of plasma oxidation [107] | Decrease in agonist-induced platelet aggregation [80,81] | Decrease in plasma leukotriene-prostacyclin ratio [82] | |
| Suppression of AP-1 [137] | Downregulation of TNF-α and IL-6 gene expression [141,142] | Suppression of postoclusion peak flow velocity of the brachial artery [51] | |
| Upregulation of GCS(H) promoter activity [143] | Downregulation of matrix metalloproteinase-9 transcription [144] | Decrease in arterial stiffness [84] | |
| Downregulation of endothelin-1 gene expression [145] | Downregulation of VCAM-1 mRNA expression [146] | Decrease in foam cell formation and extend of the atherosclerotic lesion [105-108] | |
| | | Suppression of the cholesterol deposition in the aorta [106] | |
| | | Decrease in lipid peroxidation [108] | |
| | | Upregulation of GSH-Px, SOD, catalase gene expression [139] | |
4.2. Clinical trials on the efficacy of dietary antioxidants in the prevention of LDL oxidation

4.2.1. Antioxidant Vitamins E and C

4.2.1.1. Vitamin E. When isolated LDL is oxidized by exposure to high concentrations of Cu²⁺ or aqueous ROO•, a clear initial lag phase is observed during which Vitamin E contained in LDL particle together make the LDL fraction resistant to oxidation in the standard oxidation conditions. Vitamin E acts as a chain-breaking antioxidant for LDL lipids. Consistent with this, enrichment of LDL with Vitamin E is expected to increase the lag time when the lipoprotein is exposed to these strong oxidizing conditions. Indeed, in vitro data suggest an increase in LDL oxidative resistance with the addition of Vitamin E. Andrikopoulos and co-workers have clearly shown that addition of Vitamin E (20 μM) in Cu²⁺ mediated LDL oxidation inhibits drastically the oxidative effect of the agents [26].

4.2.1.2. Vitamin C. On the effectiveness of Vitamin C in LDL oxidation, there is quite convincing evidence from in vitro studies that it strongly inhibits LDL oxidation [34]. Ascorbate prevents oxidative modification of LDL primarily by scavenging free radicals and other reactive species, thereby preventing them from interacting with LDL. Further to its potential to scavenge free radicals, Retsky and co-workers [35] have indicated that Vitamin C protects LDL against copper mediated modification also by decreased binding of Cu²⁺ to LDL. The authors suggest that decreased Cu²⁺ binding to LDL particle is owed to dehydro-l-ascorbic acid and other decomposition products of ascorbate that react with specific amino acid residues on ApoB, which normally bind Cu²⁺, thus decreasing the affinity of these amino acid residues for the metal ion. Ascorbate can also prevent the pro-oxidant activity of α-tocopherol by reducing the tocopheroxyl radical to tocopherol, thereby acting as a co-antioxidant and inhibiting LDL oxidation [36]. A number of ex vivo experimental data exist that are however insufficient to support an undisputed protective activity. Most probably, this is due to the fact that ascorbate is removed from LDL during isolation from plasma. There is a body of randomized controlled trials, which aim to link Vitamin C supplementation and reduced susceptibility of LDL to oxidation, however, clinical findings are rather confounding. Dietary Vitamin C enrichment in healthy males consuming a diet high in saturated fatty acids resulted in an increase of both plasma ascorbic acid and the lag period of in vitro LDL oxidation [36]. A modest beneficial effect on LDL oxidation was observed when Vitamin C was supplemented to healthy subjects combined with iron [37]. Measurable effects on LDL-C oxidation indices and increase in LDL-C lag time were observed when Vitamin C was supplemented with other antioxidant vitamins – namely Vitamins B₆ and B₁₂, folate, Vitamin E and β-carotene – in a multi-ingredient vitamin formula [38]. Similarly, improved lag phase was observed when Vitamin C was supplemented in...
patients with established CVD combined with Vitamin E, and β-carotene [39]. In a randomized double-blind crossover trial, supplementation with dehydrated juice concentrates from mixed fruit and vegetables resulted in a positive correlation of plasma Vitamin C with the resistance of LDL to oxidation [40]. Contrary to the above, some clinical trials show no correlation between Vitamin C intake and LDL resistance to oxidation. Tresoriere et al. [41] investigated the effects of short-term supplementation with cactus pear fruit, which is rich in ascorbate, compared with Vitamin C alone on total-body oxidative status in healthy humans. The outcome of the study was unlucky for Vitamin C alone, as no prevention of lipid hydroperoxide formation in LDL was observed. Significant prevention was shown for fruit supplementation, suggesting that components other than antioxidant vitamins play a role in the observed effect.

