

Antioxidant and anti-inflammatory mechanisms of astaxanthin in cardiovascular diseases

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Abstract

Cardiovascular disease is the most common cause of death. Oxidative stress and inflammation are pathophysiological processes involved in the development of cardiovascular diseases, so anti-inflammatory and antioxidant agents that modulate redox balance have become the targets of research to evaluate their molecular mechanisms and therapeutic properties. Astaxanthin, a carotenoid of the xanthophyll group, has potent antioxidant effects due to its molecular structure and its arrangement in the plasma membrane, factors that favor the neutralization of reactive oxygen and nitrogen species. This carotenoid also stands out for its anti-inflammatory activity, possibly interrelated with its antioxidant effect, as well as for its modulation of lipid and glucose metabolism. Considering the potential positive effects of astaxanthin on cardiovascular health evidenced by preclinical and clinical studies, this paper describes the molecular and cellular mechanisms related to the antioxidant and anti-inflammatory properties of this carotenoid in cardiovascular diseases, especially atherosclerosis.

Keywords: astaxanthin, cardiovascular disease, atherosclerosis, inflammation, oxidative stress, carotenoids, antioxidant

Introduction

According to data from the World Health Organization, cardiovascular diseases accounted for 31% of all causes of death in 2016, and they are considered the leading cause of mortality worldwide [1]. One of the risk factors for cardiovascular diseases is metabolic syndrome, a multifactorial complication that includes variables such as obesity, dyslipidemia, hypertension, and glucose metabolism dysfunctions [2]. Evidence connects these variables to increased oxidative stress, and mitochondrial dysfunction, activation of enzymes that produce reactive oxygen and nitrogen species (RONS), and impairment of the activity of antioxidant systems are the main triggering mechanisms [2-4].

Because oxidative stress and inflammation are interrelated and contribute to the initial events of cardiovascular diseases, antioxidants that modulate redox balance, such as astaxanthin, can be considered important regulators of inflammatory responses [5] and have become the focus of research to evaluate whether and how they prevent these diseases. Astaxanthin is closely linked to other well-known carotenoids, such as beta-carotene, lutein, and zeaxanthin, sharing various of the physiological and metabolic functions attributed to these compounds [6]. The two oxygenated groups, hydroxyl (OH) and carbonyl (C=O), in each of its ionone rings explains some of its unique characteristics, such as its more potent antioxidant activity and polar configuration when compared to other carotenoids [6-8]. In addition to its antioxidant action, astaxanthin has anti-inflammatory properties and the ability to modulate lipid and glucose metabolism [6, 7, 9], which promote significant benefits in the cardiovascular system, preventing disorders such as atherosclerosis, arterial hypertension, and dyslipidemia [10-20]. Given the extensive evidence supporting its health-promoting properties and safety, astaxanthin was approved as a nutraceutical by the United States Food and Drug Administration in 1999 [21].

Considering the safety of using astaxanthin as a nutraceutical and the scientific evidence of its beneficial effects on cardiovascular physiology, this review describes the molecular and cellular mechanisms involved in the antioxidant and anti-inflammatory action of astaxanthin in the prevention of cardiovascular diseases.

Mechanisms of action of astaxanthin

Antioxidant effect

Cell membrane systems are particularly vulnerable to RONS attacks due to their content of polyunsaturated fatty acids (PUFAs) and their metabolic activities, which endogenously generate other oxidizing metabolites [22].

Astaxanthin provides cell membranes with protection against RONS and oxidative damage. Given its chemical structure, its polar groups overlap the polar regions of the cell membrane, while the central nonpolar region of the molecule fits into the inner nonpolar region of the membrane. Thus, this carotenoid can take on a transmembrane alignment in biological membranes, allowing it to help maintain the membrane structure, decrease membrane fluidity, and act as an antioxidant [17, 23, 24].

Astaxanthin scavenges RONS and other reactive species (sulfur and carbon) directly, both by donating electrons and by bonding with the free radical to form a nonreactive product [25]. In addition, the presence of a series of conjugated bonds in the central nonpolar region of astaxanthin gives the molecule another antioxidant dimension, i.e., the ability to remove free radicals (high-energy electrons) from the cell interior by transporting them along its own carbon chain, as if it were a “lightning rod” for these electrons, so that these are neutralized by other antioxidants located outside the cell membrane, such as vitamin C [25].

The increased susceptibility of membrane lipids and low-density lipoprotein (LDL) to oxidation may trigger the formation of thrombi and the development of atherosclerosis [5]. One of the reactive species that induces lipid peroxidation and LDL oxidation is peroxynitrite (ONOO^-) [50, 51], which when neutralized by astaxanthin forms 15-nitroastaxanthin, a compound that also has important antioxidant action [52].

The LDL oxidation time in the presence of astaxanthin has been analyzed *in vitro* and *ex vivo*. In the *in vitro* assays, astaxanthin prolonged LDL oxidation in a dose-dependent manner, in addition to being more effective at this function than lutein and α -tocopherol. In turn, the blood samples of individuals who were supplemented daily with 1.8 mg, 3.6 mg, 14.4 mg, or 21.6 mg of astaxanthin for 14 days evidenced a significant delay in LDL oxidation when compared to samples taken before supplementation, the greatest effect being obtained with the dose of 14.4 mg (oxidation time increased by 5.0, 26.2, 42.3, and 30.7% under the doses of 1.8, 3.6, 14.4n and 21.6 mg, respectively) (Table 1) [10]. The differences in oxidation time exhibited by the different groups were not addressed by the authors. Thus, this study showed that the intake of astaxanthin prevents LDL oxidation, one of the factors involved in the process of atherosclerosis.

