REVIEW

Potential health-promoting effects of astaxanthin: A high-value carotenoid mostly from microalgae

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Received: August 30, 2010
Revised: October 13, 2010
Accepted: October 16, 2010

The ketocarotenoid astaxanthin can be found in the microalgae *Haematococcus pluvialis*, *Chlorella zofingiensis*, and *Chlorococcum* sp., and the red yeast *Phaffia rhodozyma*. The microalga *H. pluvialis* has the highest capacity to accumulate astaxanthin up to 4–5% of cell dry weight. Astaxanthin has been attributed with extraordinary potential for protecting the organism against a wide range of diseases, and has considerable potential and promising applications in human health. Numerous studies have shown that astaxanthin has potential health-promoting effects in the prevention and treatment of various diseases, such as cancers, chronic inflammatory diseases, metabolic syndrome, diabetes, diabetic nephropathy, cardiovascular diseases, gastrointestinal diseases, liver diseases, neurodegenerative diseases, eye diseases, skin diseases, exercise-induced fatigue, male infertility, and $HgCl_2$-induced acute renal failure. In this article, the currently available scientific literature regarding the most significant activities of astaxanthin is reviewed.

Keywords:
Astaxanthin / Carotenoid / *Haematococcus pluvialis* / Health-promoting effects / Microalgae

1 Introduction

The ketocarotenoid astaxanthin, 3,3′-dihydroxy-β,β-carotene-4,4′-dione, belongs to the family of xanthophylls, the oxygenated derivatives of carotenoid. Astaxanthin is ubiquitous in nature, especially in the marine environment [1], and is a red pigment common to many marine animals, such as salmonids, shrimp, lobsters, and crayfish, contributing to the pinkish-red color of their flesh [2]. Astaxanthin is biosynthesized by microalgae or phytoplankton, as the primary production level in the marine environment. Microalgae are consumed by zooplankton or crustaceans which accumulate astaxanthin and, in turn are ingested by fish which then accrue astaxanthin in the food chain [1].

Astaxanthin has been found and identified in several microorganisms including the microalgae *Haematococcus pluvialis*, *Chlorella zofingiensis*, and *Chlorococcum* sp., the red yeast *Phaffia rhodozyma*, and the marine bacterium *Agrobacterium aurantiacum* [3]. Although astaxanthin can be synthesized by plants, bacteria, a few fungi and green algae, the green microalga *H. pluvialis* is considered to have the highest capacity to accumulate astaxanthin in reported sources [4, 5]. It has been reported that *H. pluvialis* could accumulate astaxanthin up to 4–5% of dry weight [4, 6]. In addition, Roche has begun a large-scale production of synthetic astaxanthin, which consists of a mixture 1:2:1 of isomers (3S, 3S′), (3R, 3S′), and (3R, 3R) respectively, since 1990 [7].

There has been growing interest in the use of astaxanthin as a food-coloring agent, natural feed additive for the poultry industry and for aquaculture, especially as a feed supplement in the culture of salmon, trout, and shrimp. There have also been reports concerning its application in medicine due to its powerful antioxidant capacity [5]. Astaxanthin has unique chemical properties based on its molecular

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structure. Astaxanthin has two carbonyl groups, two hydroxy groups, and eleven conjugated ethylenic double bonds (Supporting Information Fig. S1). The polyene system gives astaxanthin its distinctive molecular structure, chemical properties, and light-absorption characteristics [7]. The presence of the hydroxyl and keto moieties on each ionone ring explains some of its unique features such as the ability to be esterified and a higher antioxidant activity and a more polar nature than other carotenoids [8]. Astaxanthin may act as a strong antioxidant by donating the electrons and reacting with free radicals to convert them to more stable product and terminate free radical chain reaction in a wide variety of living organisms [9, 10].

Therefore, astaxanthin has considerable potential and promising applications in human health and nutrition [9], and has been attributed with extraordinary potential for protecting the organism against a wide range of diseases [7]. This article reviews the current available scientific literatures regarding the most significant activities of astaxanthin, including its antioxidative, anticancer, antidiabetic, and anti-inflammatory properties, its protective effects on stomach, liver, the heart and the blood vessels, the nervous system, the eye, and the skin, and other activities.