4.2.2. The potential of flavonoids against LDL oxidation

Flavonoids have been found to act against LDL oxidation, and their antioxidant capacity is related to their chemical structures [42]. Flavonoids, which are mostly hydrophobic and do not bind LDL molecule, either scaveng free radicals or act as chelating agents, or protect Vitamin E, β-carotene and lycopene in the LDL particle or preserve serum paraoxonase activity, that itself promotes hydrolysis of lipid peroxides. Several in vitro studies strongly suggest that phenolics protect LDL from oxidation [26]. Also, LDL oxidisability is reduced after consumption of several natural products that are rich in flavonoids, such as olive oil and its phenolics oleuropein and hydroxytyrosol [43]. On the olive oil polyphenol effectiveness, however, Vissers et al. [44] reported that consumption of 18 mg per day of phenols from extra virgin olive oil failed to affect LDL susceptibility to ex vivo oxidation. Supplementation with Concord grape juice, a rich source of flavonoids, significantly increased LDL lag time and significantly decreased the LDL oxidation rate in healthy adults [45]. Tea polyphenols also inhibited LDL oxidation in some [46], but not all studies [47]. Most was the effect of cocoa flavonoids, present mainly in soy products, the clinical results are controversial, as some studies do show that they are effective inducers of ex vivo LDL oxidation after supplementation [50], while others do not [51].

4.2.3. Carotenoid effectiveness

Clinical trials on the carotenoid effectiveness to inhibit LDL oxidisability ex vivo have also revealed controversial results. Supplementation with tomato juice drove to significantly higher plasma lycopene levels, which was not, however, associated with any effect in susceptibility of LDL to oxidation [52]. In the study of Carroll et al. [53], supplementation with a carotene mixture or lycopene had no effect on oxidative modification of LDL in vitro, despite significant increases in plasma and LDL concentrations of lycopene, α-carotene and β-carotene. Likewise, Hininger et al. [54] showed that β-carotene, lycopene and lutein supplementation failed to enhance the resistance of LDL to oxidation or to modify the LDL polyunsaturated/saturated fatty acid ratio. β-Carotene supplementation combined with Vitamins E and C was shown to prolong LDL lag phase [39]. With reference to lycopene, in a randomised crossover dietary intervention study, healthy, non-smoker and under no medication or vitamin supplement human subjects, consumed lycopene from tomatoes and nutritional supplement for a week. Lycopene was observed to be absorbed resulting in significant increment in serum lycopene levels and in a significant decrease in serum LDL-C oxidation as the serum lycopene levels increased [55]. In patients with diabetes mellitus increased susceptibility to LDL oxidation was normalized by natural β-carotene [56] or lycopene dietary supplementation, the latter being almost as effective as Vitamin E in a high dose [57].

4.2.4. CoQ10 supplementation in humans

Two-month CoQ10 supplementation did not increase the oxidation resistance of very low density lipoprotein (VLDL) and LDL fraction, as assessed by copper-induced VLDL and LDL oxidation, haemin-induced VLDL and LDL oxidation, total antioxidative capacity of LDL, and plasma malondialdehyde measurements [58]. In mildly hypercholesterolemic subjects combined supplementation of CoQ10 and α-tocopherol or Vitamin E alone, showed a significant increase in the oxidation resistance of isolated LDL only with Vitamin E supplementation alone, while simultaneous Q10 supplementation did not increase this antioxidative effect of Vitamin E [59]. Because simultaneous supplementation with Vitamin E was shown to result in lower proportion of plasma ubiquinol of total Q10 researchers suggest the in vivo Q10-based regeneration of the tocopheryl radicals [60].