LDL oxidation is also associated with the development of endothelial dysfunction in patients with diabetes mellitus, which raises the risk of cardiovascular complications [26]. Endothelial dysfunction consists of impaired vessel relaxation dependent on endothelial factors, such as the production of nitric oxide (NO) by endothelial nitric oxide synthase (eNOS) [27]. One of the pathways responsible for this type of dysfunction is the binding of oxidized LDL with its endothelial receptor, lectin-like ox-LDL receptor 1 (LOX-1), thus favoring oxidative stress, which leads to increased lipid peroxidation and eNOS inactivation [28]. Supplementation with 10 mg/kg astaxanthin for 42 days in a diabetic rat model increased artery relaxation by significantly lowering oxidized LDL, LOX-1 receptor, and lipid peroxidation in the aorta, in addition to increasing eNOS, demonstrating that astaxanthin may have therapeutic potential for the treatment of endothelial dysfunction in diabetic patients [29].

Erythrocytes have a large amount of PUFAs and high concentrations of oxygen and ferrous ions (Fe^{2+}), which makes these cells more susceptible to oxidative changes in the lipid bilayer, leading to compromised cell stability, oxygen transport, and blood rheology [30]. The oxidation of erythrocytes is associated with both the formation of atheromas and the occurrence of intraplate hemorrhage during the development of atherosclerosis [31]. Regarding lipid peroxidation in erythrocytes, daily supplementation with 6 mg or 12 mg of astaxanthin for 12 weeks in healthy individuals showed that this carotenoid is incorporated and distributed into these blood cells. When incorporated into erythrocytes, astaxanthin exhibited antioxidant effects in the cell membrane by significantly reducing the levels of phospholipid hydroperoxides (PLOOH), which are the primary products of phospholipid oxidation (14.9 pmol/ml for the placebo group and 8.0 and 9.7 pmol/ml for the 6 and 12 mg astaxanthin groups, respectively). In that study, the two doses of astaxanthin used had similar effects when compared to placebo, suggesting that the intake of 6 mg of this carotenoid is enough to inhibit oxidative stress in erythrocytes (Table 1) [12]. A significant reduction in oxidative damage by lipid peroxidation was also observed, as the plasma levels of 12- and 15-hydroxy fatty acids ($p = 0.048$ and $p = 0.047$, respectively) decreased after three months of supplementation with 8 mg of astaxanthin in healthy men (Table 1) [13].

Blood rheology is important to cardiovascular homeostasis. Astaxanthin has reduced blood transit time in a hypertensive rat model [32] and in humans [18]. In the latter study, individuals supplemented with 6 mg of astaxanthin for ten days had significantly faster blood transit time than they did before supplementation and than the placebo group (47.6 ± 4.2 s vs. 54.2 ± 6.7 s for the treated and placebo groups, respectively; $p < 0.05$) (Table 1) [18]. One hypothesis for the improvement of blood rheology by astaxanthin is its antioxidant effect in the intra- and extracellular environment and the consequent increase in the flexibility of the erythrocyte membrane, conferred by the structural arrangement of astaxanthin in the membrane.

Other factors that affect the blood flow velocity, such as plasma viscosity and vasodilation, influence peripheral vascular resistance and may contribute to hypertension and its main cardiac complication, myocardial hypertrophy [33]. Studies of spontaneously hypertensive rats (SHR) reported that astaxanthin supplementation significantly reduced systolic pressure and induced significant histological changes in the aorta associated with decreased vascular stiffness and blood pressure [34-36]. This response was caused by increased endothelial cell-dependent vasodilation due to the greater bioavailability of NO, as well as the remodeling of the arteries. The increase in NO was caused by the reduced production of superoxide anion radicals released by NADPH oxidase, one of the antioxidant effects of astaxanthin [34, 35]. Chen et al. (2020) showed that this carotenoid also acted in the remodeling of the smooth muscle cells of the vessels, reducing their proliferation and the damage caused by oxidative stress. Astaxanthin lowered RONS by increasing the activity of antioxidant enzymes and regulating mitochondrial dynamics, mitophagy, and mitochondrial biogenesis, which are important events for the maintenance of mitochondrial and cellular metabolism [36].

The high bioavailability of NO due to lower oxidative stress promoted by astaxanthin was also associated with anti-thrombogenic effects. In a stroke-prone SHR model, astaxanthin significantly downregulated the oxidative stress marker 8-hydroxy-2'-deoxyguanosine (8-OHdG) in the urine, lowered systolic blood pressure, and lessened thrombogenesis in the cerebral veins. The observed antithrombogenic effect may have been due to vasodilation and inhibition of platelet aggregation caused by an increased bioavailability of NO [37]. In a murine model of thrombosis treated with astaxanthin in the form of the CDX-085 prodrug, an increase of approximately 20% in the blood flow of the carotid artery was observed before the occurrence of endothelial dysfunction, as was a delay in the formation of occlusive thrombi. These results were due to the increase in NO and the decrease in ONOO⁻. The authors suggested that the increase in blood flow was due to vasodilation caused by increased release of NO by endothelial cells and reduced platelet activation triggered by the antioxidant effect [38].

