REVIEW

Cardiovascular Therapeutics

Nutraceuticals and Prevention of Atherosclerosis: Focus on ω-3 Polyunsaturated Fatty Acids and Mediterranean Diet Polyphenols

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Keywords
Atherosclerosis; Cardiovascular disease; Mediterranean diets; Nutraceuticals; Omega-3 fatty acids; Polyphenols.

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SUMMARY

Nutraceuticals are potentially healthful foods that play a role in maintaining human well being, enhancing health and preventing, or even treating, specific diseases. More than for any other diseases, cardiovascular diseases occur in association with risk factors that are amenable to prevention or treatment by nutraceutical interventions. Several ingredients marketed for use in dietary supplements address such risk factors. The ability of nutraceuticals to favorably influence cardiovascular risk factors and atherosclerotic vascular disease should be recognized as an enormous opportunity for the prevention or treatment of this common condition. In this review, we attempt at summarizing some of the recent research findings on ω-3-polyunsaturated fatty acids and antioxidant polyphenols that have beneficial cardiovascular effects to update the practicing clinicians on the potential benefits of nutraceuticals in this area.

doi: 10.1111/j.1755-5922.2010.00211.x

> "Let food be thy medicine and medicine be thy food"
> Hyppocrates (460–377 B.C.)

Introduction

Cardiovascular diseases (CVD), of which atherosclerotic vascular disease is by far the largest component, remain the leading cause of morbidity and mortality worldwide. Although genetic factors and aging are important in determining the overall risk, a substantial proportion of CVD occur in conjunction with a series of modifiable risk factors, such as hypercholesterolemia, hypertension, obesity, insulin resistance, and diabetes, susceptible to lifestyle modifications, which include diet and physical exercise [1]. Such awareness has spurred, in the last 50 years, a growing worldwide interest on the prospect that more appropriate eating habits can promote and maintain a healthy life. In the past, health-focused dietary approaches have mostly focused on the curtailment of dietary sodium, refined sugars, and saturated fats. In more recent years, however, attention of researchers and of the media has expanded on trying to promote the consumption of "curative nutrients." This approach has gained large support because people in general appear to prefer consuming increased amounts of specific foods rather than restricting themselves from something they like. Such a renewed hyppocratic perception of "food as a medicament" has encouraged pharmaceutical and biotechnology companies toward the search and the marketing of a growing number of new substances, from dietary supplements to nutraceuticals (Table 1), with the aim of improving human health, making us fitter and more resilient to disease. Because the scientific characterization and commercial regulation of such substances vary from country to country [2,3], their popularity imposes the medical and scientific community a constant revision of their therapeutic potentials.

On this background, this short review will focus on summarizing the main mechanistic evidence supporting the consumption of selected nutraceuticals and functional foods generically labeled as "cardioprotective" to prevent CVD.

The Pathogenesis of Atherosclerotic Vascular Disease—Focus on the Orchestrating Role of the Vascular Endothelium

Because a description of the cellular and molecular events underlying atherosclerosis is important for the understanding of the

Table 1 Definitions of dietary supplements, nutraceuticals, and functional foods

Dietary supplements (United States Government Office, 1994)	A product (other than tobacco) in the form of a capsule, powder, softgel, or gelcap intended to supplement the diet to enhance health that bears or contains one or more of the following dietary ingredients: a vitamin, mineral, amino acid, or other botanical or dietary substance.
Phytochemical (Bloch and Thomson, 1995)	Substances found in edible fruit and vegetables that can be ingested daily (in quantities of grams) by humans and that exhibit a potential to favorably modulate human metabolism to prevent cancer and other diseases (isoflavones, resveratrol, garlic allyl-sulphides, tomato lycopene, onion quercetin, etc.).
Nutraceuticals (De Felice, 1995)	Food or part of food that provides medical or health benefits, including the prevention and/or treatment of a disease.
Nutraceuticals (Zeisel, 1999)	A diet supplement that delivers a concentrated form of a biologically active component of food in a nonfood matrix to enhance health.
Functional food (Zeisel, 1999)	Nutrient consumed as part of a normal diet but delivering one or more active ingredients (that have physiologic effects and may enhance health) within the food matrix.
Functional food (Hardy, 2000)	Any food or ingredient that has a positive impact on an individual's health, physical performance, or state of mind, in addition to its nutritive value.

mode of action of cardiovascular nutraceuticals, a short review of current concepts of inflammation and atherosclerosis gives the proper background to place their effects in context.