5. Dietary antioxidants and vascular endothelial dysfunction

The vascular endothelium plays a key role in the regulation of vascular tone by the release of vasodilator substances, mainly NO and prostacyclin, and vasocostricter substances, mainly TXA2, free radicals and endothelin. In particular, in normal endothelium, factors such as NO mediate vasodilation, platelet desegregation and antiproliferation and inhibits inflammation; lipoprotein lipase induces lipolysis, and IPA, von WF, and protein C act as thrombolytic agents. In endothelial dysfunction vasoconstriction, inflammation, proliferation mediated by several growth factors (i.e. MGFI and PDGF), platelet aggregation mediated by adhesion molecules, such as selectins, and thrombosis, result in detrimental functional consequences and adverse long-term effects, including vascular remodeling. In CVD,
endothelium dysfunction of the coronary arteries may cause or contribute to myocardial ischemia. Numerous clinical trials have been carried out to evaluate dietary interventions to improve endothelial dysfunction, amongst which many on the potential of the antioxidants derived by the diet. The degree of the dysfunction correlates with elevated LDL oxidation, dyslipidemias, mainly elevated LDL and decreased high-density lipoprotein levels, hypertension and diabetes.

5.1. Clinical trials on the efficiency of dietary antioxidants in endothelial function

5.1.1. Antioxidant Vitamins E and C

Early in 1988, two randomized controlled trials were carried out regarding the effect of Vitamin E supplementation on platelet function, however, results were contradictory. Colette and co-workers [61] reported that high doses of Vitamin E (1 g/day) diminish ADP-induced platelet aggregation in diabetic patients and suggested that this effect is partly mediated through a diminution of the COX activity. On the contrary, Stamper et al. [62] failed to associate Vitamin E supplementation in similar dose (727 mg/day) with changes in COX products or in prostacyclin levels, despite the significant increase in plasma and erythrocyte Vitamin E levels. Although the dose supplemented was alike in both studies, the number of participants was rather small (nine and 20, respectively) and only in that of Colette et al. [61] all participants were with diagnosed diabetes mellitus. Since then the efficacy of the antioxidant vitamin in platelet function is still a matter of controversy. Supplementation of 40 healthy subjects with 300 mg/day of Vitamin E was shown to significantly decrease platelet as revealed by the decreased platelet aggregation in response to ADP and arachidonic acid, the increased sensitivity to inhibition by PGE1, the decreased plasma β-thromboglobulin concentration and the decreased ATP secretion [63]. Supplementation with Vitamin C did not affect platelet function significantly although a trend towards decreased platelet aggregability and an increased sensitivity to the inhibitor PGE1 were observed. Likewise, modest doses of Vitamin E (100 IU/day) significantly lowered blood TxB2 in 40 diabetic patients [64], while platelet aggregation decreased in 20 heart transplant recipients receiving 500 IU/day of Vitamin E for 2 months in response to either thrombin or ADP, thus decreasing the high thrombotic risk associated with heart transplantation [65]. In 20 patients with established coronary artery disease 400 IU/day supplementation for 8 weeks resulted in significant increase in plasma vitamin levels, however, did not exert any effect in endothelial function assessed by brachial artery infusion of acetylcarnitine and nitroprusside [66]. Similarly, in hypercholesterolemic and chronic smoking subjects, intra-arterial forearm infusions of acetylcarnitine did not augment endothelium-dependent relaxation [28].

Oral mono-treatment with Vitamin C in doses equal to 2 g of healthy male subjects resulted in significant reduction of ADP-induced platelet aggregation and in the arterial stiffness in such short length of time as 6 h after administration [67]. Although not confirmed, the authors speculated that the mechanism responsible for this activity is likely to involve protection of NO from inactivation of free radicals. Equal to the previous dose, not oral, but intravenous administration of 10 patients with coronary heart failure enhanced the inhibition of platelet aggregation by the NO donor nitroprusside and tended also to increase responses to glyceryl trinitrate, as well as increased flow-mediated dilatation in the brachial artery [68]. Enhancement of the platelet responsiveness in acute intravenous administration of ascorbic acid was confirmed also by Schindler and co-workers [69], this time in a dose equal to 3 g and in healthy non- or chronic smokers. Several other studies in which administration of ascorbic acid was acute [70], or short-term [71] or long-term [72] converge at the protective role of the nutrient in the endothelium function. The activity however is doubtful, since other researchers failed to associate short-term [73] or long-term [71] or acute [74] administration with endothelial protection.

