Astaxanthin: A Novel Potential Treatment for Oxidative Stress and Inflammation in Cardiovascular Disease

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Oxidative stress and inflammation are implicated in several different manifestations of cardiovascular disease (CVD). They are generated, in part, from the overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that activate transcriptional messengers, such as nuclear factor–κB, tangibly contributing to endothelial dysfunction, the initiation and progression of atherosclerosis, irreversible damage after ischemic reperfusion, and even arrhythmia, such as atrial fibrillation. Despite this connection between oxidative stress and CVD, there are currently no recognized therapeutic interventions to address this important unmet need. Antioxidants that provide a broad, "upstream" approach via ROS/RNS quenching or free radical chain breaking seem an appropriate therapeutic option based on epidemiologic, dietary, and in vivo animal model data. However, human clinical trials with several different well-known agents, such as vitamin E and β-carotene, have been disappointing. Does this mean antioxidants as a class are ineffective, or rather that the "right" compound(s) have yet to be found, their mechanisms of action understood, and their appropriate targeting and dosages determined? A large class of potent naturally-occurring antioxidants exploited by nature—the oxygenated carotenoids (xanthophylls)—have demonstrated utility in their natural form but have eluded development as successful targeted therapeutic agents up to the present time. This article characterizes the mechanism by which this novel group of antioxidants function and reviews their preclinical development. Results from multiple species support the antioxidant/anti-inflammatory properties of the prototype compound, astaxanthin, establishing it as an appropriate candidate for development as a therapeutic agent for cardiovascular oxidative stress and inflammation. **© 2008 Elsevier Inc. All rights reserved.** **(Am J Cardiol 2008;101[suppl]:58D–68D)**

Atherosclerosis is an inflammatory disease of the arterial wall that remains a principal cause of death and disability, despite the application of statin and antiplatelet therapies. The severe clinical manifestations of the disease—myocardial infarction (MI) and stroke—are mainly caused by the abrupt obstruction of the vessel lumen by a thrombus triggered by the rupture or erosion of an atherosclerotic plaque.[1] Existing data strongly suggest that immunoinflammatory–related mechanisms are the major determinants of these atherothrombotic plaque sequelae.[2] Thus, most of the important advances in the comprehension of the mechanisms of atherothrombosis come from studies of the critical components involved in the modulation of the immunoinflammatory balance within the plaque. Despite an increasing understanding of these processes, there have been no approved therapeutic interventions in vascular biology that fully incorporate the current understanding of oxidative stress and inflammation.[3]

Role of Reactive Oxygen and Nitrogen Species in Cardiovascular Inflammation

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are well recognized for functioning as both potentially harmful and beneficial cell-signaling molecules. Normally generated by tightly regulated enzymes, such as nicotinamide adenine dinucleotide phosphate oxidase (NADPH) and nitric oxide synthase (NOS), excessive and/or chronic overproduction of ROS/RNS either from the mitochondrial electron transport chain or various other ROS/RNS–generating enzymes, NADPH or NOS, results in oxidative stress, a harmful process that can be an important source of damage to cellular components, including lipids, proteins, and DNA. In contrast, beneficial effects of ROS/RNS (eg, superoxide radical and nitric oxide) occur transiently at low-to-moderate concentrations and mediate physiologic roles in cellular responses to oxygen deprivation: defense against infectious agents, modulation of cellular signaling pathways, and the induction of cellular proliferation. Paradoxically, various ROS-mediated actions, in

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fact, may protect cells against ROS-induced oxidative stress and reestablish or maintain "redox balance" or "redox homeostasis."[4]

In postischemic myocardium, elevated levels of exogenous ROS are generated in cardiomyocytes, endothelial cells, and infiltrating neutrophils that can lead to cellular dysfunction and necrosis. ROS most likely contribute to the pathogenesis of MI and serve as mediators of the reversible ventricular dysfunction (stunning) that often accompanies reperfusion of ischemic myocardium.

Oxidative Stress and Endothelial Dysfunction Are Common among Risk Factors and All Manifestations of Cardiovascular Disease

During the past 25 years, the literature has established the pivotal role of the endothelium in preserving vascular homeostasis. Nitric oxide produced in endothelial cells and platelets is believed to be the main factor responsible for endothelial functional integrity (ie, vasorelaxation). Reduced nitric oxide bioavailability causes so-called "endothelial dysfunction," which constitutes the molecular dysfunction common to both the "stable" lesions of atherosclerotic narrowing and acute plaque rupture leading to unstable angina or MI. Substantial evidence is accumulating on the role of oxidative stress in reducing nitric oxide bioavailability and subsequent increased endothelial dysfunction as well as alterations of cell signaling pathways. The role of endothelial cell apoptosis as a possible end stage of endothelial dysfunction and plaque disruption has also been proposed. In vitro and in vivo evidence suggest a role of oxidative stress as a putative mechanism, ultimately leading to plaque erosion and activation through increased endothelial cell apoptosis. Thus, oxidative stress, regardless of the stage of atherosclerotic disease, likely represents a key mechanism in vascular disease progression and a possible clinical target for prevention.[5–7]

Importance of the Nuclear Factor–κB Transcription Family

The nuclear factor–κB (NF-κB)/Rel pathway has been shown to be critical to the inflammatory process and worthy of targeting for medicinal intervention. When activated by signals sourced from pathogens, oxidative stress, or other physiologic stresses, the NF-κB/Rel family of effector proteins translocate to the nucleus, resulting in the increased transcription of proinflammatory genes.[8] As a molecular nexus point in the inflammatory process, NF-κB is frequently found to be activated at sites of inflammation in diverse diseases and is particularly important in cardiovascular disease (CVD), where increased transcription of proinflammatory cytokines, chemokines, cell adhesion molecules, matrix metalloproteinases, cyclooxygenase-2, and inducible nitric oxide synthase play a critical role in the disease etiology.[9]

Important activators of NF-κB and oxidative stress are closely associated with many of the various risk factors for atherosclerosis: hypercholesterolemia, diabetes mellitus and its associated advanced glycation end products, uncontrolled hypertension, and elevated plasma homocysteine levels. Activated NF-κB has been identified in the smooth muscle cells, macrophages, and endothelial cells of human atherosclerotic lesions. Additionally, enhanced activation of this transcription factor has been shown to occur very early after administration of a high-fat diet in the low-density lipoprotein (LDL) receptor–deficient atherosclerotic mouse model.[10] In patients with unstable angina, enhanced levels of oxidized LDL increased NF-κB activation, suggesting ties between oxidative stress and inflammation in this pathway.[11]

