Antioxidant and anti-inflammatory mechanisms of action of astaxanthin in cardiovascular diseases (Review)

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Abstract. Cardiovascular diseases are the most common cause of mortality worldwide. Oxidative stress and inflammation are pathophysiological processes involved in the development of cardiovascular diseases; thus, anti-inflammatory and antioxidant agents that modulate redox balance have become research targets so as to evaluate their molecular mechanisms of action and therapeutic properties. Astaxanthin, a carotenoid of the xanthophyll group, has potent antioxidant properties 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 has prominent anti-inflammatory activity, possibly interrelated with its antioxidant effect, and is also involved in the modulation of lipid and glucose metabolism. Considering the potential beneficial effects of astaxanthin on cardiovascular health evidenced by preclinical and clinical studies, the aim of the present review was to describe the molecular and cellular mechanisms associated with the antioxidant and anti-inflammatory properties of this carotenoid in cardiovascular diseases, particularly atherosclerosis. The beneficial properties and safety profile of astaxanthin indicate that this compound may be used for preventing progression or as an adjuvant in the treatment of cardiovascular diseases.

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1. Introduction

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

As oxidative stress and inflammation are interrelated and contribute to the initial events of cardiovascular diseases, antioxidants that modulate redox balance, such as astaxanthin, may be considered as 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 associated with 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 the unique characteristics of astaxanthin, 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 are beneficial 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, the aim of the present review was to describe the molecular and cellular mechanisms underlying the antioxidant and anti-inflammatory role of astaxanthin in the prevention of cardiovascular diseases.

2. 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 protects cell membranes against RONS and oxidative damage. Due to its chemical structure, its polar groups overlap the polar regions of the cell membrane, while the central non-polar region of the molecule fits into the inner non-polar region of the membrane. Thus, this carotenoid may take on a transmembrane alignment in biological membranes, helping to maintain the membrane structure and decrease membrane fluidity, and acting 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 non-reactive product (25). In addition, the presence of a series of conjugated bonds in the central non-polar region of astaxanthin enables the molecule to remove free radicals (high-energy electrons) from the cell interior by transporting them along its own carbon chain, resembling 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 ($ONOO^-$) (26,27), which is neutralized by astaxanthin to form 15-nitroastaxanthin, a compound that also has important antioxidant action (28).

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 compared with lutein and α-tocopherol. In turn, the blood samples of individuals who were supplemented daily with 1.8, 3.6, 14.4, or 21.6 mg astaxanthin for 14 days evidenced a significant delay in LDL oxidation when compared to samples collected 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% with 1.8, 3.6, 14.4 and 21.6 mg astaxanthin, respectively) (Table I) (10). Thus, it was demonstrated that the intake of astaxanthin delayed LDL oxidation, one of the key factors involved in the process of atherosclerosis.

LDL oxidation is also associated with the development of endothelial dysfunction in patients with diabetes mellitus, which increases the risk of cardiovascular complications (29). Endothelial dysfunction consists of impaired vessel relaxation dependent on endothelial factors, such as the production of nitric oxide (NO) by endothelial NO synthase (eNOS) (30). One of the pathways responsible for this type of dysfunction is the binding of oxidized LDL to its endothelial receptor, lectin-like ox-LDL receptor 1 (LOX-1), thus favoring oxidative stress, which leads to increased lipid peroxidation and eNOS inactivation (31). Supplementation with 10 mg/kg astaxanthin for 42 days in a diabetic rat model increased artery relaxation by significantly lowering oxidized the levels of 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 (32).

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 rheological properties (33). The oxidation of erythrocytes is associated with both the formation of atheromas and the occurrence of intraplate hemorrhage during the development of atherosclerosis (34). As regards lipid peroxidation in erythrocytes, daily supplementation with 6 or 12 mg of astaxanthin for 12 weeks in healthy individuals demonstrated that this carotenoid is incorporated and distributed into these blood cells. When incorporated into erythrocytes, astaxanthin exerted antioxidant effects on the cell membrane by significantly reducing the levels of phospholipid hydroperoxides, 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 sufficient to inhibit oxidative stress in erythrocytes (Table I) (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 3 months of supplementation with 8 mg astaxanthin in healthy men (Table I) (13).