Singh et al. [75] found that acute intra-arterial Vitamin C had no major effect on endothelial reactivity in healthy older subjects, whereas a healthy “Mediterranean-type” diet for 6 weeks improved vasodilator function in the forearm, contrary to the supplementation of Vitamin C as tablets, that had no major effect. This suggests that direct effects of Vitamin C alone are not sufficient to account for the improved vasodilatation seen with the ‘healthy’ diet. These findings suggest that either Vitamin C had pro-oxidant effects within the concentration range achieved in the study or contributed to vascular relaxation in synergy with other antioxidants in the “healthy” diet.

5.1.2. The effect of flavonoids on endothelial function

The clinical trials carried out as to the potential of dietary flavonoids to counter heart disease risk by inhibiting platelet aggregation result to contrasting evidence, to this moment. Hence, no effectiveness was shown after dietary supplementation with genistein and daidzein rich soy in males in platelet aggregation [76], neither after supplementation with quercetin [77] and apigenin in platelet aggregation, despite the significant increase in nutrient plasma levels. Duffy and co-workers [78] observed a dose-dependent platelet aggregation in response to respective agonists in coronary artery disease patients, however, no alterations by acute or chronic tea consumption, regardless of the fact that plasma flavonoids increased with both acute and chronic tea consumption. Black tea in comparison to hot water did not inhibit collagen or ADP-induced postprandial platelet aggregation in the study of Hodgson et al. [79], even though flavonoids were proven to be absorbed. Contrary to these findings, cocoa supplementation in healthy subjects for 28 days significantly increased plasma epicatechin and catechin levels and decreased platelet function [80], and chocolate consumption inhibited collagen-induced platelet aggregation [81], and induced increases in plasma prostacyclin and decreases in plasma leukotrienes.
levels in humans [82], evident of an anti-thromboembolic activity. Further to ex vivo agonist-induced latelet aggregation, diverse evidence on the flavonoid effectiveness in endothelial function has come out by a large body of clinical intervention studies. In postmenopausal women, supplementation with isoflavones (<100 mg/day) for a short period ranging from 4 to 6 weeks, isoflavones were found to significantly increase forearm vascular resistance following L-NMMA [83], to significantly lower postocclusion peak flow velocity of the brachial artery [51] and to reduce arterial stiffness with improved systemic arterial compliance attributable to a reduction in total peripheral resistance and a corresponding reduction in central pulse wave velocity [84]. Contrary, Nikander and co-workers [85] failed to show any effect of flavonoids in endothelial function of postmenopausal women, as serum levels of C-reactive protein and E-selectin and plasma levels of NO(\textsubscript{x}) did not differ after isoflavone supplementation compared to placebo. This lack of effect was in agreement with previous studies in postmenopausal women [86]. Nevertheless, the encouraging results of short-term flavonoid interventions in endothelial function are confirmed by larger scale longer-term trials [87] in postmenopausal women as well.

5.1.3. The endothelium response to CoQ10 administration

Only two intervention trials do exist concerning the potential of CoQ10 in endothelial function. Watts et al. [88] revealed that oral administration of 200 mg of CoQ10 for 12 weeks improved endothelial function of conduit arteries of the peripheral circulation in 40 dyslipidemic patients with Type II diabetes, but did not alter plasma F(2)-isoprostane concentrations. The mechanism could involve increased endothelial release and/or activity of nitric oxide due to improvement in vascular oxidative stress, an effect that might not be reflected by changes in plasma isoprostane levels. Contrary, administration of the same dose of CoQ10 for the same period and in dyslipidemic patients with Type II diabetes as well, however in 80 subjects, did not improve endothelial forearm vasodilator function when given alone, while combined with fenofibrate, the effectiveness was significant [89]. The authors suggested that the effect of this combination could be due to an increase in the bioactivity of and/or responses to endothelium-derived relaxing factors, including nitric oxide, and this might entail synergistic stimulation of peroxisome proliferator-activated receptors.

5.1.4. The endothelium response to phytosterol administration

In a double-blind crossover trial, 41 children with familial hypercholesterolemia were fed with spreads rich in sitosterol and campesterol. Lipid levels and endothelial function were assessed after a 4-week treatment period. Reduction of LDL cholesterol in children did not improve endothelial function assessed as flow-mediated dilation of the brachial artery [90].