Some of the vascular benefits promoted by the antioxidant effect of astaxanthin were reported in an open study of 20 postmenopausal women who had a high rate of oxidative stress [14]. After eight weeks of supplementation with 12 mg of astaxanthin, there was a significant reduction of 4.64% and 6.93% in the systolic and diastolic pressure values, respectively, possibly resulting from the reduction in the vascular tone due to the action of the carotenoid in the endothelium. Reduced vascular resistance in the lower limbs (3.7% increase in the ankle-brachial pressure index), a 4.58% increase in antioxidant capacity, and improvement of some physical and mental symptoms, such as tired eye sensation and difficulty sleeping, were also observed [14].

In addition to the protective action of astaxanthin in the lipid oxidation process, this carotenoid influences the activity of antioxidant enzymes involved in lipid metabolism, such as thioredoxin reductase (TrxR) and paraoxonase-1. TrxR is an antioxidant enzyme involved in the reduction of thioredoxin, lipid hydroperoxides, and hydrogen peroxide [39]. One study showed that thioredoxin in its oxidized form was associated with the degree of severity of chronic heart failure and with the oxidative stress resulting from this pathology [40]. Paraoxonase-1 is an enzyme that binds to serum high-density lipoprotein (HDL) and is responsible for protecting both LDL and HDL from oxidation processes, as well as for breaking down oxidized lipids [41]. The effect of astaxanthin on these two enzymes was evaluated in rabbits fed a diet rich in cholesterol [42]. The authors found that astaxanthin (100 and 500 mg/100 g of feed) reduced the amount of oxidized protein, possibly due to changes in the activities of TrxR and paraoxonase-1, but the *in vitro* evaluation showed only the direct action of this molecule on TrxR [42].

Once again demonstrating the antioxidant effects of astaxanthin, Choi et al. [11, 16] conducted two randomized and double-blind clinical studies with overweight or obese individuals (Table 1). In one study, volunteers supplemented with 5 mg and 20 mg of astaxanthin for three weeks showed lower oxidative stress biomarkers associated with lipid peroxidation than before treatment, with 34.6% and 35.2% reductions in malondialdehyde (MDA) levels and 64.9% and 64.7% in the isoprostane (ISP) levels, respectively. An increase in the activity of the antioxidant defense system was also observed,

with 193% and 194% increases in superoxide dismutase (SOD) and 121% and 125% in total antioxidant capacity at the doses of 5 mg and 20 mg, respectively, compared to the data before treatment [16]. In that study, no significant differences were observed between the results obtained under the two doses, showing that the clinical effects of this carotenoid are not proportional to the dose. Later, the same authors analyzed lipid profile, oxidative stress, and antioxidant system parameters [11]. After 12 weeks of supplementation with 20 mg of astaxanthin, the same results were observed in oxidative stress and the antioxidant system as in the previous study. Regarding the lipid profile, there was a significant reduction of 10.4% in the LDL concentration, 7.59% in ApoB, and 8.22% in the ApoA1/ApoB ratio (considered an index of the risk of heart attack) compared to the placebo group values [11]. Therefore, these studies show that astaxanthin reduces oxidative stress and modulates the lipid profile in overweight and obese individuals, mitigating the risk of developing cardiovascular diseases.

Astaxanthin also has potent detoxifying and antioxidant effects in smokers [15]. The free radicals induced by smoking have been strongly associated with increased oxidative stress, contributing to the increased susceptibility of smokers to the pathogenesis of cardiovascular diseases. This group of individuals requires a higher daily intake of antioxidants than nonsmokers to reduce the consequences of prolonged exposure to toxins present in cigarettes. After three weeks, supplementation with different doses of astaxanthin (5 mg, 20 mg, and 40 mg) in active smokers prevented oxidative damage by suppressing lipid peroxidation and stimulating the activity of the antioxidant system (Table 1) [15]. This effect was confirmed by the significant reductions in serum MDA and ISP and increased SOD and total antioxidant capacity in the three astaxanthin groups compared to the indices before treatment [15]. The authors also observed that the serum concentration of astaxanthin in the groups treated with 20 mg and 40 mg was similar, showing that there was saturation of its absorption and that smaller doses, such as 5 mg, may have the necessary antioxidant effect for these individuals. However, placebo-controlled studies with larger groups and longer interventions may help determine the best dosage for smokers.

Several preclinical studies have shown that astaxanthin also exerts an indirect antioxidant effect by activating transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), increasing the expression of its antioxidant target genes, such as phase II biotransformation enzymes [43-49]. A study with a model of coronary microembolization in rats showed that supplementation with astaxanthin drastically attenuated the induction of cardiac dysfunction, myocardial infarction, and cardiomyocyte apoptosis, which was associated with the suppression of oxidative stress via activation of Nrf2/HO-1 signaling [44].