Blood rheology is important for cardiovascular homeostasis. Astaxanthin was shown to reduce blood transit time in a hypertensive rat model (35) as well as in humans (18). In the latter study, individuals receiving supplementation with 6 mg astaxanthin for 10 days had a significantly faster blood transit time compared with that before supplementation and with that in the placebo group (47.6±4.2 vs. 54.2±6.7 sec for the treated and placebo groups, respectively; P<0.05; Table I) (18). One hypothesis for the improvement of blood rheology by astaxanthin is its antioxidant effect on 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, affect peripheral vascular resistance and may contribute to hypertension and its main cardiac complication, myocardial hypertrophy (36). 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 (37-39). 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, which is one of the antioxidant effects of astaxanthin (37,38). Chen *et al* (2020) reported that this carotenoid also participated in the remodeling of the smooth muscle cells of the vessels, reducing their proliferation and the damage caused by oxidative stress. Astaxanthin lowered RONS levels by increasing the activity of antioxidant enzymes and regulating mitochondrial dynamics, mitophagy and mitochondrial biogenesis, which are

Table I. Clinical studies that have demonstrated the potential beneficial effects of oral astaxanthin supplementation on cardiovascular physiology.

Study type	Subjects (age)	Intervention (no. of subjects per group)	Mechanism of action evaluated	Main findings	(Refs.)
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/m²) (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, particularly 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 39 non-smokers (21-43 years)	Control (n=39)[b]; AST 5 mg/day (n=13); AST 20 mg/day (n=13); AST 40 mg/day (n=13); 3 weeks	Antioxidant effect	Reduced MDA and ISP; increased SOD and TAC	(15)
Randomized, double-blind	23 overweight (25<BMI≤29.9 kg/m²) and obese (BMI>30 kg/m²) subjects (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 subjects with triglyceride levels 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 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)

Table I. Continued.

Study type	Subjects (age)	Intervention (no. of subjects per group)	Mechanism of action evaluated	Main findings	(Refs.)
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)

aIndividuals without supplementation. bNon-smoking individuals without supplementation. cIndividuals with normal body weight ($20 < BMI \leq 24.9$ kg/m^2) and without supplementation; AST, astaxanthin; BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; VLDL, very low-density lipoprotein; MDA, malondialdehyde; SOD, superoxide dismutase; ISP, isoprostane; TAC, total antioxidant capacity.

important for the maintenance of mitochondrial and cellular metabolism (39).

The high bioavailability of NO due to lower oxidative stress promoted by astaxanthin was also associated with its antithrombogenic 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 suppressed 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 (40). In a murine model of thrombosis treated with astaxanthin in the form of the CDX-085 prodrug, an increase of ~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 (41).

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 8 weeks of supplementation with 12 mg 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 role of astaxanthin in the lipid oxidation process, this carotenoid affects 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 (42). A previous study demonstrated that thioredoxin in its oxidized form was associated with the degree of severity of chronic heart failure and with the resulting oxidative stress (43). Paraoxonase-1 binds to serum high-density lipoprotein (HDL) and is responsible for protecting both LDL and HDL from oxidation, as well as for breaking down oxidized lipids (44). The effect of astaxanthin on these two enzymes was evaluated in rabbits fed a cholesterol-rich diet (45). 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 only demonstrated the direct action of this molecule on TrxR (45).

Choi *et al* (11,16) also conducted two randomized and double-blind clinical studies on overweight or obese individuals to demonstrate the antioxidant effects of astaxanthin (Table I). In one study, volunteers who received 5 and 20 mg astaxanthin for 3 weeks exhibited lower oxidative stress biomarkers associated with lipid peroxidation compared with prior to treatment, with a 34.6 and 35.2% reduction in malondialdehyde (MDA) levels, and 64.9 and 64.7% reduction in isoprostane (ISP) levels, respectively. An increase in the