5.1.5. Combined administration of dietary antioxidants; a promising undertaking?

A matter of research has also been the effectiveness of a combination of antioxidants in endothelial function, yet the efficacy of the combination is questionable. Thus, pretreating 76 patients with Vitamins E and C prior to elective coronary artery bypass resulted in no reduction of the myocardial perfusion defect determined by thallium-201 uptake [91]. In a short and limited scale trial (12-week, 18 subjects) in non-smoking and non-diabetic patients with established coronary artery disease, antioxidant supplementation consisting of Vitamins E and C, and ß-carotene did not improve endothelial function evaluated on the basis of percent and absolute changes in brachial artery diameter [39]. Kinlay and co-workers [92] subjected 30 subjects with coronary artery disease in Vitamin E (800 IU per day) and Vitamin C (1000 mg per day) supplementation and evaluated the coronary artery endothelial function measured as the change in coronary artery diameter to acetylcholine infusions and brachial artery endothelial function assessed by flow-mediated dilation at baseline and 6 months. Although plasma u-tocopherol and ascorbic acid increased with active therapy, there was no improvement in coronary and brachial endothelial vasomotor function over 6 months. Contrary, in the long-term, large scale ASAP study, combined supplementation of E and C vitamins was shown to retard the progression of common carotid atherosclerosis defined as the linear regression slope of ultrasonographically assessed common carotid artery mean IMT [93]. Combined administration of Vitamins C and E at high dosages, also improved endothelial function and decreased plasma levels of plasminogen activator inhibitor-1 (PAI-1), von Willebrand factor and PAI-1/PA ratio in chronic smokers [94]. Monotherapy with Vitamin C alone was ineffective in reducing serum levels of IL-1b, IL-6, soluble VCAM-1, and soluble ICAM-1, and improving forearm vasodilatory response to reactive hyperemia in 43 healthy young chronic smokers, whereas in combination with Vitamin E the opposite results were obtained [95]. Indicative of the reduced endothelial dysfunction with combined antioxidant supplement, including Vitamins E and C, in patients treated prior to cardiopulmonary bypass, was the better-maintained endothelium-dependent vasodilation after cardiopulmonary bypass [96].

6. Evidence from experimental animal models of atherosclerosis

Evidence linking dietary antioxidants to atherosclerosis in humans is still circumstantial and although in some studies the association of antioxidant intake and low risk for atherosclerosis is perceptible, in others this association cannot be established. The inconsistency of the results reflects the limitations of human studies. The diet differences, the pre-existing total antioxidant status, the stage of disease, the interaction between dietary modulation and genetic composition
of individuals, the dosage and duration of supplementation, the age, as well as sex, are dynamics to read between the lines in order to explain the inconsistent clinical findings. Studies in experimental animal models of atherosclerosis allow us to minimize the confounding factors during the pathogenesis of the disease; therefore, the results outcome are considered indicative and more reliable.

6.1. Antioxidant vitamins

Supplementation of cholesterol-fed rabbits with α-tocopherol increased both the resistance of LDL to oxidation and agonist-induced relaxation of thoracic aortas, whereas supplementation with β-carotene had no effect on LDL oxidizability yet did enhance agonist-induced vasodilatation [97]. These results suggest that β-carotene and α-tocopherol act by increasing vascular antioxidant status rather than LDL antioxidant status. In addition, α-tocopherol may act by non-antioxidant mechanisms. For example, Keane et al. [98] proposed that α-tocopherol acts in the vascular wall by inhibiting protein kinase C (PKC) activation by oxidized LDL, hence inhibiting PKC-mediated phosphorylation of endothelial cell muscarinic receptors and enhancing agonist-induced NOS activation. Several studies have convincingly demonstrated that dietary Vitamin E inhibits atherosclerosis in animal models [99,100]. In this of Terasawa et al. [101], mice with genetically engineered Vitamin E deficiency exhibited 30% more atherosclerosis and a moderate two-fold increase in tissue levels of F2-isoprostanes, markers of lipid oxidation compared to wildtype mice. While in humans a dose of Vitamin E corresponding to 300 mg significantly increased aortic Vitamin E and the lag-time of ex vivo LDL oxidation under strong oxidative conditions, yet failed to inhibit atherosclerosis and reduce the macrophage content of atherosclerotic lesions in homozygous WHHL rabbits [102]. In this study, only probucol at low dose, but not Vitamin E or CoQ10, significantly reduced the size of atherosclerotic lesions after 1 year of treatment. However, on the CoQ10 effect, the aortic and coronary artery plaque sizes, coronary atherosclerosis index, aortic and coronary atherosclerosis scores were significantly lower in rabbits receiving a tran fatty acid rich diet supplemented with CoQ [103]. Using mice that are unable to synthesize ascorbic acid and are prone to develop atherosclerosis, tested has been whether altered levels of Vitamin C in the diet influence the initiation or maturation of atherosclerotic plaques [104]. Although Vitamin C did alter neither foam cell formation nor the size of atherosclerotic plaques, however significantly influenced their collagen content and collagen in the adventitia surrounding vessels with plaques. Since ascorbic acid is important in connective tissue metabolism, where it acts as the reducing cofactor in the reactions catalyzed by prolyl and lysyl hydroxylases, and the content and networking of collagen fibers in the plaques were less in the low Vitamin C animals, these results show a crucial role for Vitamin C during the maturation of atherosclerotic plaques.