In its initial stages, atherosclerosis is characterized by the intimal recruitment of selected populations of white blood cells, especially monocytes, and by the gradual accumulation of lipids and extracellular matrix. The endothelium and the underlying collagen layers overlying the enlarged intima—the so-called fibrous cap—for a long time during atherosclerosis progression prevent the contact of the highly thrombogenic lipid core of the plaque with the circulating blood. The sudden rupture of the eventually thin fibrous cap, from the outside or from the inside (in the latter case due to the rupture of *vasa vasorum* penetrating the intima), transforms this slow process into an acute event, with the sudden development of thrombosis [4]. When this occurs in a coronary artery, the consequent myocardial ischemia, in appropriate conditions, may precipitate an acute coronary syndrome, that is, acute myocardial infarction or unstable angina. In the cerebral circulation or in the cerebro-afferent arteries this may lead to an acute stroke.

The recruitment of monocytes reflects specific molecular changes in the adhesive properties of the endothelial surface, due to the surface expression of specific endothelium-leukocyte adhesion molecules (ELAMs) ("athero-ELAMs"). One of the most relevant such proteins is vascular cell adhesion molecule-1 (VCAM-1). Pathophysiologically, relevant stimuli for VCAM-1 expression include several causes of increased oxidative stress, such as oxidized low density lipoproteins (LDL), many cytokines, the advanced glycation end-products (AGEs) associated with diabetes, and possibly also high glucose and high insulin [5]. Once adhered, monocytes transmigrate, through the action of chemotactic stimuli also deriving from the activated endothelium, from the blood into the subendothelial intima, where, upon internalization of modified lipoproteins, they become macrophages able to secrete proinflammatory cytokines. These contribute to maintaining a state of vascular inflammation, further augmenting the expression of adhesion molecules, chemoattractants, and macrophage-activating factors, thus initiating a vicious circle leading to lesion progression.

Later on, the production of matrix metalloproteinases (MMPs)—by the same macrophages as well as by endothelial cells—may promote the erosion of the collagen layers of the fibrous cap and ultimately lead to plaque rupture [6]. The stability of the atherosclerotic plaque is indeed thought to reflect the balance and interplay of various dynamic factors, including endothelial dysfunction, the proliferation of smooth muscle cells (which are mainly responsible for the synthesis of collagen), and the degradation of collagen and other elements of the extracellular matrix by MMPs, mostly produced by activated macrophages. The much sought-after prevention of acute vascular events therefore requires interventions that affect such mechanisms leading on the one hand to the slow formation of atherosclerotic lesions and on the other to the sudden precipitation of plaque rupture.

Antiatherogenic Functional Food and Nutraceuticals

Functional foods and related nutraceuticals claimed as "vasculoprotective" are a very large, heterogeneous, and ever-increasing number of substances. A synoptic table reporting the claimed cardiovascular benefits of a wide array of such bioactive compounds is provided in Table 2. We will here focus only on the mechanistic evidence supporting the consumption of two well-known vasculoprotective classes of foods, namely:

- fish and fish oil, as a source of ω-3 polyunsaturated fatty acids (ω-3 FA);
- red wine and olive oil as a source of antioxidant phenols.

ω-3 PUFAs Eicosapentaenoic (EPA) and Docosahexaenoic (DHA) in Fish and Fish Oil

Since the early studies in Greenland Eskimos, dietary intake of ω-3 FA, introduced with fish, fish-derived products or supplements, has been consistently associated with cardiovascular protection [7]. Randomized intervention trials have also by-and-large shown that ω-3 FA may reduce relevant cardiovascular endpoints, such as sudden death and fatal myocardial infarction [8,9], with estimated benefits that exceed the potential vascular risk associated with methylmercury, dioxins, polychlorinated biphenyls and

Table 2 Potential cardiovascular benefits of different functional foods and nutraceuticals