activity of the antioxidant defense system was also observed, with a 193 and 194% increase in superoxide dismutase (SOD) and a 121 and 125% increase in total antioxidant capacity at the doses of 5 and 20 mg, respectively, compared with the data prior to treatment (16). No significant differences were observed between the results obtained with the two doses, indicating that the clinical effects of this carotenoid are not dose-dependent. Later, the same authors analyzed lipid profile, oxidative stress and antioxidant system parameters (11). After 12 weeks of supplementation with 20 mg astaxanthin, the same results in oxidative stress and the antioxidant system as in the previous study were observed. 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 demonstrated 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 compared with non-smokers to reduce the consequences of prolonged exposure to toxins present in cigarettes. After 3 weeks, supplementation with different doses of astaxanthin (5, 20 and 40 mg) in active smokers prevented oxidative damage by suppressing lipid peroxidation and stimulating the activity of the antioxidant system (Table I) (15). This effect was confirmed by the significant reduction in serum MDA and ISP levels and the increased SOD activity and total antioxidant capacity in the three astaxanthin groups compared with the indices prior to treatment (15). The authors also observed that the serum concentration of astaxanthin in the groups treated with 20 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 optimal dosage for smokers.

Several preclinical studies have demonstrated that astaxanthin also exerts an indirect antioxidant effect by activating transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), and increasing the expression of its antioxidant target genes, such as phase II biotransformation enzymes (46-52). A study with a model of coronary microembolization in rats revealed 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/heme oxygenase-1 signaling (47).

Thus, astaxanthin may accumulate in the blood plasma and, through its antioxidant action, it helps reduce the levels of RONS responsible for LDL oxidation and lipid peroxidation; it increases the bioavailability of NO, enabling its vasodilator and antithrombogenic effects; it increases the activity of antioxidant enzymes; and it ensures the stability of blood rheological properties, thus avoiding the loss of erythrocyte flexibility and the increase in plasma viscosity, factors that affect the blood flow velocity. These actions of astaxanthin against early events of atherosclerotic plaque formation and arterial dysfunction may delay the progression of cardiovascular diseases (Fig. 1).

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 (53). Astaxanthin exerts a marked anti-inflammatory effect, which may be interrelated with its antioxidant effect and contributes to physiological changes that benefit cardiovascular function (Fig. 1) (53-58).

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 (59). 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 non-enzymatic oxidation of LDL are the main damage-associated molecular patterns 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 (59). The expression of these receptors is controlled by nuclear factor-κB (NF-κB), the main mediator of the inflammatory response, which is activated by pattern recognition receptors and pro-inflammatory cytokines (60). 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 (54-58,61-82). Supplementation with 10 µM 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, as reflected by reduced protein expression of MMP-9 and MMP-2 and of the mRNA levels of five MMPs (62). Astaxanthin at this concentration also reduced the gene expression of pro-inflammatory markers, such as interleukin (IL)-1β, IL-6, tumor necrosis factor-α (TNF-α), inducible nitric oxide synthase (iNOS) and COX-2 (62). These results corroborate those of other studies indicating that astaxanthin reduces the expression of pro-inflammatory mediators in macrophages (62-65) and other cell types, such as microglia, endothelial vascular cells and human neutrophils (66-69).