Potential Impact of Upstream Reduction in Inflammatory Activity and the Potential Significance of Balanced Reactive Oxygen Species

Enzymatic antioxidants (eg, superoxide dismutase and glutathione peroxidase, and chemical antioxidants, such as carotenoids, vitamin E, etc.) scavenge or quench excess lipid and aqueous phase ROS/RNS, allowing signaling molecules and cellular pathways, such as NF-κB, to operate normally in homeostasis with the associated downstream products from these pathways, which results in a balanced ROS/RNS redox environment. However, in response to commonly encountered inflammatory stimuli (eg, cytokines, pathogens, etc.), ROS/RNS production transiently increases dramatically, temporarily overwhelming normal cellular enzymatic and chemical antioxidants. Cell signaling pathways are now activated, which in the case of NF-κB results in large increases in the previously normal levels of downstream inflammatory mediators, enzymes, and cytokines, such as cyclooxygenase-2, prostaglandin E_2, and tumor necrosis factor–α (TNF-α), which at their higher levels now drive potentially curative inflammation (Figure 1). However, in the case of many prolonged disease pathologies, including many forms of CVD, ROS/RNS levels are chronically elevated, resulting in a state of prolonged oxidative stress and inflammation because of the depletion of natural and dietary antioxidants, ultimately leading to a persistently malevolent redox homeostasis.

Connexins, Gap Junction Communication, and Cardiovascular Disease

Gap junction proteins called connexins mediate gap junction intercellular communication (GJC) and are a group of ≥20 highly conserved proteins with developmental and tissue-specific expression patterns. GJC allows the direct

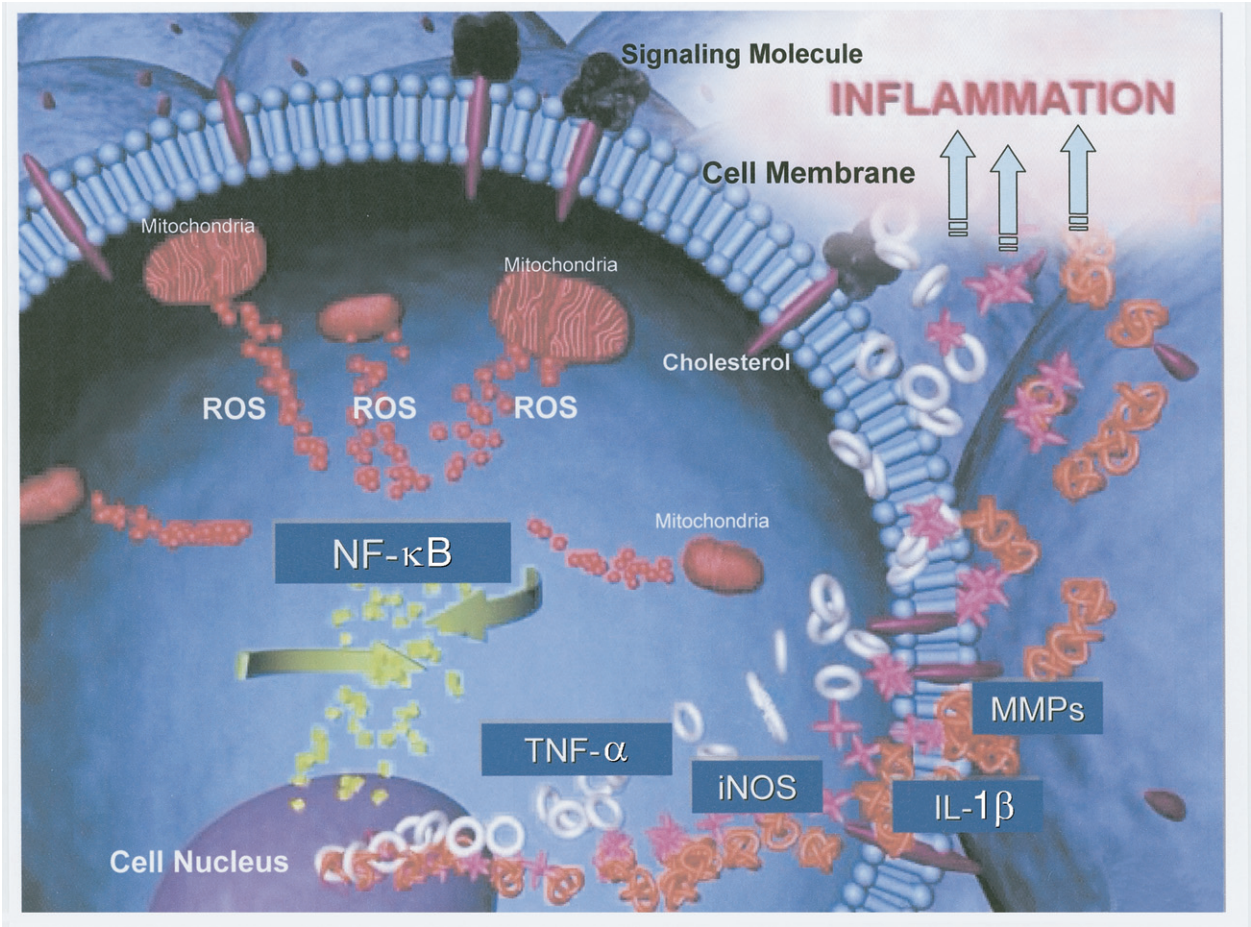

Figure 1. Balanced reactive oxygen species (ROS) environment and normal cellular health. Transcription factors, such as nuclear factor–κB (NF-κB) upregulate the production of downstream inflammatory mediators, including inducible nitric oxide synthase (iNOS), matrix metalloproteinases (MMPs), cyclooxygenase-2 (COX-2), tumor necrosis factor–α (TNF-α), interleukin-1β (IL-1β), and others. During normal cellular function, ROS allow the production of appropriate amounts of signaling molecules to operate normally along with the downstream products of these pathways, creating a balanced ROS environment and normal cellular health. (Courtesy of Cardax Pharmaceuticals, Inc.)

transmission among neighboring cells of ions, small hydrophilic metabolites, and messengers <1–2 kDa in size. GJC plays an important role in normal development and physiology, with a loss of function implicated in various human diseases and pathologies.