Bioactive compound	Examples	Sources	Putative vasculoprotective effects
Flavonols	Quercetin, kaempferol, catechin	Onion, apple, tea, berries, olives, broccoli, lettuce, red wine, cocoa/chocolate	↓TC, ↓LDL-C oxidation↑HDL-C, AOx, ↓platelet aggregation, ↓eicosanoid synthesis, ↓athero-ELAMs, ↓angiogenesis, ↓MMPs
Flavonols	Epicatechin, epigallocatechin, epicatechin-3-gallate, epigallocatechin-3-gallate	Green/black tea, cocoa/chocolate	AOx, ↓apoptosis, ↓LDL-C oxidation, ↓platelet aggregation, ↓athero-ELAMs, ↓angiogenesis, ↓MMPs
Lignans	Enterolactone, enterodiol	Flaxseed oil, lucerne, clover	↓LDL-C, AOx, estrogen/antiestrogen; ↓atherosclerosis *in vivo* but may show adverse CVD effect (pro-oxidant activity with partially defatted flaxseed)
Isoflavones	Genistein, daidzein	Soybeans, legumes	↓TC and LDL-C, ↓LDL-C oxidation, ↓TG, ↑HDL-C, ↓thrombosis, AOx, estrogen/antiestrogen, ↓athero-ELAMs, ↓angiogenesis, ↓atherosclerosis *in vivo*, ↓MMPs
Stilbenoids	Resveratrol	Grapes, red wine, peanuts	↓LDL-C oxidation, ↓platelet aggregation/thrombosis, ↓eicosanoid synthesis, AOx, ↓athero-ELAMs, ↓angiogenesis but promotes angiogenesis in the ischemic heart, ↓atherosclerosis *in vivo*, ↓MMPs
Carotenoids	Lycopene	Tomatoes, tomato products	↓LDL-C and LDL-C oxidation, Aox, ↓athero-ELAMs, ↓MMPs, but no effects was shown in animal models of ATS and and dietary intervention studies using well-defined subjects population did not provided a clear evidence of lycopene in the prevention of CVD
Carotenoids	α-Carotene β-carotene, γ-carotene, δ-carotene	Carrots, pumpkins, maize, tangerine, orange and yellow fruits and vegetables	Inconsistent data. β-carotene have shown adverse CVD effect because its prooxidant activity
Organosulfur compounds	Allicin, diallyl sulfide, diallyl disulfide, allyl mercaptan	Garlic, onion, leek	↓TC and LDL-C, ↓TG, ↓cholesterol and FA synthesis, ↓BP, ↓thrombosis, AOx, ↓athero-ELAMs, ↓angiogenesis, ↓atherosclerosis *in vivo*, ↓MMPs
Soluble dietary fibers	Glucan, pectin, psyllium	Oats, barley, yeast, fruit, vegetables, psyllium seed, fortified cereals and grains	↓TC, ↓TG, ↓LDL-C
Isothiocyanates	Phenethyl (PEITC), benzyl (BITC), sulforaphanes	Cruciferous vegetables (e.g., watercress, broccoli)	no relevant effects
Monoterpenes	d-Limonene, perillic acid	Essential oils of citrus fruit, cherries, mint, herbs	↓TC and LDL-C, ↓HMGCoAR, ↓angiogenesis
Plant sterols	Sitostanol, stigmasterol, campesterol	Tall oil, soybean oil, rice bran oil	↓TC and LDL-C, AOx, ↓cholesterol absorption; adverse effect: ↓carotenoid absorption
Phenolic acids	Tyrosol, hydroxytyrosol, oleoeuropeine, caffeic acid, cumaric acid	Extra virgin olive oil	↓LDL-C oxidation, ↓platelet aggregation/thrombosis, ↓eicosanoid synthesis, AOx, ↓athero-ELAMs, ↓atherosclerosis *in vivo*, ↓MMPs
ω-3 PUFA	DHA, EPA, αLA	Fish and fish oil, green leaves	↓TC, suppression of cardiac arrhythmias, ↓BP ↓platelet aggregation, ↓eicosanoid synthesis, ↓athero-ELAMs, ↓angiogenesis; ↓MMPs
Prebiotics	Inulin-type fructans	Fruit and vegetable, purified extract from chicory root	↓TC and ↓TG
Probiotics	Selected strains of *Lactobacillus acidophilus, Bifidobacterium bifidum and Lactobacillus bulgaricus*	Fermented milk products	↓TC, LDL-C and BP

AOx, antioxidant activity; BP, blood pressure; CVD, cardiovascular disease; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; MMPs, metalloproteinases; ELAMs, endothelial leukocyte adhesion molecules.

other environmental contaminants that frequently pollute marine food products [10,11].