Thus, astaxanthin can accumulate in the blood plasma and, by its antioxidant action, help reduce the levels of RONS responsible for LDL oxidation and lipid peroxidation; increase the bioavailability of NO, enabling its vasodilator and anti-thrombogenic effects; increase the activity of antioxidant enzymes; and ensure the stability of blood rheological properties, thus avoiding the loss of erythrocyte flexibility and the increase in plasma viscosity, factors that influence the blood flow

velocity. These actions of astaxanthin against early events of atherosclerotic plaque formation and arterial dysfunction can slow the progression of cardiovascular diseases (Figure 1).

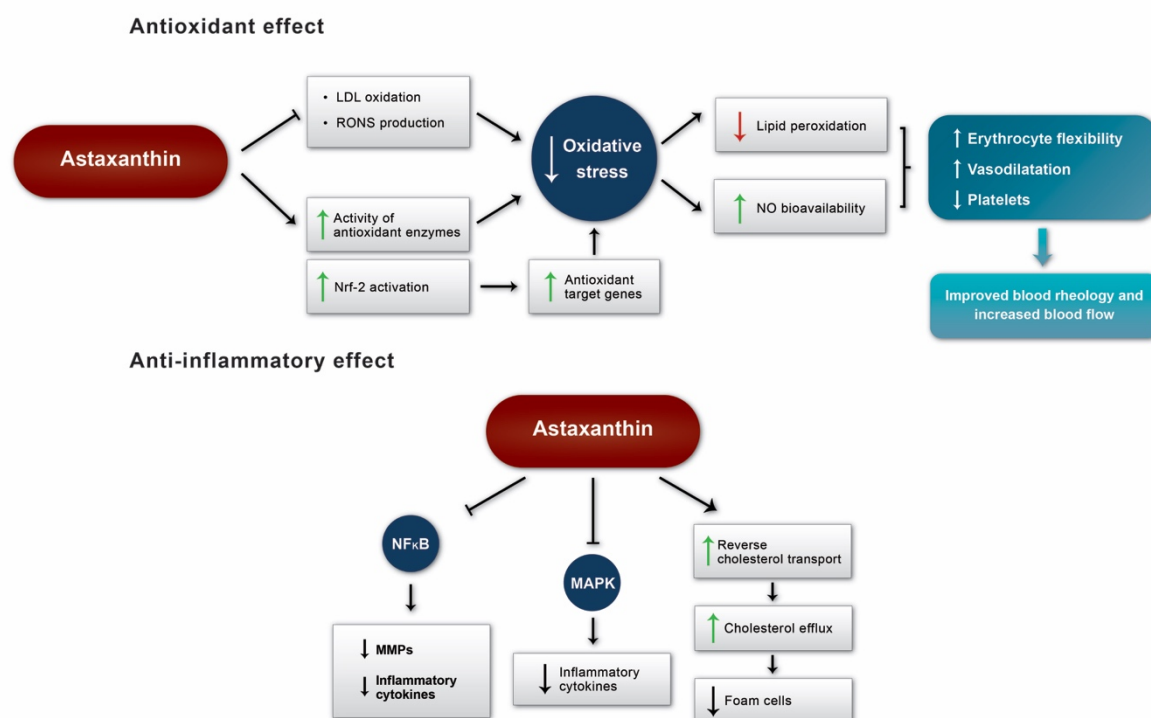


Figure 1. Scheme of the antioxidant and anti-inflammatory mechanisms of astaxanthin in cardiovascular diseases.

Anti-inflammatory effect

Inflammation plays an important role in the development of cardiovascular diseases and other comorbidities, such as hypertension, hypercholesterolemia, type 2 diabetes, chronic kidney disease, and obesity [50]. Astaxanthin exerts a marked anti-inflammatory effect, which may be interrelated with its antioxidant effect and contributes to physiological changes that benefit cardiovascular function (Figure 1) [50-55].

Atherosclerosis is a degenerative and chronic disease that affects large- and medium-caliber arteries. Atherogenesis, the initial phase of the atherosclerotic process, is characterized by the accumulation of LDL in the subendothelial layer of the vascular wall, which is responsible for inflammation mediated by the innate and adaptive immune responses [56]. The anti-inflammatory effects promoted by astaxanthin are evidenced in its role in atherosclerosis prevention, as will be detailed below.

The epitopes generated from enzymatic or nonenzymatic oxidation of LDL are the main damage-associated molecular patterns (DAMPs) recognized by macrophages and are responsible for the onset of the inflammatory cascade, with the release of cytokines and chemokines that recruit more resident vascular macrophages and monocytes from the blood. Macrophages bind to oxidized LDL via scavenger receptors, such as SR-A, SR-B2 (CD36), and LOX-1 [56]. The expression of these receptors is controlled by nuclear factor- κ B (NF- κ B), the main mediator of the inflammatory response, which is activated by pattern recognition receptors (PRRs) and pro-inflammatory cytokines [57]. In inflammatory states, macrophages produce excessive amounts of pro-inflammatory mediators, such as cytokines, chemokines, NO, cyclooxygenase-2 (COX-2), and matrix metalloproteinases (MMPs). MMPs are responsible for the degradation of most extracellular matrix proteins and mediate the tissue remodeling associated with atherosclerosis [5].