In the heart, electrical activation of the myocardium is necessary to produce effective pumping of blood and depends on the orderly coordinated spatial and temporal transfer of current (ions) from one cell to another. In normal ventricular myocytes, this is accomplished through extensive cell–cell coupling via gap junctions. Cells have the capacity to rapidly regulate the function (posttranslational regulation), quantity (transcriptional regulation), and composition (assembly regulation) of connexins within the gap junctional structures to accommodate physiologic demands for altered cell–cell communication. Specifically, connexin 43 is the most ubiquitously expressed connexin in tissues and is essential for normal cardiac function and contraction. Connexin 40, a related protein, is primarily localized in the atria and exhibits altered levels and localization in cardiac tissue after atrial fibrillation. Figure 2A[12] shows a schematic of the hexameric oligomerization of connexins to assemble a connexon (gap junction hemichannel) that when paired with a connexon from an adjoining cell, forms a water-filled channel allowing transfer of ions and molecules via GJC. Figure 2B depicts the transmembrane organization of individual connexin proteins in the plasma membrane.

This communication infrastructure is involved in the pathophysiology of multiple cardiovascular disorders.[13–15] Connexin levels and localization during cardiac tissue remodeling after atrial fibrillation has been a focus of recent interest and thus constitutes a plausible therapeutic target for the treatment of dysrhythmias.[16,17] In mice, genetic depletion of connexin 43 localized in both mitochondrial and plasma membranes abolishes the protection of ischemia and diazoxide-induced preconditioning.

Mechanistically, this tissue protective effect stems from ROS formation in response to the transport of diazoxide to the inner mitochondrial membrane.[18] Mitochondrial con-

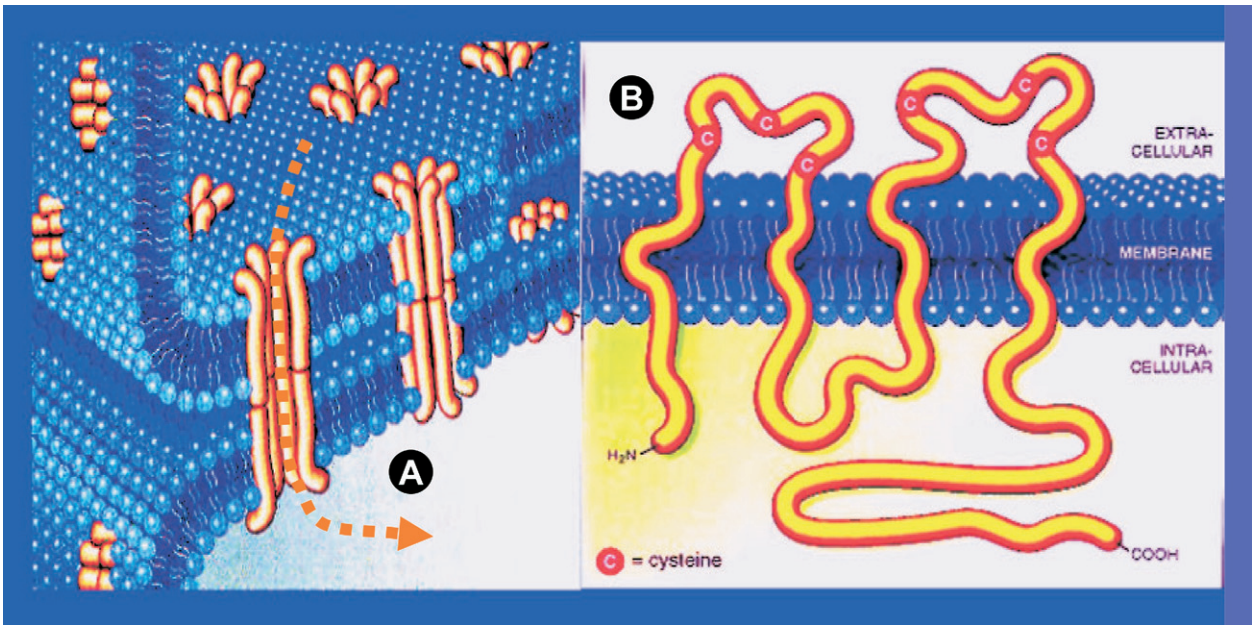

Figure 2. Organization of connexins in the plasma membrane. Connexin proteins organize into connexon cylinders in the plasma membrane. (*A*) Diagrammatic cross-section through an area of cell–cell contact containing gap junctions. Connexin proteins are shown (*yellow*) traversing the phospholipid bilayer in the plasma membrane (*blue*). Each cell contributes 6 connexins to form a cylinder enclosing a central water-filled pore seen in cross section (*foreground*). (*B*) A single connexin molecule traverses the plasma membrane 4 times with both N- and C-terminal ends in the cytoplasm. Connexins assemble to form a connexon by forming 3 sulfhydryl bonds between the highly conserved cysteine residues present in each opposing loop. Thus, each connexon is bound by 18 sulfhydryl bonds to produce a tight seal blocking the entry of extracellular ions, such as Ca^{2+}. (Adapted from *Science and Medicine*.[12])

nexin 43 content is also decreased in aged mouse hearts, and reduced levels of connexin 43 may contribute to the loss of cardioprotection.[19] Several carotenoids have been shown to upregulate connexin 43 protein levels and GJC.[20]

Single Pathway/Enzyme Drug Targeting versus Broader Upstream Therapeutic Approaches

Despite the association among oxidative stress, inflammation, and CVD, no therapies have been successfully verified through randomized clinical trials.[21] Individual "lock and key" therapies targeted to specific pathways and proteins, such as kinases, although mechanistically attractive, may produce off-target side effects or, when the obstruction of a pathway is complete, potentially severe drug-related adverse events. Free radical–scavenging antioxidants may reduce the overall burden of ROS/RNS and a broad upstream downregulation of oxidative stress, but studies examining the pharmacologic potential of antioxidants that show promise in epidemiology and in vivo animal studies have failed in prospective human trials. A better understanding of therapeutic mode of action, uptake, distribution, pharmacokinetics, and metabolism of all proposed antioxidants is a prerequisite to the successful discovery and development of therapeutic agents combating oxidative stress.