6.2. Atherosclerosis animal models fed on natural polyphenol-rich extracts

Total extracts from traditional natural plants, rich in polyphenolic compounds, have been shown to inhibit the development of atherosclerosis in animal models [105,106]. Proanthocyanidins are naturally occurring polyphenolic compounds widely available in fruits, vegetables, nuts, seeds, flowers and bark. Grape seed proanthocyanidins (GSPE), a combination of biologically active polyphenolic flavonoids, were examined for their anti-atherosclerotic activity in cholesterol-fed rabbits. [107]. Feeding proanthocyanidin-rich extracts (0.1 and 1% in the diet) to rabbits significantly reduced severe atherosclerosis in the aorta, almost the same of that of probucol. Immunohistochemical analysis revealed a decrease in the number of oxidized LDL-positive macrophage-derived foam cells in atherosclerotic lesions in the aorta of rabbits fed proanthocyanidin-rich extract. Apparently, the anti-atherosclerotic activity was due to the prevention of LDL oxidation in the arterial wall. Similarly, in a spontaneous familial hypercholesterolemic model, the Kurosawa and Kusanagi-hypercholesterolemic rabbits, cacao liquor polyphenols (CLP) suppressed the development of atherosclerotic lesions [108]. Because the researchers did not show a decrement in plasma cholesterol levels, though a decrement in plasma TRAPS and an increment in the resistance of LDL to oxidation, the results might easily be attributed to the antioxidant properties of polyphenols on the one hand, while on the other indicate that they are absorbed into the bloodstream in order to finally act as antioxidants.

7. Modulation of gene transcription

Nutrients can influence gene expression directly or via gene promoters, via control of regulatory signals in nontranslated regions, and via post-transcriptional pathways. All the genes encoding proteins and the genes associated with transcriptional activation and signals modulating the transcription of the respective genes are potential candidates for gene–diet interaction. The capacity of dietary antioxidants to modulate gene expression has been investigated chiefly during the last decade. Knowledge about the cellular effects of dietary antioxidant compounds has been obtained mostly by studies on cell tissue cultures in vitro and on experimental animal models.