In vitro and *in vivo* studies have evaluated the effect of astaxanthin on the formation of atherosclerotic plaques [51-55, 58-79]. Supplementation with 10 μ M astaxanthin significantly reduced the expression of the SR-A and CD36 scavenger receptors in the THP-1 macrophage line and reduced the total activity of MMPs, represented by reduced protein expression of MMP-9 and MMP-2 and of the mRNA levels of five MMPs [59]. Astaxanthin at this concentration also reduced the gene expression of pro-inflammatory markers such as interleukin (IL)-1 β , IL-6, tumor necrosis factor (TNF)- α , inducible nitric oxide synthase (iNOS), and COX-2 [59]. These results corroborate other studies that indicated that astaxanthin reduces the expression of pro-inflammatory mediators in macrophages [59-62] and in other cell types, such as microglia, endothelial vascular cells, and human neutrophils [63-66].

The significant decreases in MMPs and proinflammatory cytokines may have resulted from the suppression of the NF- κ B transcription factor by astaxanthin [51-55, 59, 61, 65, 67, 68]. NF- κ B is frequently activated at inflammation sites associated with various pathologies, especially cardiovascular diseases, in which the increased expression of its pro-inflammatory target genes plays a critical role in the etiology of these diseases [69]. The inflammatory pathway of NF- κ B is, at least in part, regulated by oxidative stress [69]. Astaxanthin inhibits the activity of I κ B kinase (IKK), a complex responsible for the control of NF- κ B activation. This keeps NF- κ B inactive in the cell cytoplasm, so its pro-inflammatory target genes, such as TNF- α , IL-1 β , and iNOS, are downregulated [61].

Cholesterol uptake is balanced by the transfer of this molecule from macrophages to free apolipoproteins A1 or to HDL, the latter being responsible for the reverse cholesterol transport process. When cholesterol uptake exceeds cholesterol efflux in macrophages, lipid droplets accumulate in the cytoplasm, forming foam cells, the main markers of atherosclerosis (Figure 2) [56]. The progression of cholesterol accumulation can lead to its precipitation in the form of crystals, which activate the inflammasome, leading to cell death by apoptosis or necrosis [80]. The atherosclerotic plaque is

separated from the bloodstream by a fibrous layer, which upon rupture initiates intraluminal thrombosis, the initial event of stroke and other coronary syndromes [56].

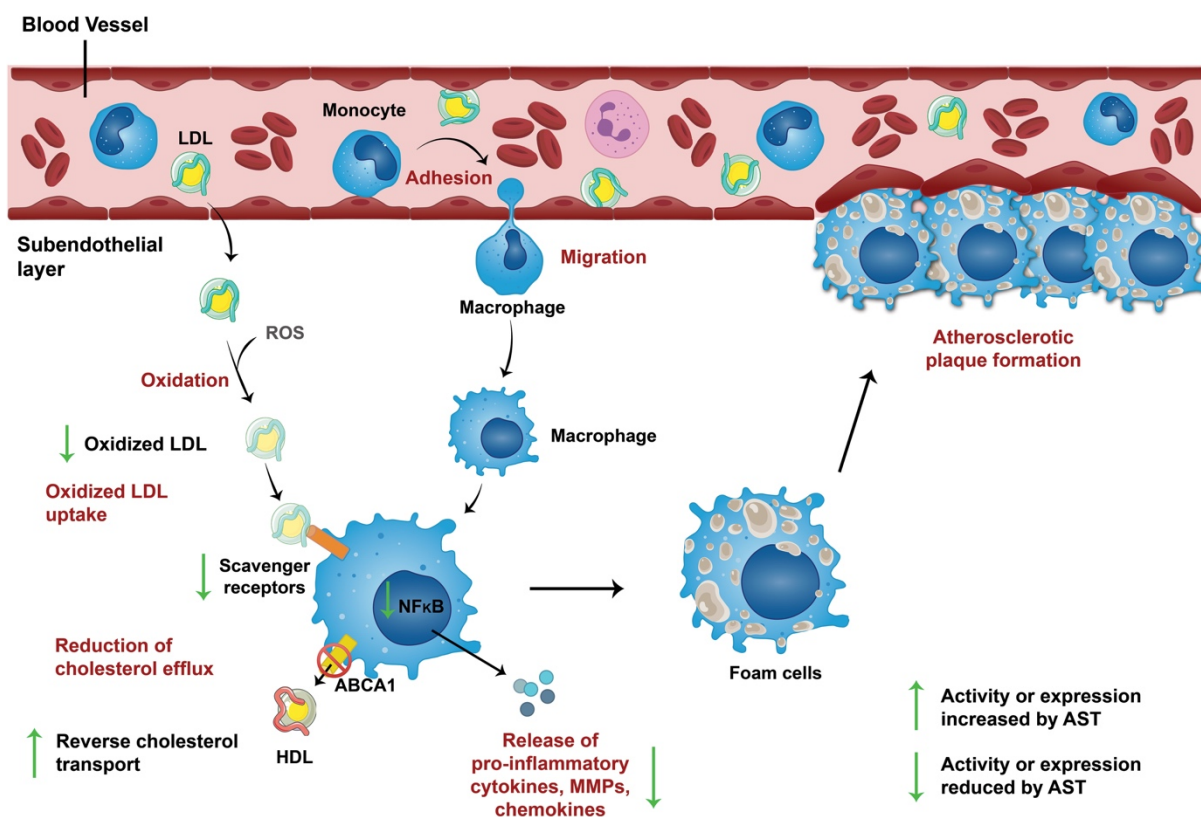


Figure 2. Mechanism of atherosclerotic plaque formation in the subendothelial layer of the vascular wall and the action of astaxanthin. AST = astaxanthin (adapted from [5]).