Membrane Alignment of Antioxidant Compounds Predicts Activity

Recent work by McNulty et al[22] suggests that conformational differences in membrane alignment of antioxidants may help explain their biologic activity and disparate study results. In particular, the antioxidant, anti-inflammatory activity of these molecules appears, in part, to be a consequence of their precise transmembrane alignment in the lipid bilayer of cellular membranes. In addition to preventing lipid-based oxidation, this alignment provides a variation in the ability of the hydrophilic biophores in the end pieces to be adequately exposed to interact with reactive species in the aqueous environment, potentially facilitating ROS shuttling along the double bonds of the carbon backbone of the compound (Figure 3). The transmembrane alignment of the molecule also likely provides proximity to cofactors, such as vitamin C, which serve as a sink for accepting the radical cations, effectively recharging the electron transfer capacity.[23]

The Xanthophyll Carotenoid Astaxanthin Targets Oxidative Stress

The most effective compound in these membrane positional studies is the xanthophyll carotenoid astaxanthin. A reddish-colored C-40 compound, astaxanthin is a powerful

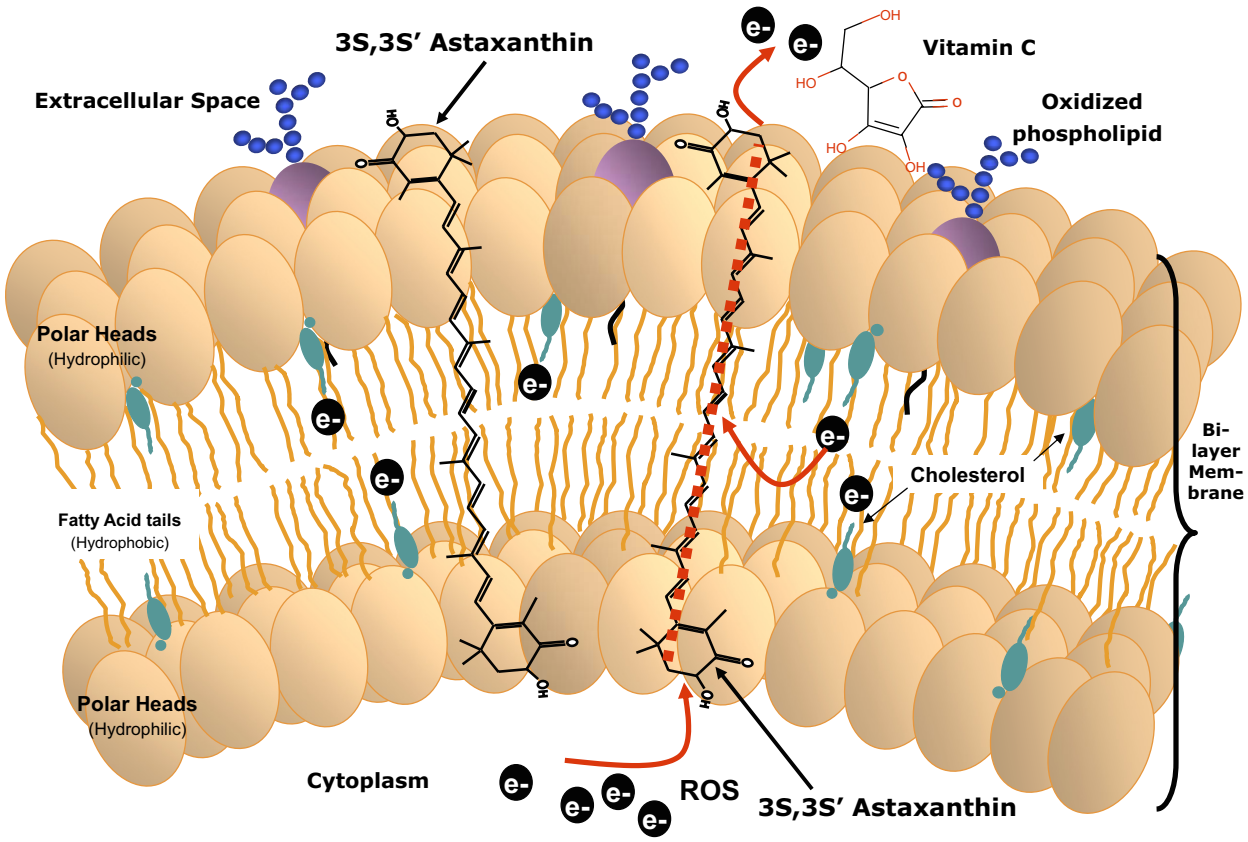

Figure 3. Transmembrane orientation of polar carotenoids facilitates electron shuttling. Specific physicochemical interactions of antioxidant compounds with membranes is likely responsible for their antioxidant properties and their biologic benefits. The transmembranous alignment provides exposure of the polar (hydrophilic) ends of the molecule to the internal cytoplasm and to the aqueous environment external to the cell (or the mitochondrial matrix and the intermembrane space of mitochondria), potentially facilitating electron transfer via the double bonds of the carbon scaffold of the compound. The intramembranous alignment of the molecule also likely provides proximity to vitamin C, which serves as a "sink" for accepting the radical cations generated effectively "recharging" the capacity of the degraded carotenoid. RNS = reactive nitrogen species; ROS = reactive oxygen species.

broad-ranging antioxidant that occurs naturally in a wide variety of living organisms, such as microalgae, fungi, complex plants, and crustaceans.[24] It is a quencher of ROS/RNS single- and 2-electron oxidants as well as a chain-breaking scavenger of free radicals. The potent antioxidant activity of astaxanthin has been observed to modulate biologic functions ranging from lipid peroxidation to tissue protection against light damage.[22,25] A water-dispersible astaxanthin derivative has demonstrated efficacy in both in vitro and in vivo experimental animal models.[26] After an extensive US Food and Drug Administration (FDA) review of its safety profile, a synthetic, racemic form of astaxanthin was approved as an animal food additive in 1987 (Roche Vitamins; DSM, Heerlen, Netherlands). Natural microalga or shrimp shell extracts containing esterified astaxanthin compounds have been approved by the FDA as nutraceuticals.[27] For various biologic, strategic, and business-related reasons, including bioavailability, solubility, and manufacturing standards, among others, neither the synthetic, racemic astaxanthin nor the nutraceutical extracts are likely to be developed or commercialized as pharmaceutical products. In contrast, biocompatible and bioavailable derivatives of astaxanthin are being developed for pharmaceutical applications in human health where oxidative stress-mediated inflammation may be important, including CVD, prostate and other cancers, hepatitis, Alzheimer disease, and age-related macular degeneration.[24]

Safety and Metabolism of the Xanthophyll Carotenoids

In general, xanthophyll carotenoids are highly lipophilic and water insoluble. However, several of the xanthophyll carotenoid derivatives under development demonstrate significantly improved solubility and oral bioavailability. Given orally, these compounds enter the circulation via the lymphatic system, are processed in the liver, and are then distributed to targeted tissues via plasma lipids.[28] Therefore, the strong safety profile of the un-derivatized molecules is both relevant and important.