The significant decreases in the levels of MMPs and proinflammatory cytokines may result from the suppression of the NF-κB transcription factor by astaxanthin (54-58,62,64,68,70,71). NF-κB is frequently activated
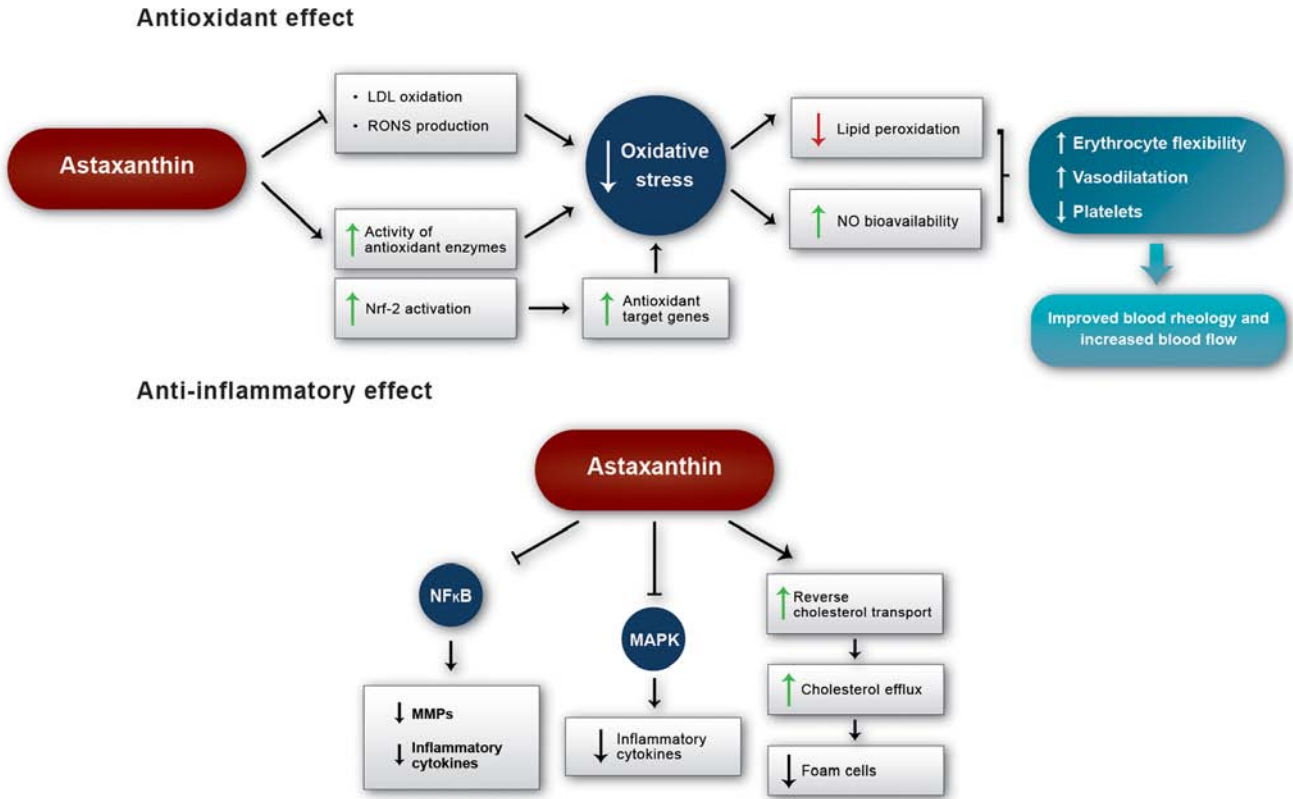
Figure 1. Scheme of the antioxidant and anti-inflammatory mechanisms of action of astaxanthin in cardiovascular diseases. LDL, low-density lipoprotein; RONS, reactive oxygen and nitrogen species; NF-κB, nuclear factor-κB; MMP, matrix metallopeptidase; MAPK, mitogen-activated protein kinase; NO, nitrogen oxide.
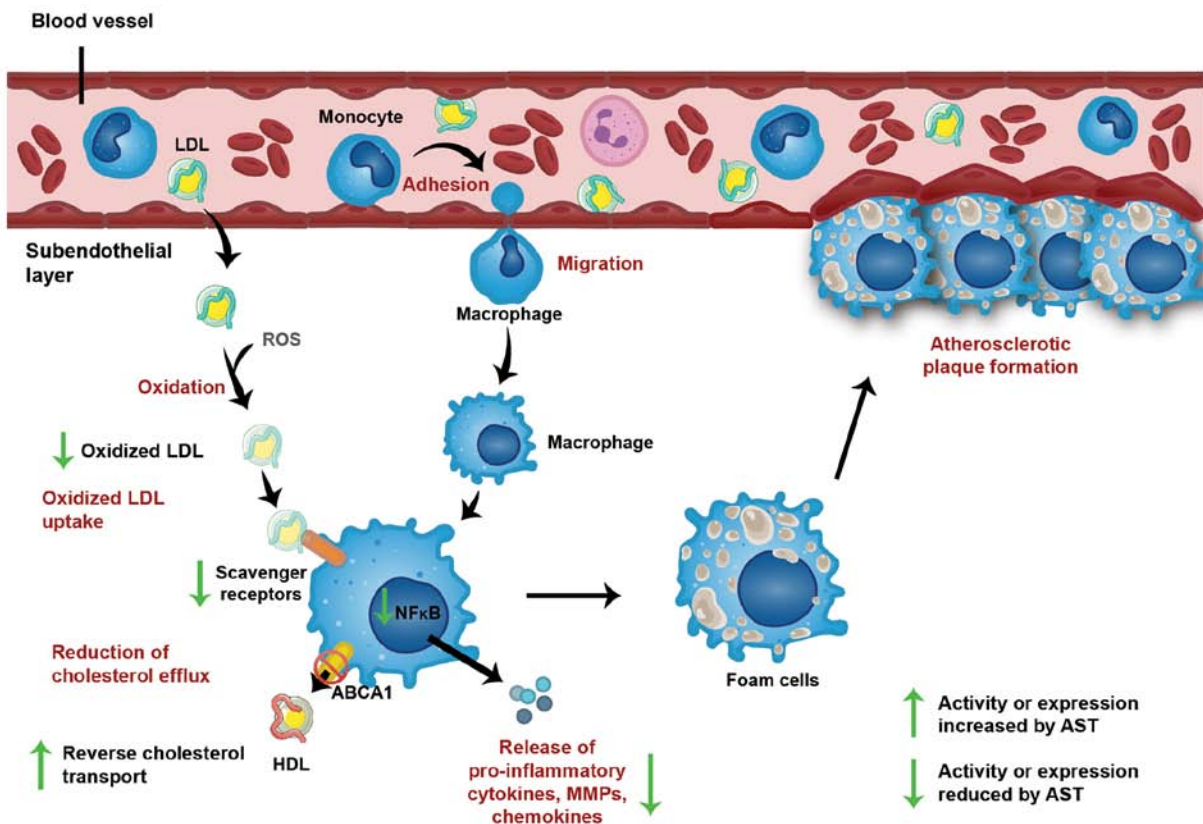