7.1. Studies in cell cultures and experimental animal models

7.1.1. Antioxidant vitamins

7.1.1.1. Vitamin E. CD36 scavenger receptor expression is a key factor in atherogenesis. In ApoE−/− mice, when CD36 is missing, mice do not develop atherosclerosis [109]. Inhibition of class A scavenger receptors and CD36 expression at the transcriptional level by α-tocopherol in aortic smooth
P450 1A1 (CYP1A1) is one of the key detoxifying enzymes ing the resulting damage in cells and tissues. Cytochrome intake of ascorbic acid might be a useful means of prevention extremely low. Therefore, it is suggested that supplementalsis. Under oxidant conditions, plasma Vitamin C status is influences genes with possible involvement in atherosclerosis. It downregulates VCAM-1 gene expression. Part of its potential to protect against atherosclerotic plaque formation may be due to the protection of T-cells from apoptosis by inhibition of CD95 ligand expression [118]. The inhibitory activity of Vitamin E at the level of gene transcription has been reported for over 30 genes, amongst which, several play a major role in atherosclerosis. It downregulates VCAM-1 gene transcription in human vascular endothelial cells [119], and in macrophage cultures [120], as well as protects against monocyte Mac-1-dependent adhesion to endothelial cells induced by oxLDL [121]. Kim and co-workers [122] showed that Vitamin E upregulates the expression of hepatic α-tocopherol transfer protein (α-TTP) gene in rats. The significance of this is that a correct regulation of α-TTP may maintain adequate Vitamin E levels in plasma, thus reducing the risk for atherosclerosis. Treatment of NZW rabbits with Vitamin E combined with probucol significantly decreased VCAM-1 mRNA and reduced atherosclerosis in adjacent segments of the thoracic aorta in vivo [123]. Vitamin E had no effect on cycloxygenase (COX) mRNA and protein levels, indicating a post-translational regulation of COX. However, further experiments indicated that Vitamin E reduces formation of peroxynitrite, a hydroperoxide shown to be involved in the activation of COX-2 [124]. Other homologues of tocopherols were also effective in inhibiting COX activity, but their degree of inhibition varied. The variable potency to inhibit COX activity was not explained totally by differences in their antioxidant capacity. In human ventricular cardiomyocytes, α-tocopherol increased glutathione peroxi-dase (GSH-Px) activity and although mRNA levels mirrored the changes in enzyme activity, the de novo nuclear GSH-Px-I transcript synthesis was unaffected by α-tocopherol [125], meaning a posttranscriptional modulation in GSH-Px mRNA by α-tocopherol.

7.1.1.2. Vitamin C. On the effect of Vitamin C on gene regulation, many studies have shown that this it potentially influences genes with possible involvement in atherosclerosis. Under oxidant conditions, plasma Vitamin C status is extremely low. Therefore, it is suggested that supplemental intake of ascorbic acid might be a useful means of prevent-ing the resulting damage in cells and tissues. Cytochrome P450 1A1 (CYP1A1) is one of the key detoxifying enzymes catalizing cigarette smoking derived toxins and is relevant to smoking-induced atherogenesis. Induced CYP1A1 gene expression is decreased by ascorbic acid administration [126]. Similarly, ascorbic acid normalizes CuZnSOD, MnSOD, and catalase gene expressions, and suppresses the expression of protein disulfide isomerase, a protein associated with abnormal vascular smooth muscle cell growth [127]. In high doses it has been proven to suppress P450 gene expression [127], thus inhibiting the generation of vasoconstric-tor eponoxidosatrinenic acids that produce oxygen-derived free radicals. Elastin is a dominant extracellular matrix pro-tein product of the elastin gene synthesized by vascular smooth muscle cells in the form of tropoelastin. Elastin is implicated in vascular development and disorders, such as discrete fibrocellular stenoses in the aorta, coronary arteri-ies, carotid arteries, pulmonary arteries, and other peripheral arteries [128]. Often, individuals with these disorders are susceptible to peripheral vascular disease and myocardial infarctions. Vitamin C has been shown to decrease elastin mRNA stability and stabilize collagen I mRNA to a great extent [129]. Transcription of elastin was reduced 72% by exposure of vascular smooth muscle cells to ascorbate. Vitamin C reduces constitutive monocye ICAM-1 expression when supplemented in normal subjects [130], attenuates iNOS mRNA [131] and TNF-α induced ICAM-1 expression in endothelial cells [132], enhances cytoprotection by the induction of B-cell lymphoma-2 gene expression (Bcl-2) [133], and suppresses VEGF mRNA levels in thrombin or CoCl2 stimulated smooth muscle cells [134]. 8-Oxoguanine-DNA glycosylase 1 (hOGG1) and human MutT homologue (hMTH1) are two representative enzymes for DNA repair under oxidative damage. Tarng and co-workers [135] showed that Vitamin C supplementation upregulates hOGG1 and hMTH1 gene expressions in peripheral blood lymphocytes obtained from chronic hemodialysis patients.