As part of an FDA feed additive petition filed in 1987, Roche Vitamins selected the xanthophyll carotenoid astaxanthin to conduct both good laboratory practices and non–good laboratory practices safety pharmacology and toxicology studies.[29] Acute and subacute (10-day) toxicology

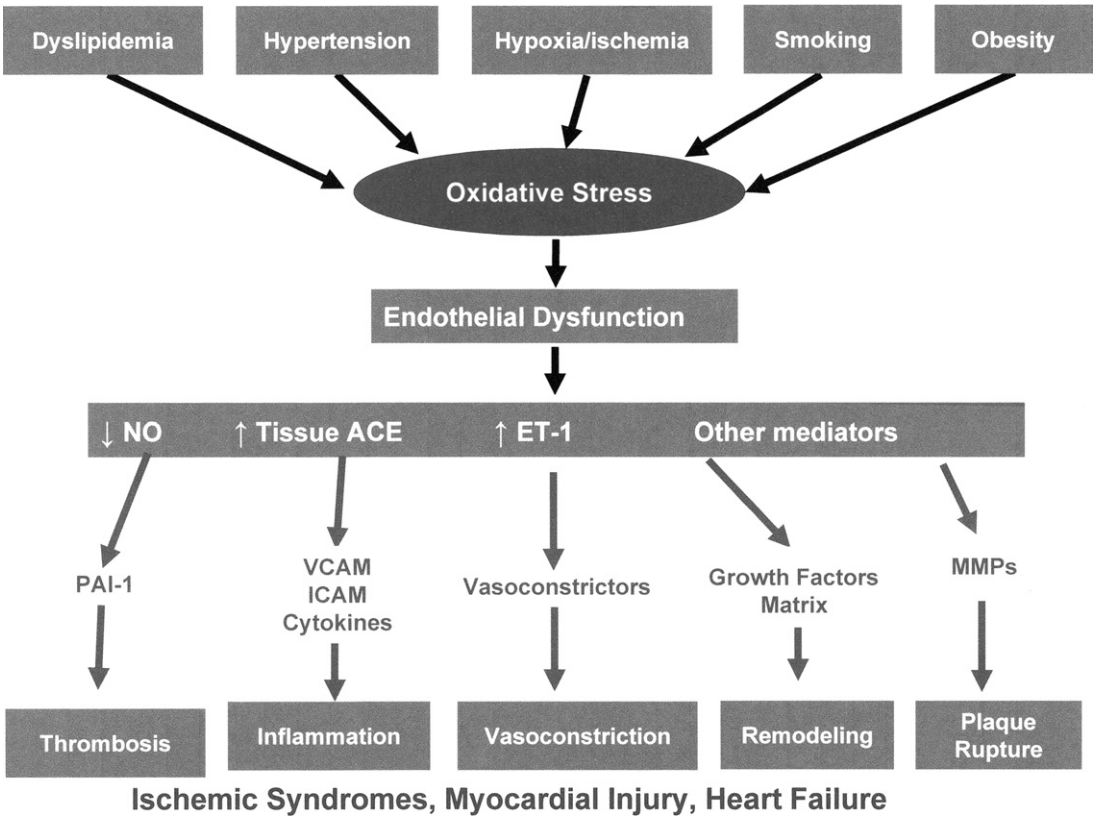

Figure 4. Oxidative stress is the prime, common mediator of endothelial dysfunction. When triggered by risk factors, such as hypertension, dyslipidemia, diabetes mellitus, and obesity, increased oxidative stress results in decreased bioavailability of nitric oxide, increased production of endothelin-1 (ET-1), increased angiotensin-converting enzyme (ACE), and increased local tissue generation of angiotensin II, which in turn, further increases oxidative stress. ICAM = intracellular adhesion molecule; MMPs = matrix metalloproteinases; NO = nitric oxide; PAI-1 = plasminogen activator inhibitor–1; VCAM = vascular cell adhesion molecule. (Adapted from *Circulation*.[35])

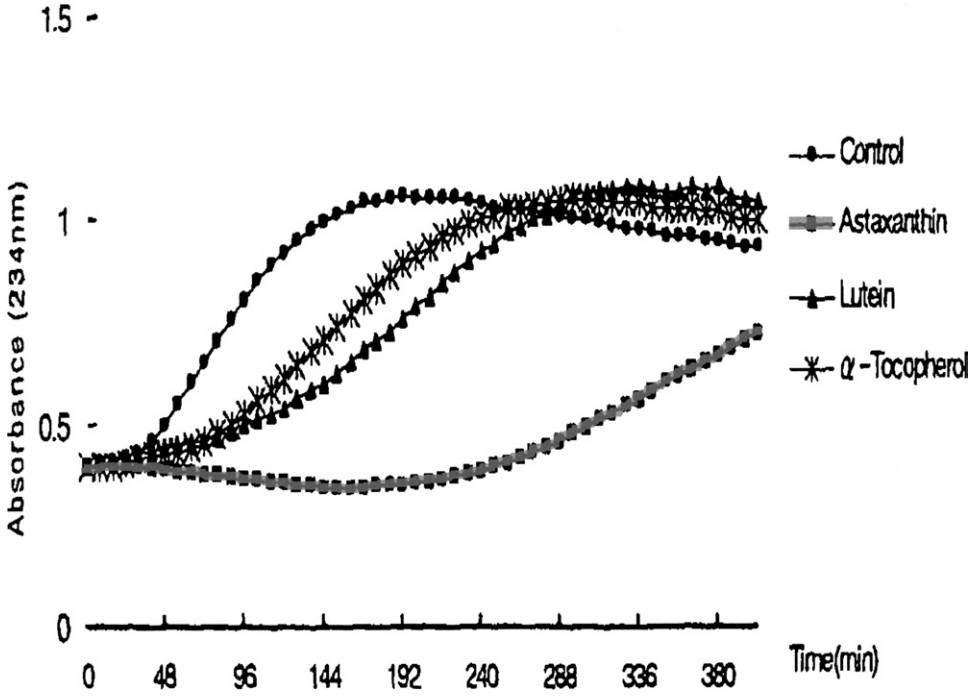

Figure 5. Astaxanthin delays formation of oxidized low-density lipoprotein (LDL). Conjugated dienes are formed in the LDL fraction in the presence of 10 μg/mL of various carotenoids. Oxidation of LDL (35 μg/mL protein) catalyzed by the addition of V-70 (final 200 μmol/L) was monitored continuously by the diene formation at 234 nm. Astaxanthin clearly delays the formation of conjugated dienes as well as the amount of conjugated dienes formed, when compared with active controls, including α-tocopherol. (Reprinted with permission from *J Atheroscler Thromb.*[36])