Reverse cholesterol transport consists in HDL removing excess cholesterol from peripheral tissues and transporting it to the liver, where it is degraded by bile juice and excreted in the feces, thus preventing the accumulation of cholesterol in macrophages [76]. Both the liver and intestine synthesize apolipoprotein A-I (apoA-I) and apoA-II in plasma, which incorporate free cholesterol and phospholipids through the ATP-binding cassette A1 (ABCA1) hepatic transporter, originating from nascent HDL. In peripheral tissues, nascent HDL molecules recruit free cholesterol from foam cells via the macrophage ABCA1 transporter [77]. It is noteworthy that this reverse cholesterol transport was also observed in the lymphatic system, which is responsible for much of the removal of cholesterol from different tissues [76]. Finally, mature HDL can transport cholesterol directly to the liver via the SR-B1 scavenger receptor or can transfer cholesteryl esters to (very) low-density lipoproteins [(V)LDL] through the CETP (cholesteryl ester transfer protein) [78]. These lipoproteins are absorbed in the liver by their specific receptors, which is probably the predominant pathway in humans. Once in the liver, cholesterol is secreted into the bile via the ABCG5 and ABCG8 transporters. Some of these molecules

can be reabsorbed by the intestine and reach the bloodstream again, while the rest is excreted in the feces [77].

In atherosclerosis, apolipoproteins are oxidized by the enzyme myeloperoxidase, which is expressed in macrophages during the inflammatory process, compromising cholesterol efflux via ABCA1 [78]. In individuals with heart disease, elevated levels of apoA-I modified by myeloperoxidase were identified, and their HDL molecules were dysfunctional in performing reverse cholesterol transport. In addition, an association was observed between increased cholesterol efflux via ABCA1 and reduced risk of cardiovascular diseases (OR: 0.30; 95% CI, 0.14-0.66; $p < 0.003$) [79]. Thus, the oxidation of apolipoproteins by macrophage myeloperoxidase is a determining factor in HDL dysfunction in cholesterol transport and therefore in the risk of cardiovascular diseases.

The effects of astaxanthin on reverse cholesterol transport have been demonstrated *in vivo*. In wild-type and ApoE^{-/-} mice, astaxanthin increased cholesterol efflux from peripheral tissues to the liver and its subsequent excretion in feces [58]. In addition, in the ApoE^{-/-} model, this carotenoid promoted a significant decline in plasma total cholesterol, triglycerides, and non-HDL cholesterol and reduced the atherosclerotic plaque area of the aortic sinus and the cholesterol concentration in the aorta compared to controls. Thus, astaxanthin exerts anti-atherosclerotic effects by increasing the reverse cholesterol transport pathway, but the molecular mechanisms of this action are still unknown [58].

In addition to acting in reverse cholesterol transport, astaxanthin acts in some lipid metabolism steps, a finding corroborated by a randomized, placebo-controlled clinical study of 61 adult individuals with moderate hyperlipidemia [20] (Table 1). In that study, daily supplementation with 6, 12, or 18 mg of astaxanthin for 12 weeks promoted an improvement in the lipid profile of the patients. Triglycerides were reduced 25.2% and 23.8% ($p < 0.05$) by doses of 12 and 18 mg, respectively. HDL was increased 10.6% ($p < 0.05$) and 15.4% ($p < 0.01$) by doses of 6 and 12 mg, respectively. The doses of 12 and 18 mg also significantly increased serum adiponectin ($p < 0.01$ and $p < 0.05$, respectively), a protein secreted by adipocytes with important functions in the cardiovascular and endocrine systems associated with its anti-inflammatory, atheroprotective, and insulin-sensitizing actions [20].

Inflammation is also involved in the pathophysiology of metabolic syndrome, a multifactorial disorder associated with glucose and lipid metabolism disorders. This disease has risk factors that are also strongly associated with the development of cardiovascular complications, including type 2 diabetes, dyslipidemia, hypertension, and abdominal fat deposition [81]. In this context, astaxanthin has been promising in the improvement of glucose and lipid metabolism in a randomized, placebo-controlled clinical study with 43 diabetic patients aged between 46 and 62 years [19] (Table 1). In agreement with a previous clinical study [20], supplementation with astaxanthin (8 mg/day for 8 weeks) significantly increased serum adiponectin ($47 \pm 14 \mu\text{g/mL}$ vs. $45 \pm 13 \mu\text{g/mL}$ and $36 \pm 15 \mu\text{g/mL}$ compared to placebo and baseline, respectively; $p < 0.05$) and improved the lipid profile of patients, as shown by the reductions in triglycerides ($128 \pm 52 \text{ mg/dL}$ vs. $150 \pm 85 \text{ mg/dL}$ and $156 \pm 90 \text{ mg/dL}$ compared to placebo and baseline, respectively; $p < 0.05$) and VLDL ($27 \pm 16 \text{ mg/dL}$ vs. $31 \pm 16 \text{ mg/dL}$