Figure 2. Mechanism of atherosclerotic plaque formation in the subendothelial layer of the vascular wall and the action of astaxanthin (adapted from Fig. 2 in ref. 5). AST, astaxanthin; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ROS, reactive oxygen species; NF-κB, nuclear factor-κB; MMP, matrix metallopeptidase; ABCA1, ATP-binding cassette A1.

at inflammation sites associated with various pathologies, particularly cardiovascular diseases, in the etiology of which the increased expression of its pro-inflammatory target genes plays a key role (72). The inflammatory pathway of NF-κB is, at least in part, regulated by oxidative stress (72). Astaxanthin inhibits the activity of IκB kinase, a complex responsible for the control of NF-κB activation. This maintains NF-κB inactive in the cell cytoplasm, and its pro-inflammatory target genes, such as TNF-α, IL-1β and iNOS, are downregulated (64).

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 (Fig. 2) (59). The progression of cholesterol accumulation may lead to its precipitation in the form of crystals, which activate the inflammasome, leading to cell death by apoptosis or necrosis (83). 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 (59).

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 (79). Both the liver and intestine synthesize apolipoprotein A-I (apoA-I) and apoA-II in the 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 (80). Of note, this reverse cholesterol transport was also observed in the lymphatic system, which is largely responsible for the removal of cholesterol from different tissues (79). 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 lipoprotein (VLDL) through the cholesteryl ester transfer protein (81). These lipoproteins are absorbed in the liver by their specific receptors, which is likely 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 (80).

In atherosclerosis, apolipoproteins are oxidized by the enzyme myeloperoxidase, which is expressed in macrophages during the inflammatory process, compromising cholesterol efflux via ABCA1 (81). 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) (82). 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 (61). 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 may exert antiatherosclerotic effects by increasing the activity of the reverse cholesterol transport pathway, but the molecular mechanisms underlying this action remain elusive (61).