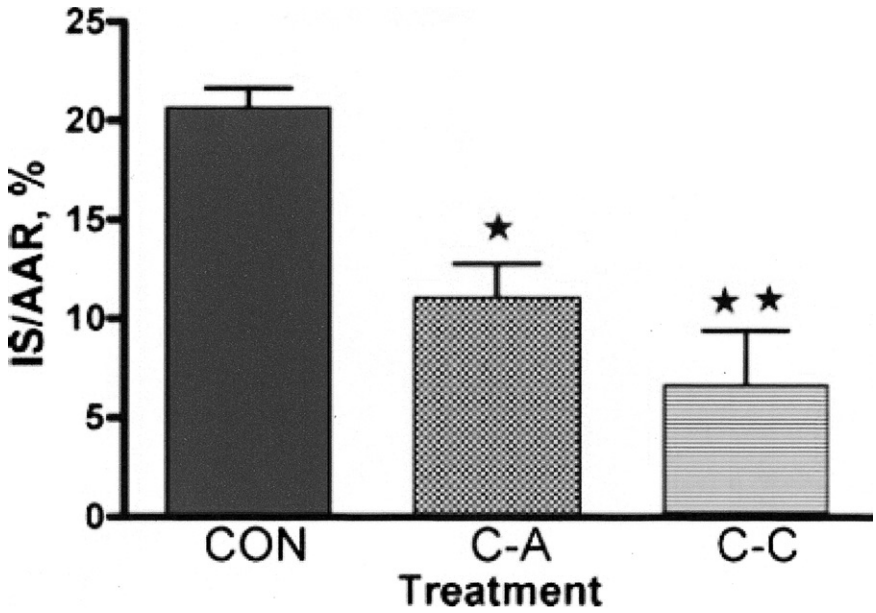

Figure 6. Astaxanthin derivative reduces mean infarct size. Mean infarct size expressed as a percentage of infarct size/area at risk (IS/AAR%). Control animals (CON) received vehicle for 4 days before ischemia and reperfusion. Acutely treated animals (C-A) received 50 mg/kg of astaxanthin disuccinate disodium salt (ADD) 2 hours before ischemia-reperfusion. Chronically treated animals (C-C) received ADD 50 mg/kg for 4 days before ischemia-reperfusion on day 5. ★$p < 0.01$; ★★$p < 0.001$. (Reprinted with permission from *Mol Life Sci*.[38])

studies (oral and parenteral routes) in rats (up to 1.2 g/kg per day for 91 days) and dogs (up to 162 mg/kg per day for 91 days) demonstrated no effects in dogs at the highest dose, with only minor findings in rats. Ames and micronucleus assays also demonstrated no mutagenicity profile at the doses tested. Reproductive toxicology (teratology and embryotoxicity) was evaluated in both rats and rabbits; again, no effects were seen with astaxanthin doses of up to 1,000 mg/kg.[29] Since the approval by the FDA of this feed additive petition in 1987, astaxanthin has been used in the animal feed market (primarily salmon), with no evidence of toxicity. The safety of astaxanthin in humans is supported by published human clinical trials using astaxanthin-containing dietary supplements.[30]

Astaxanthin for the Potential Treatment of Reactive Oxygen Species–Mediated Inflammation and Oxidation in Cardiovascular Disease

Oxidative stress and inflammation play an important role in a number of aspects of CVD, including endothelial dysfunction,[31] functional lipid disorders,[32] periprocedural myocardial damage,[33] and atrial fibrillation.[34]

When triggered by risk factors, such as hypertension, dyslipidemia, diabetes, and obesity, increased oxidative stress results in decreased bioavailability of nitric oxide, increased production of endothelin-1, increased angiotensin-converting enzyme, and increased local tissue generation of angiotensin II, which, in turn, further increases oxidative stress and endothelial dysfunction (Figure 4).[35]

Astaxanthin reduced measurements of LDL oxidation in humans in contrast to other antioxidants, such as vitamin E (α-tocopherol) and lutein, compared with controls (Figure 5).[36] In a separate study conducted by Mason and colleagues,[37] astaxanthin eliminated the lipid peroxidation caused by rofecoxib in cellular membrane models. The ability of astaxanthin to protect LDL from oxidation was further supported by ex vivo analysis of LDL particles from humans fed astaxanthin. In this study, astaxanthin conferred increased resistance to copper-induced oxidation seen as increased LDL oxidation lag times.[36]

Studies of Ischemia-Reperfusion Injury with an Astaxanthin Derivative

Ischemia-reperfusion injury follows the restoration of blood flow to ischemic tissue and is mediated by ROS/RNS. Within the myocardium, this occurs with mechanical or pharmacologic reperfusion in the setting of MI or at the time of flow restoration in coronary artery bypass grafting or heart transplantation. Reperfusion, after a period of ischemia, is thought to result in part from a brisk inflammatory response and the massive production of ROS from various sources, especially neutrophils. Studies in animal models indicate that redox-sensitive transcription factors are activated, including NF-κB and the mitogen-activated protein kinases. In response, there are numerous antioxidant systems produced by the cell that attenuate the damage done by ROS.[4] Many pharmacologic interventions to limit ischemia-