compared to placebo; $p < 0.05$). Furthermore, astaxanthin marginally reduced fasting glucose (8.3 ± 2.7 mmol/L vs. 9.4 ± 3.2 mmol/L compared to placebo; $p = 0.057$) and significantly increased serum fructosamine (5.8 ± 3.8 μ mol/L vs. 7.32 ± 4.31 μ mol/L and 7.36 ± 4.2 μ mol/L compared to placebo and baseline, respectively; $p < 0.05$), an important marker in the control of diabetes that signals the mean concentration of blood glucose. Patients supplemented with astaxanthin also showed lower visceral fat deposition ($11.2 \pm 3.4\%$ vs. $11.85 \pm 3.8\%$ compared to placebo; $p < 0.05$) and systolic blood pressure (132 ± 18 mmHg vs. 133 ± 19 mmHg and 143 ± 27 mmHg compared to placebo and baseline, respectively; $p < 0.05$) [19].

Disorders characterized by ischemia/reperfusion, including myocardial infarction, stroke, and peripheral vascular disease, are among the most frequent causes of morbidity and mortality worldwide [81]. Ischemia/reperfusion is a complex inflammatory process associated with high levels of oxidative stress in the affected tissue [82]. In rodents with hepatic lesions induced by ischemia/reperfusion, astaxanthin not only reduced oxidative stress and histopathological damage [83, 84] but also exerted a significant anti-inflammatory effect, attenuating the release of inflammatory cytokines through the mitogen-activated protein kinase (MAPK) pathway [84, 85]. Furthermore, astaxanthin promoted an anti-inflammatory and antioxidant effect in the context of myocardial injury due to ischemia/reperfusion in rabbits by significantly reducing the activation of the complement system associated with the reduced deposition of C-reactive protein and the membrane attack complex in the injured area of the myocardium [86].

In mice with nonalcoholic steatohepatitis (NASH) induced by a hyperlipidic diet, supplementation with astaxanthin (0.02% in the diet, approximately 20 mg/kg body weight) significantly improved several liver parameters: It reduced liver inflammation, decreased the proportion of pro-inflammatory M1-type macrophages, reduced stellate cells activation, and attenuated liver fibrosis, the accumulation and peroxidation of hepatic lipids, and insulin resistance [87]. Additionally, astaxanthin was more effective in preventing and treating NASH and improving liver inflammation and fibrosis than vitamin E (standard NASH treatment). The same study also corroborated the potential of astaxanthin to improve NASH in 12 individuals supplemented with this carotenoid (12 mg/day; control: placebo) for 24 weeks [87].

The anti-inflammatory and antioxidant effects of astaxanthin were also confirmed by a randomized clinical trial in 42 healthy young women supplemented with placebo or astaxanthin at 2 or 8 mg/day [17] (Table 1). In comparison to placebo, after eight weeks of treatment, the 2 mg dose significantly lowered the plasma inflammatory marker C-reactive protein (unspecified values; $p < 0.05$). Astaxanthin improved the immune responses of the participants, as evidenced by the increased cytotoxic activity of natural killer cells (8 mg dose, $67.9 \pm 3.0\%$ vs. $57.8 \pm 2.7\%$ lysis; $p < 0.05$), levels of T and B lymphocytes (2 mg dose, $75.7 \pm 1.6\%$ vs. $70.6 \pm 1.5\%$ and $13.1 \pm 0.5\%$ vs. $10.7 \pm 0.5\%$, respectively; $p < 0.05$), and production of interferon- γ and IL-6 (dose of 8 mg, 9.55 pg/mL vs. 4.68 pg/mL and 25.2 pg/mL vs. 13.6 pg/mL, respectively; $p < 0.05$). Finally, starting at 4 weeks, both doses

drastically reduced plasma 8-OHdG (unspecified values; $p < 0.01$), a biomarker of oxidative DNA damage [17].

In summary, inflammation plays an essential role in the pathophysiology of cardiovascular diseases and their risk factors, while astaxanthin has therapeutic effects stemming from its anti-inflammatory action. The mechanism of action of this carotenoid involves the inhibition of the NF- κ B and MAPK signaling pathways, which reduces the inflammatory process and stimulates reverse cholesterol transport, thereby attenuating the formation of foam cells (Figure 1).

Clinical evidence of the prevention of cardiovascular diseases by astaxanthin

Potential positive effects of oral astaxanthin supplementation on cardiovascular physiology were evidenced in eleven clinical studies, summarized in Table 1. Of these eleven studies, six were randomized placebo-controlled studies [11-13, 17, 19, 20], one was single-blinded [18], two were open-label [10, 14], and two were randomized but lacked a placebo group [15, 16]. Six studies evaluated the metabolic and oxidative changes promoted by astaxanthin in healthy individuals, while five of them investigated individuals who had one dimension of metabolic syndrome, such as obesity, dyslipidemia, and type 2 diabetes. In addition, the dose of astaxanthin ranged between 1.8 mg and 40 mg, and the duration of supplementation varied between 10 days and 12 weeks, reflecting a wide variety of these two parameters. Despite encompassing a small population with a total of 417 individuals, their results show that the action of astaxanthin in promoting cardiovascular health is mainly due to its antioxidant and anti-inflammatory effects, its ability to modulate lipid and glucose metabolism, and its role in the maintenance of blood rheology.