In addition to its function in reverse cholesterol transport, astaxanthin is involved in certain lipid metabolism steps, a finding corroborated by a randomized, placebo-controlled clinical study of 61 adult individuals with moderate hyperlipidemia (Table I) (20). In that study, daily supplementation with 6, 12 or 18 mg astaxanthin for 12 weeks led to an improvement in the lipid profile of the patients. Triglycerides were reduced by 25.2 and 23.8% (P<0.05) by doses of 12 and 18 mg, respectively, whereas HDL was increased by 10.6% (P<0.05) and 15.4% (P<0.01) by doses of 6 and 12 mg, respectively. 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 (84). In this context, astaxanthin has been found to be promising in the improvement of glucose and lipid metabolism in a randomized, placebo-controlled clinical study with 43 diabetic patients aged 46-62 years (Table I) (19). In agreement with a previous clinical study (20), supplementation with astaxanthin (8 mg/day for 8 weeks) significantly increased serum adiponectin (47±14 vs. 45±13 and 36±15 µg/ml compared with placebo and baseline, respectively; P<0.05) and improved the lipid profile of the patients, as shown by the reductions in the levels of triglycerides (128±52 vs. 150±85 and 156±90 mg/dl compared with placebo and baseline, respectively; P<0.05) and VLDL (27±16 vs. 31±16 mg/dl compared with placebo; P<0.05). Furthermore, astaxanthin marginally reduced fasting glucose levels (8.3±2.7 vs. 9.4±3.2 mmol/l compared with placebo; P=0.057) and significantly increased serum fructosamine levels (5.8±3.8 vs. 7.32±4.31 and 7.36±4.2 µmol/l compared with placebo and baseline, respectively; P<0.05), an important marker in the control of diabetes that reflects the mean concentration of blood glucose. Patients receiving astaxanthin supplementation also exhibited lower visceral fat deposition (11.2±3.4 vs. 11.85±3.8% compared with placebo; P<0.05) and systolic blood pressure (132±18 vs. 133±19 and 143±27 mmHg compared with 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 (85). Ischemia/reperfusion is a complex

inflammatory process associated with high levels of oxidative stress in the affected tissue (86). In rodents with hepatic lesions induced by ischemia/reperfusion, astaxanthin not only reduced oxidative stress and histopathological damage (87,88) but also exerted a significant anti-inflammatory effect, attenuating the release of inflammatory cytokines through the mitogen-activated protein kinase (MAPK) pathway (88,89). Furthermore, astaxanthin exerted 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 (90).

In mice with non-alcoholic steatohepatitis (NASH) induced by a high-lipid diet, supplementation with astaxanthin (0.02% in the diet, ~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 cell activation, and attenuated liver fibrosis, the accumulation and peroxidation of hepatic lipids and insulin resistance (91). Additionally, astaxanthin was more effective in preventing and treating NASH and improving liver inflammation and fibrosis compared with vitamin E (standard NASH treatment). The same study also corroborated the potential of astaxanthin to improve NASH in 12 individuals receiving this carotenoid as a supplement (12 mg/day; control: placebo) for 24 weeks (91).

The effects of astaxanthin on the relief of liver injury was shown to be correlated to its positive effects on the intestinal microbiota and consequent reduction of inflammation (92). In fact, a growing body of evidence indicates that gut microbiota plays a key role in the pathogenesis of inflammatory disorders and cardiovascular diseases, and alterations in its composition (dysbiosis) have been associated with heart failure, hypertension, atherosclerosis and metabolic syndrome (93-95). Several recent *in vivo* studies revealed that astaxanthin supplementation improved gut microbiota composition, which may contribute to its local and systemic anti-inflammatory and antioxidant effects (92,96-99). The beneficial effect of astaxanthin on gut microbiota has been shown to be correlated with the mitigation of cardiovascular disease-related pathologies and risk factors, such as obesity (100), insulin resistance (99) and alcoholic fatty liver disease (92).

The anti-inflammatory and antioxidant effects of astaxanthin were also confirmed by a randomized clinical trial in 42 healthy young women receiving placebo or astaxanthin at 2 or 8 mg/day (Table I) (17). Compared with placebo, after 8 weeks of treatment, 2 mg astaxanthin significantly lowered the levels of 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±3.0 vs. 57.8±2.7% lysis; P<0.05), levels of T and B lymphocytes (2 mg dose, 75.7±1.6 vs. 70.6±1.5 and 13.1±0.5 vs. 10.7±0.5%, respectively; P<0.05), and the production of interferon (IFN)-γ and IL-6 (dose of 8 mg, 9.55 vs. 4.68 and 25.2 vs. 13.6 pg/ml, respectively; P<0.05). Finally, starting at 4 weeks, both doses drastically reduced the plasma levels of 8-OHdG (unspecified values; P<0.01), a biomarker of oxidative DNA damage (17).

Although macrophages are the main type of immune cell found in atherosclerotic plaques, T lymphocytes also contribute to the development of the disease (101). In fact, the inflammatory response mediated by T lymphocytes plays a crucial role in the etiology of cardiovascular diseases, contributing to atherosclerosis, heart failure and myocardial infarction (102-108). For example, T helper cells can be activated by LDL particles in the arterial wall and trigger inflammation through an autoimmune response, contributing to the development of atherosclerotic plaques (106,108,109). Similarly, self-reactive T helper cells may target cardiomyocytes, contributing to the development of heart failure (103).