The reduced morbidity and mortality from CVD by ω-3 FA can explained by two main basic mechanisms: the suppression of cardiac arrhythmias [12]; and reduced athero-thrombotic events, through the modulation of specific risk factors—reducing platelet aggregation [13], decreasing plasma triglycerides [14], and blood pressure [15,16]; and the direct regulation of systemic and local inflammation underlying plaque inception, progression, and instability. The anti-inflammatory and anti-atherogeneic properties of ω-3 FA have been largely investigated in *in vitro*, animal and human studies. Cellular models of early atherogenesis based on cultured endothelial cells challenged with various pro-atherogenic stimuli have shown that both DHA and EPA significantly inhibit most of the critical events connected with endothelial activation, including the expression of VCAM-1. Such regulatory effects occur in a range of DHA concentrations compatible with nutritional supplementation to a normal Western diet and are strictly related in magnitude to the extent of incorporation into total cell lipids [17–19]. Such "anti-inflammatory" effects are not limited to the expression of transmembrane molecules involved in leukocyte recruitment, but also appear to occur for other cytokine-activated products, such as the pro-inflammatory and pro-angiogenic enzyme cyclooxygenase (COX)-2 [20], as well as the pro-inflammatory and chemoattractant soluble proteins interleukin (IL)-6 [21], IL-8 [18], macrophage-colony stimulating factor (M-CSF) [22], and monocyte chemoattractant protein (MCP)-1 [23]. Such effects are accompanied by reduced monocyte adhesion to cytokine-activated endothelium [17,18]. In parallel with the modulation of endothelial activation, an antioxidant effect can also be documented in cultured endothelial cells supplemented with DHA [20,22,24], mostly attributable to the reduced membrane assembly and activation of the reactive oxygen species (ROS)-producing enzyme complex nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase, likely as a consequence of the plasma membrane changes occurring upon DHA incorporation [20] (Figure 1).

Cell treatment with ω-3 FA also favorably influences other correlates of atherosclerotic risk. It was observed that both EPA and DHA, when supplemented to macrophages in culture, significantly reduce the stimulated release of MMPs [23,25], thus likely contributing to the plaque-stabilizing effects observed in humans [26]. In addition, fish oils prevent serotonin-induced smooth muscle cells proliferation [27], an effect that may play a role in preventing restenosis after an endovascular intervention.

While cellular models of atherosclerosis essentially indicate a protective role of fish oil, results from animal studies carried out in nonhuman primates, pigs, rabbits, and mice, although mostly pointing out to the existence of an anti-aterogenic effect, are somewhat contradictory, probably due to differences in study