7.1.1.3. Phenolic antioxidants. Polyphenolic compounds have been found to: (i) modulate the expression of COX-2 and of antioxidant enzymes [139], (ii) to downregulate VEGF expression in human aortic endothelial cells [140], IFN-γ and IL-4 gene expression in normal peripheral blood mononuclear [141] and in macrophages [142]. Myhrstad and co-workers [143] have reported that quercetin, kaempferol, and apigenin increased gene expression of a reporter driven by the glutamylcysteine synthetase (GCS) heavy subunit (GCS(h)) promoter, while a fourth flavonoid, myricetin and sugar conjugates of quercetin were unable to increase reporter expression. Quercetin was also able to induce a distal part of the GCS(h) promoter containing only two antioxidant-response/electrophile-response elements (ARE/EpRE). Inappropriate expression of matrix metal-llopeptinases contributes to atherogenesis. The flavonoid resveratrol is believed to exert antiatherogenic activity by...
inhibiting matrix metalloproteinase-9 mRNA expression through suppression of activation of nuclear factor AP-1 [144] and also endothelin-1 gene expression, partially by interfering with the ERK1/2 pathway through attenuation of reactive oxygen species formation [145]. Using electrophoretic mobility shift assays, polyphenols, including oleuropein, hydroxytyrosol, tyrosol, and resveratrol, have been found to inhibit VCAM-1 mRNA expression by NF-κB and AP-1 molecular pathways [146]. NF-κB pathway seems to interfere also in the modulation of iNOS mRNA expression by flavonoids [147], as well as TNF-α expression [148].

Apart from antioxidant Vitamins E and C and flavonoids, other dietary antioxidant compounds have been reported to influence gene expression related to CVD development. α-Lipoic acid suppresses expression of endothelial genes relevant in diabetes, such as tissue factor and endothelin-1 by inhibiting activation of NF-κB [149], and expression of E-selectin, VCAM-1 and ICAM-1 [150]. Nutritional supplementation of CoQ10 renders the hearts resistant to ischemia-reperfusion injury by inducing significantly the expression of ubiquitin mRNA in the hearts of the experimental animals [151]. The extract obtained from a Mediterranean culinary resin, namely Pistacia lentiscus, known to be rich in triterpenoids and contain few polyphenols [152], was shown to modulate CD36 expression in oxLDL treated peripheral blood mononuclear cells [153]. Researchers observed that the extract inhibited oxLDL-triggered apoptosis and necrosis and intracellular GSH downregulation and regulated CD36 mRNA and protein levels. Because the triterpenoid fraction alone was remarkably effective, the authors suggest that the extract exerts an anti-atherogenic effect mainly due to the contained triterpenes.

8. Conclusive marks and future prospects

In the past decade, considerable progress has been made concerning our knowledge of bioactive components in foods and their contribution to homeostasis and disease prevention. Bioactive components of food, which are of special interest, include the Vitamins E and C, polyphenols, carotenoids—mainly lycopene and β-carotene, and CoQ10, featured by antioxidant properties. In the above discussion regarding these dietary antioxidants and their protective effect on atherosclerosis, critically reviewed is the research evidence linked to the prevention of LDL oxidation, to the endothelial function and to gene regulation. Observations that occur in bibliography are chiefly derived from in vitro studies. Evidence that these compounds augment antioxidant defense in vivo is not compelling. In human clinical trials, the doses of antioxidants supplemented do not always show to inhibit lipid peroxidation convincingly. Results are contrasting and not always consistent with the in vitro findings. On the other hand, studies in animal models of atherosclerosis most clearly show an anti-atherogenic effect of dietary antioxidants, however, they focus mainly on early atherosclerotic events and not in advanced atherosclerosis as in humans. Since there are not accepted animal models for severe disease, such as plaque rupture, the potential of natural antioxidants in prevention of human atherosclerosis remains to be answered. Human clinical trials should focus on subjects with increased oxidative stress rather than the general population (Fig. 1).

The application of genomic tools to study the integrated effects of nutrients on gene regulation, namely nutrigenomics, holds great promise in clarifying or increasing the understanding of how nutrients affect the whole organism in health and disease. The fact that clinical trials drive to

![Fig. 1. The atherogenic process. The potential role of antioxidants.](image-url)
controversial observations enhances the thesis that each subject can display apart from general traits, particular susceptibility to the dietary factor due to genetic polymorphisms. However, the interactions of dietary antioxidants with genetic variations of genes regulatory on a phenotype with high risk for atherosclerosis have been a subject of research for only a few years and only a limited number of studies exist [154]. Because the new tools recently developed allow easy determination of most gene polymorphisms from a blood sample, especially single nucleotide polymorphism, the beneficial effects of antioxidants can potentially be established in complete organisms with known polymorphisms. This advance could as well open a new horizon into the mechanisms of prevention of CVD by personalized diets based upon dietary antioxidants.

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