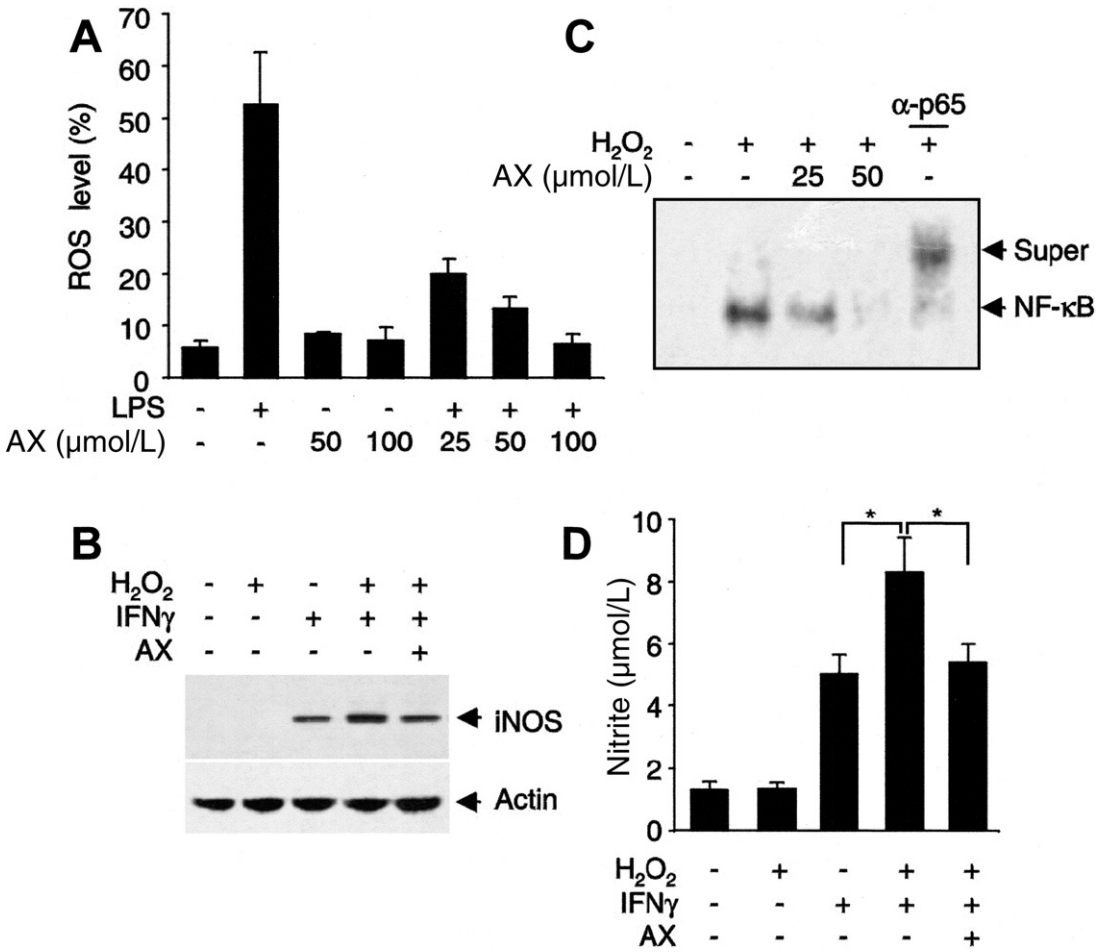

Figure 7. Effect of astaxanthin on reactive oxygen species (ROS) generation, nuclear factor–κB (NF-κB) activation, and inducible nitric oxide synthase (iNOS) expression. (*A*) RAW264.7 cells were treated with lipopolysaccharides (LPS) in the presence or absence of astaxanthin for 30 minutes, incubated with DCFH2-DA (5 μmol/L) for an additional 30 minutes, and intracellular levels of ROS were determined by fluorescence-activated cell sorter (FACS) analysis. (*B*) RAW264.7 cells were treated with 1 μmol/L of hydrogen peroxide (H_2O_2) in the presence or absence of astaxanthin for 2 hours, and NF-κB activity was analyzed by electrophoretic mobility shift assay (EMSA). Supershifting was performed with antibody against the NF-κB p65 subunit (α-p65). (*C*) Peritoneal macrophages were treated with H_2O_2 and/or interferon-γ (10 units/mL) in the presence or absence of astaxanthin (50 μmol/L) for 14 hours, and levels of iNOS were measured by Western blotting. (*D*) Nitrite was measured in the culture medium after 24-hour culture. Data shown are means ± SD (n = 3). *p <0.05. AX = astaxanthin. (Reprinted with permission from *Mol Cells*.[50])

reperfusion injury have been evaluated, and this field continues to be an area of intense research interest.[38,39]

Significant cardioprotection has been demonstrated in multiple animal models (rat, rabbit, dog) of reperfusion-ischemia damage with a derivative, astaxanthin disuccinate disodium salt (ADD). These studies show up to 70% protection from ischemic damage in dogs using standard measures of myocardial salvage.[40]

When evaluated in a rat model of ischemia-reperfusion, parenteral administration of ADD was found to significantly reduce infarct size compared with control animals. In these experiments, rats were treated with 1 of 3 doses of ADD for 5 days and then subjected to 30 minutes of ischemia and 2 hours of reperfusion. The animals that received ADD had smaller infarctions in a dose-dependent manner than control animals. An inverse correlation was observed between infarct size and the concentration of deesterified free astaxanthin in plasma.[41] Later, ADD was evaluated in a canine model of ischemia-reperfusion. In these experiments, ADD was administered parenterally 2 hours before the ischemic challenge, or daily for 5 days before ischemia and compared with control animals. In each case, 1 hour of ischemia was followed by 2 hours of reperfusion. Animals treated for 2 hours had significantly smaller infarcts than the control animals, and the animals treated for 5 days had still smaller infarcts. Again, an inverse correlation between plasma levels of free astaxanthin and tissue salvage was noted (Figure 6).[40]

ADD has been shown to reduce infarct size as well. New Zealand white rabbits received ADD for 4 days before induction of ischemia for 30 minutes followed by 3 hours of reperfusion. When evaluated histologically, animals treated with ADD had significantly smaller infarcts than the control animals. There was also a strong trend of reduced serum troponin levels in the ADD-treated animals. Measurements of tissue levels of ADD revealed the compound accumulates in myocardial tissue. After 4 days of therapy, myocardial

tissue levels were orders of magnitude higher than plasma levels at the time of the ischemia-reperfusion experiment. Immunofluorescence studies indicated the ADD treatment resulted in less deposition of C-reactive protein and membrane attack complex in the ischemic tissue than in the controls, indicating a less robust inflammatory response in these treated animals.[42]

Astaxanthin Reduces Rethrombosis After Vascular Thrombotic Occlusion

Intracellular generation of ROS is also thought to be required for rethrombosis and platelet aggregation.[43] The astaxanthin derivative ADD has demonstrated efficacy in a canine model of carotid artery rethrombosis and platelet aggregation.[44] After forming an occlusive thrombus in the carotid artery, dogs were administered recombinant tissue plasminogen activator intra-arterially to achieve thrombolysis in the presence of either 0.9% sodium chloride solution or ADD (10, 30, or 50 mg/kg via intravenous infusion). The results indicated a dose-dependent reduction in the incidence of carotid artery rethrombosis. In addition, ex vivo platelet aggregation and thrombus weights were dose-dependently inhibited by ADD. No change was recorded in bleeding time among the treatment groups. The data demonstrate that ADD significantly reduces the incidence of secondary arterial thrombosis in a clinically relevant model of thrombolysis, while maintaining normal hemostasis.