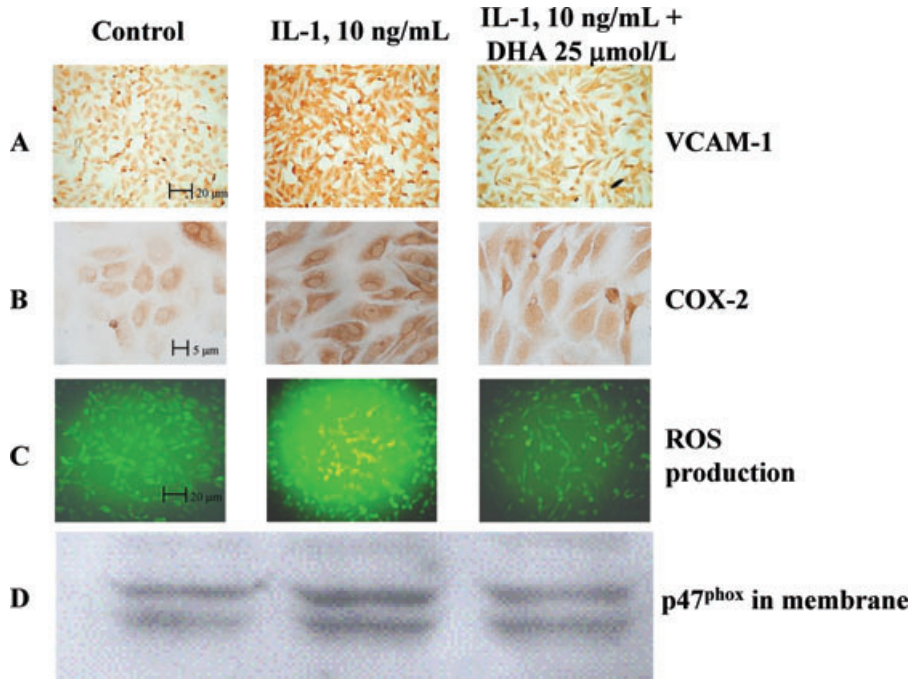

Figure 1 The effects of the ω-3 fatty acid DHA on endothelial VCAM-1 and COX-2 expression, the generation of reactive oxygen species and the activation of NAD(P)H oxidase. Immunocytochemical analysis of the effect of DHA on the stimulated expression of VCAM-1 (A) and COX-2 (B) in endothelial cells. Human umbilical or saphenous vein endothelial cell (HUVEC or HSVEC) were treated with 25 μmol/L DHA for 48 h before stimulation with IL-1β 10 ng/mL for 16 h. After incubations, monolayers were immunostained with anti-VCAM-1 and anti-COX-2 monoclonal antibodies, respectively. (C) HUVEC (or HSVEC) were treated as in A. ROS generation was assessed by the oxidation of dichloro-fluoresceine diacetate to a highly fluorescent compound. (D) Subcellular soluble and particulate (membrane) fractions were isolated from HUVEC treated as in A, and Western blots were performed with an antibody specific for the NAD(P)H oxidase subunit p47^{phox} (unpublished data).

designs and species [23,28,29]. Furthermore, it is not yet clear whether it is the absolute low amounts of ω-3 FA in the diet or is the high ratio of ω-6 FA to ω-3 FA—another typical feature of the pro-atherogenic Western diets—to predispose individuals to increased CVD disease [30]. In the LDL receptor-null mice, a commonly used model of atherosclerosis, results point to a relevance of the ratio [31].

Finally, complementary studies on angiogenesis have highlighted a potential anti-angiogenic activity by ω-3 FA *in vitro* and *in vivo* [32,33], an effect interpreted as promoting plaque stabilization [34].

Phenolic Compounds in Red Wine and Extra-Virgin Olive Oil: Resveratrol and Hydroxytyrosol as Potential Cardiovascular Nutraceuticals

By the end of the 1970s findings from epidemiological and clinical studies had indicated that consumption of fruit and vegetables prevents several major diseases, including cancer and CVD [35], prompting health authorities to promote consumption of these foods and clinicians to carry out supplementation trials. The primary nutrients responsible for providing such protection were thought to be antioxidant vitamins, which are abundant in fruit and vegetables [36]. However, when the role of individual antioxidants, such as vitamins C, E, and carotenoids, was examined in controlled trials, the results were clearly disappointing [37]. It was therefore proposed that only the whole fruit and vegetables, containing a very vast array of antioxidant components in optimally balanced amounts, may provide the optimal "polypharmacy" against the development of atherosclerosis.