Table 1. Clinical studies that have shown the potential positive effects of oral astaxanthin supplementation on cardiovascular physiology.

Study type	Individuals	Intervention (number of individuals per group)	Mechanism of action evaluated	Main findings	Reference
Open-label	24 healthy volunteers (mean 28.2 ± 7.8 years)	Control (n=6) ^a ; AST 1.8 mg/day (n=5); AST 3.6 mg/day (n=5); AST 14.4 mg/day (n=3); AST 21.6 mg/day (n=5) 14 days	Antioxidant effect	Delayed LDL oxidation time	[10]
Randomized, double-blind, placebo-controlled	27 overweight and obese adults (BMI > 25 kg/m ²) (20 - 55 years)	Placebo (n=13); AST 20 mg/day (n=14) 12 weeks	Antioxidant effect	Reduced LDL, ApoB, and ApoA1/ApoB ratio relative to baseline; Increased TAC and SOD compared to baseline; Reduced lipid peroxidation biomarkers (MDA and ISP) compared to baseline.	[11]
Randomized, double-blind, placebo-controlled	30 healthy individuals (50-69 years)	Placebo (n=10); AST 6 mg/day (n=10); AST 12 mg/day (n=10) 12 weeks	Antioxidant effect	Reduced phospholipid hydroperoxide levels in erythrocytes	[12]
Randomized, double-blind, placebo-controlled	39 healthy men (19-33 years)	Placebo (n=19); AST 8 mg/day (n=20) 3 months	Antioxidant effect	Reduced plasma lipid peroxidation, especially 12-hydroxy and 15-hydroxy fatty acids	[13]
Open-label, uncontrolled study	20 healthy postmenopausal women with high oxidative stress levels (mean 55.7 ± 4.8 years)	AST 12 mg/day (n=20) 8 weeks	Antioxidant effect	Lowered blood pressure; Increased antioxidant capacity; Reduced vascular resistance in lower limbs and serum adiponectin	[14]
Randomized	39 smokers (≥ 20 cigarettes per day) and	Control (n=39) ^b ; AST 5 mg/day (n=13); AST 20 mg/day (n=13);	Antioxidant effect	Reduced MDA and ISP; Increased SOD and TAC	[15]

	39 nonsmokers (21-43 years)	AST 40 mg/day (n=13) 3 weeks			
Randomized, double-blind	23 overweight (25 kg/m ² < BMI ≤ 29.9 kg/m ²) and obese (BMI > 30 kg/m ²) individuals (mean 25.1 ± 3.7 years)	Control (n=10) ^c ; AST 5 mg/day (n=12); AST 20 mg/day (n=11) 3 weeks	Antioxidant effect	Reduced MDA and ISP; Increased SOD and TAC	[16]
Randomized, double-blind, placebo-controlled	61 healthy individuals with triglyceride levels between 120-200 mg/dl (25 - 60 years)	Placebo (n=15); AST 6 mg/day (n=15); AST 12 mg/day (n=15); AST 18 mg/day (n=16) 12 weeks	Lipid metabolism	Reduced triglyceride levels; Increased HDL; Increased adiponectin	[20]
Randomized, double-blind, placebo-controlled	42 healthy young women (20 - 22 years)	Placebo (n=14); AST 2 mg/day (n=14); AST 8 mg/day (n=14) 8 weeks	Anti-inflammatory and antioxidant effect	Increased total number of T and B lymphocytes; Increased cytotoxic activity of natural killer cells; Reduced the biomarkers of oxidative damage 8-hydroxy-2'-deoxyguanosine and C-reactive protein	[17]
Single-blind	20 healthy adult men (37 - 67 years)	Placebo (n= 10); AST 6 mg/day (n=10) 10 days	Blood rheology	Reduced blood transit time	[18]
Randomized, double-blind, placebo-controlled	43 participants with type 2 diabetes (46 - 62 years)	Placebo (n=21); AST 8 mg/day (n=22) 8 weeks	Lipid and glucose metabolism	Increased adiponectin; Reduced visceral fat mass, triglycerides, VLDL, fructosamine, and systolic blood pressure	[19]

AST = astaxanthin; ^a individuals without supplementation; ^b nonsmoking individuals without supplementation; ^c individuals without overweight and without supplementation; BMI = body mass index; LDL = low-density lipoprotein; ApoA1 = apolipoprotein A1; ApoB = apolipoprotein B; SOD = superoxide dismutase; MDA = malondialdehyde; ISP = isoprostane; TAC = total antioxidant capacity; HDL = high-density lipoprotein; VLDL = very low-density lipoprotein.

Conclusion

Based on preclinical and clinical evidence, the antioxidant and anti-inflammatory effects of astaxanthin slow the progression of cardiovascular diseases. As an antioxidant, astaxanthin reduces oxidative stress, increases the bioavailability of NO and the activity of antioxidant enzymes, and maintains the rheological properties of blood. Its anti-inflammatory properties involve modulating the NF- κ B and MAPK signaling pathways, reducing the release of pro-inflammatory cytokines, and increasing reverse cholesterol transport by HDL, which attenuates the accumulation of cholesterol in foam cells and thus the formation of atherosclerotic plaques. These properties of astaxanthin, together with its demonstrated safety profile, make this compound an important agent in the prevention and/or adjuvant treatment of cardiovascular diseases.

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