Astaxanthin was shown to be effective not only in preventing oxidative stress in T lymphocytes (110-115), but also in modulating their activity (115-123). In the aforementioned clinical study on healthy young women (17), astaxanthin supplementation stimulated mitogen-induced lymphoproliferation and increased the subpopulation of T lymphocytes, without changing the populations of T killer or T helper cells, as well as increased the response to tuberculin, an indicator of T lymphocyte function. In a mouse model of NASH, astaxanthin reduced T helper and T killer cell recruitment to the liver, contributing to the improvement of inflammation and insulin resistance (91). Supplementation for 45 days with fish oil containing astaxanthin (1 mg/kg of body weight) significantly reduced the proliferative capacity of T lymphocytes in response to mitogens and RONS production when compared with fish oil alone (115). In *in vitro* studies with peripheral blood mononuclear cells from patients with asthma and allergic rhinitis, it was demonstrated that astaxanthin significantly suppressed the activation of T lymphocytes induced by phytohemagglutinin (116,122). Another *in vitro* and *ex vivo* study with cultured lymphocytes demonstrated that astaxanthin stimulated their immune response and increased the production of IL-2 and IFN-γ, without inducing cytotoxicity (117). The administration of astaxanthin in mice prevented renal fibrosis by mechanisms involving stimulation of T killer cell recruitment and increased production of IFN-γ (118). In cats, astaxanthin increased the immune response mediated by total T lymphocytes and T helper cells (119).

Therefore, astaxanthin was shown to exert a clear modulatory effect on T lymphocytes, overall improving their immune response or downregulating their potentially pathological immune activation. However, the role of T lymphocyte modulation by astaxanthin in the risk and progression of cardiovascular diseases remain to be fully elucidated.

In summary, inflammation plays a key role in the pathophysiology of cardiovascular diseases and their risk factors, while astaxanthin exerts beneficial anti-inflammatory effects. The mechanism of action of this carotenoid involves inhibition of the NF-κB and MAPK signaling pathways, which suppresses the inflammatory process and stimulates reverse cholesterol transport, thereby attenuating the formation of foam cells (Fig. 1).

3. Clinical evidence of the prevention of cardiovascular diseases by astaxanthin

The potential beneficial effects of oral astaxanthin supplementation on cardiovascular physiology were evidenced in

11 clinical studies, summarized in Table I. Of these 11 studies, 6 were randomized placebo-controlled studies (11-13,17,19,20), 1 was single-blinded (18), 2 were open-label (10,14), and 2 were randomized but lacked a placebo group (15,16). A total of 6 studies evaluated the metabolic and oxidative changes promoted by astaxanthin in healthy individuals, while 5 investigated individuals who had one element of the metabolic syndrome, namely obesity, dyslipidemia or type 2 diabetes. In addition, the dose of astaxanthin ranged between 1.8 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, the results of those studies indicated that the beneficial effect of astaxanthin on cardiovascular health was mainly due to its antioxidant and anti-inflammatory properties, its ability to modulate lipid and glucose metabolism, and its role in the maintenance of blood rheological properties.

4. Conclusion

Based on preclinical and clinical evidence, the antioxidant and anti-inflammatory effects of astaxanthin appear to delay 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 the blood. Its anti-inflammatory properties involve modulating the NF-κB and MAPK signaling pathways, reducing the release of pro-inflammatory cytokines and increasing reverse cholesterol transport by HDL, thereby attenuating the accumulation of cholesterol in foam cells and the formation of atherosclerotic plaques. These properties of astaxanthin, together with its favorable safety profile, make this compound a promising option for the prevention and/or adjuvant treatment of cardiovascular diseases.

Availability of data and materials

Not applicable.

Authors' contributions

CPMP and JJN contributed to the conception of the study and critically reviewed the article. CPMP contributed to the design of the manuscript and figure preparation and editing. CPMP, ACRS, ARV, PSP and JJN contributed to the data acquisition and analysis and drafted the manuscript. All authors have approved all aspects of the present study and agree to be fully accountable for ensuring the integrity and accuracy of the work. All the authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

All the authors declare that they have no competing interests.

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