Mediterranean diets, known for being associated with cardiovascular protection [38], are characterized by a low consumption of butter and red meat, a high intake of antioxidant phenol-rich vegetables and fruits, but also the olive-related derivative olive oil, as well as red wine. Hydroxytyrosol and resveratrol are the two major antioxidant phenols contained in olive oil and red wine, respectively. Several human studies have pointed out to a vasculoprotective effect for both resveratrol and hydroxytyrosol [39,40], and many efforts have been made to characterize their biological mechanisms of action. Although studies in animal models of atherosclerosis overall agree on an anti-atherogenic effect of both red wine and resveratrol [41–43], the use of olive oil and hydroxytyrosol has produced conflicting results, with olive oil usually reducing [44–46], while hydroxytyrosol sometimes promoting the development of atherosclerotic lesions [47]. These data support the concept that phenolic components-enriched products, out of the original matrix, might not only be useless but also harmful, and suggest that the formulation of functional foods should approximate—as much as possible—the natural environment in which active molecules are found. Results obtained in cellular models of atherosclerosis conversely confirm the protective role for both hydroxytyrosol and resveratrol furnishing plausible mechanistic explanations for the protective effects observed in humans. Both resveratrol and hydroxytyrosol inhibit lipid peroxidation, while resveratrol also enhances the cholesterol efflux by upregulating the ATP-binding cassette, sub-family A [ABCA]-1 receptors [48,49]. Both compounds inhibit platelet aggregation [50,51] and the synthesis of eicosanoids, but only resveratrol suppresses the induction of tissue factor (TF) in endothelial cells and monocytes [52]. Finally, hydroxytyrosol and resveratrol reduce the expression of VCAM-1 in endothelial cells stimulated by various proinflammatory and proatherogenic stimuli [53,54], an effect largely mediated by the reduced activation of the redox-sensitive transcription factor nuclear factor(NF)-κB [53]. Furthermore, recent findings from our laboratory indicate that hydroxytyrosol may improve plaque stability by reducing the stimulated release of MMP-9 by macrophages in culture (Figure 2). Interestingly, such effect was even more evident in the presence of oleic acid, a typical macro-component of olive oil, in agreement with the concept that functional foods may exert a better protective effect when the natural food matrix is maintained. It must however be remarked that clinical trials with selected Mediterranean diet antioxidants are missing, and that these compounds are therefore mostly seen as acting in concert with the nutritional matrix and other components.

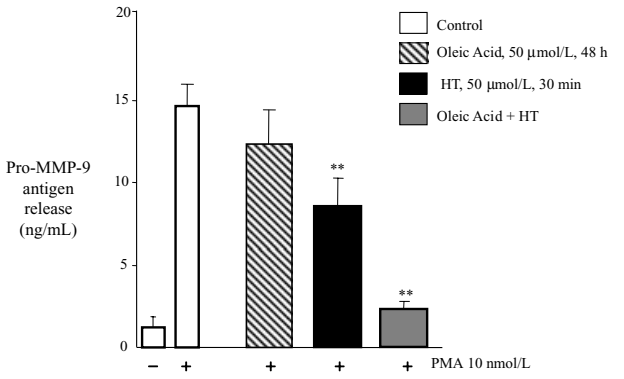

Figure 2 Hydoxytyrosol inhibits the PMA-induced expression of MMP-9 in U937 cells. Fifty μmol/L hydroxytyrosol or 50 μmol/L oleic acid (or both) were added to monocytoid U937 cells for the indicated time before adding phorbol myristate acetate (PMA) for further 24 h, after which matrix metalloproteinase (MMP)-9 released in the medium was determined by a soluble-phase EIA (**$P < 0.001$) (unpublished data).

Conclusions and Perspectives

More than for any other disease, CVD appear to be highly sensitive to nutritional intervention. However, while for decades nutritional recommendations have focused on negative messages – *what not to eat* –, today the other side of the nutritional coin—*what to eat*—is equally, if not more, importantly emphasized. Impressive progress has been made over the past three decades in defining the role of bioactive compounds in reducing the risk of atherosclerotic vascular disease, also unraveling some of the underlying biological mechanisms. Here we have summarized some of the evidence linking ω-3 FA and selected antioxidant polyphenols with protection from atherosclerosis. For them, the concerted transcriptional control of several pro-inflammatory genes implicated in atherosclerosis likely plays a key role. Such quantitatively minor bioactive dietary components appear overall to fine-tune the

response of our genes to dangerous environmental challenges, curbing physiological responses without abrogating them totally. By decreasing the endothelial responsiveness to pro-inflammatory, pro-atherogenic, and pro-angiogenic stimuli, they appear to impact molecular events not targeted by any other drugs or interventions. Their potential preventive or therapeutic role appears therefore complementary to those of already enacted pharmacological treatments. Overall, one has, however, to remark that clinical evidence for the health benefits of these compounds is much stronger for ω-3 FA than for antioxidant polyphenols of the Mediterranean diet. Clearly, more clinical research is necessary, especially with these latter compounds.

Conflict of Interest

The authors declare no conflict of interests.

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