Astaxanthin Modifies Oxidative Stress Messengers and Inflammatory Mediators

As discussed earlier, the NF-κB inflammatory pathway has been shown to be at least partially regulated by ROS and has been implicated in various forms of CVD.[45] Higuchi et al[46] have shown that TNF-α–induced cardiomyocyte hypertrophy is mediated through ROS-induced NF-κB activation, with oxidative species serving as a hypertrophic signaling transducer. This result is consistent with the observation of Molkentin and Dom[47] of an emerging paradigm whereby multiple signaling pathways operate together to orchestrate a hypertrophic response. The role of inflammatory mediators in the failing heart is evidenced by immunomodulation of cytokines in experimental models of heart failure.[48] The potential of NF-κB as a therapeutic target for treating cardiac hypertrophy has been suggested because experimental inhibition of NF-κB signaling can attenuate cardiac hypertrophy without sensitizing cardiomyocytes to apoptotic cell death.[49]

Astaxanthin has been shown to inhibit inflammation-induced nitric oxide production and inflammatory gene expression by suppressing I-κB kinase–dependent NF-κB activation.[50] This inhibition of the expression or formation of proinflammatory mediators and cytokines was observed in both a lipopolysaccharide–stimulated macrophage cell line (RAW264.7) and isolated primary macrophages. Figure 7A shows the results of a cell culture experiment quantifying ROS levels in RAW264.7 macrophages treated with lipopolysaccharide in the presence or absence of various astaxanthin concentrations. Astaxanthin inhibited the intracellular accumulation of ROS in lipopolysaccharide–stimulated RAW264.7 cells in a dose-dependent manner, decreased hydrogen peroxide–induced NF-κB activation in RAW264.7 cells (Figure 7B), and lowered expression of inducible nitric oxide synthase in primary macrophages (Figure 7C/D). Moreover, astaxanthin blocked nuclear translocation of the activated NF-κB p65 subunit and I-κBα degradation, which correlated with its inhibitory effect on I-κB kinase activity. These results suggest that astaxanthin, most likely because of its antioxidant activity, inhibits the production of inflammatory mediators by blocking NF-κB pathway activation, possibly resulting from upstream suppression of I-κB kinase activity, leading to decreased I-κBα degradation.[50] In the same study, astaxanthin was also shown to suppress serum levels of nitric oxide, prostaglandin E_2, TNF-α, and interleukin 1-β in lipopolysaccharide-treated mice. A separate study demonstrated the ability of astaxanthin to suppress the development of inflammation in an endotoxin-induced uveitis model.[51] Here, they showed that in vivo astaxanthin (100 mg/kg) was as strong an anti-inflammatory as prednisolone (10 mg/kg), and in RAW264.7 macrophage cells, again decreased nitric oxide production, inducible nitric oxide synthase activity, and prostaglandin E_2 and TNF-α production.

Summary of Astaxanthin Preclinical Studies

Preclinical studies of the xanthophyll carotenoid astaxanthin and its derivatives demonstrate anti-inflammatory properties and potential efficacy in the setting of ischemia-reperfusion, the reduction of lipid peroxidation, and the reduction of rethrombosis after thrombolysis. It remains to be seen if the results of these ex vivo and animal studies can be reproduced in human subjects. The long history of astaxanthin use in the aquaculture industry and the known safety profile of the nutraceutical compound make it an ideal choice for clinical study in settings such as elective percutaneous coronary interventions, acute MI, and coronary artery bypass grafting. Past experiences with the transition from animal models of ischemia-reperfusion to human studies are fraught with disappointment likely because of difficulty in delivering compounds to acutely infarcting tissue and in separating acute injury from irreversibly infarcted tissue. Distribution and localization of astaxanthin in myocardium clearly support further development of the preclinical program.

Conclusion

ROS and RNS are produced as a consequence of normal cellular metabolism. However, under certain conditions, excessive amounts are produced, resulting in increased oxidative stress. Excess ROS/RNS can activate numerous pathways, leading to increased expression of proinflammatory genes and elevated production of proinflammatory cytokines, mediators, and enzymes. The transcriptional activator NF-κB—an important component of one of these inflammatory pathways—directly upregulates the production of inflammatory mediators, including inducible nitric oxide synthase, cyclooxygenase-1 and cyclooxygenase-2, TNF-α, and interleukin 1-β. Although inhibition of this key inflammatory factor may have therapeutic potential, attempts to chronically inhibit NF-κB or other components of this pathway have led to undesirable side effects and/or so-called off-target effects. Elucidation of critical intercellular and intracellular signaling mechanisms can help in the determination of mechanisms through which oxidative stress, inflammation, and pathologic precursors produce potentially clinically relevant cardiovascular dysfunction and subsequent disease.

Evidence suggests that the xanthophyll carotenoids, through their unique physicochemical properties, may be able to dampen or modulate excessive ROS/RNS, with a consequent impact on inflammatory processes in the absence of the negative effects demonstrated with traditional pharmaceutical lock and key interventional strategies. A basic understanding of the mode of action of astaxanthin and its potential role in the regulation of important inflammatory messengers as well as other modes of cellular communication is currently being investigated. Multiple ex vivo and in vivo studies have shown the effectiveness of astaxanthin in several potentially relevant cardiovascular pathologies driven by oxidative stress that results in inflammation. Thus, there may be a potential therapeutic role for astaxanthin derivatives in the management of myocardial injury, oxidized LDL, and rethrombosis after thrombolysis, as well as other cardiac diseases, such as atrial fibrillation. However, the potential effect of the xanthophyll carotenoids, specifically astaxanthin, on signal transduction pathways, cellular homeostasis, and biologic effects as potential clinical targets remains to be clarified.

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Author Disclosures

The authors who contributed to this article have disclosed the following industry relationships:

Fredric J. Pashkow, MD, is a salaried employee, corporate officer, and stockholder in Cardax Pharmaceuticals, Inc., a company involved in the discovery and development of drugs targeted to treat oxidative stress and inflammation. Compounds in development by the company include derivatives of the antioxidant xanthophyll corotenoid family that are discussed in this supplement.

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Charles L. Campbell, MD, is a member of the Speakers' Bureau for Novartis Pharmaceuticals; and serves on the Scientific Advisory Board for Cardax Pharmaceuticals, and Medicure Pharmaceuticals, and sanofi-aventis.

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