2 Chemistry of astaxanthin

2.1 Astaxanthin profiles in microalgae

Free astaxanthin is particularly susceptible to oxidation [8]. Therefore, astaxanthin in nature is either conjugated with proteins or esterified with one or two fatty acids to form monoester and diester forms [8, 11]. In *H. pluvialis*, astaxanthins exist mainly as various astaxanthin esters formed by combining various fatty acids with different isomers of astaxanthin [6]. Various astaxanthin isomers have been characterized on the basis of the configuration of the two hydroxyl groups on the molecule [8]. Considering that each molecule has two chiral centers in C-3 and C-3′, astaxanthin may present three configurational isomers, two enantiomers (3R, 3′R and 3S, 3′S) a meso form (3R, 3′S). The 3S, 3′S stereoisomer is the main form found in *H. pluvialis* [7].

For different algal strains, the compositions and profile of astaxanthin were different [6]. Peng *et al.* [11] showed that the green microalga *C. zofingiensis* had a remarkably higher percentage of astaxanthin diesters in comparison with *H. pluvialis* with a higher percentage of astaxanthin monoesters. The esters of astaxanthin and adonixanthin, and free canthaxanthin were the major carotenoids in the alga *Chlorococcum* cells [3]. In *C. zofingiensis*, the major carotenoids were astaxanthin (about 70%) and canthaxanthin (about 30%) [12]. On the contrsry, astaxanthin alone was the major carotenoid in *H. pluvialis* [6]. Boussiba *et al.* [4] showed that the esterified astaxanthin accounts for more than 99% of the total carotenoids.

The esterification of the hydroxyl groups of astaxanthin increases its hydrophobicity and therefore its solubility in globules made of triacylglycerols. The fatty acid composition of the astaxanthin esters is very close to that of triacylglycerols, consisting mostly of oleic ($C_{18:1}$), palmitic ($C_{16:0}$), and linoleic acids ($C_{18:2}$), and oleic acid constitutes 51% of the fatty acids of astaxanthin esters [13]. Miao *et al.* [14] indicated that astaxanthin $C_{18:1}$ and astaxanthin $C_{16:1}/C_{18:1}$ were the main astaxanthin monoester and diester, respectively, in *H. pluvialis*. Zhekisheva *et al.* [13] suggested that the accumulation of astaxanthin was accompanied and perhaps preceded by that of oleate-rich triacylglycerols and the ability to fit the composition of astaxanthin esters with that of triacylglycerols was one of the reasons for *H. pluvialis* being the richest natural source of astaxanthin.

2.2 Isomerization of *trans*-astaxanthin

In the astaxanthin molecule (Supporting Information Fig. S1), each double bond from the polyene chain may exist in two configurations as geometric isomers *cis* or *trans* [7, 15, 16]. Most carotenoids found in nature are predominantly all *trans*-isomers [7]. *trans*-Astaxanthin is readily isomerized to *cis*–*trans* mixtures, especially the 9-*cis* and 13-*cis* unhindered isomers for steric reasons [17]. Although astaxanthin exists mainly as *trans*-astaxanthin esters of various fatty acids, *cis*-astaxanthin esters are also detected in the algal pigment extracts. A high-yielding astaxanthin ester-producing strain of the microalga *H. pluvialis*, which can accumulate astaxanthin up 5.02% of cell dry weight, is found to contain 36.7 mg/g of *trans*-astaxanthin (73.1%) and 13.5 mg/g of *cis*-astaxanthins (26.9%) [6].

The isomerization of *trans*-astaxanthin to *cis*-isomers in different organic solvents has been investigated [17]. The isomerization rate of *trans*-astaxanthin is dependent on the solvent. Although the relative contents of 9-*cis*- and 13-*cis*-astaxanthins formed during isomerization are different in different solvents, 13-*cis*-astaxanthin is the main *cis*-isomer from *trans*-astaxanthin. The results also indicate that a higher temperature can promote markedly the isomerization rate of *trans*-astaxanthin [17]. The fact that *trans*-astaxanthin cannot be isomerized completely to its *cis*-isomers indicates that the isomerization reaction of *trans*-astaxanthin is a reversible reaction [17, 18]. Studies have revealed that *cis*-astaxanthins can also be isomerized to produce *trans*-astaxanthin and other *cis*-astaxanthins, and the isomerization of *trans*-astaxanthin follows first-order reversible reaction kinetics [18].

2.3 Bioavailability and safety of astaxanthin

Carotenoid absorption strongly depends on a number of factors that are not entirely understood. Bioavailability of carotenoids also depends on their structures; in general,

polar carotenoids (*e.g.* free astaxanthin) tend to be of higher bioavailability than apolar species (*e.g.* β-carotene and lycopene) [19]. It has been reported that astaxanthin from *H. pluvialis* shows better bioavailability than β-carotene from *Spirulina platensis* and lutein from *Botryococcus braunii* [10]. In addition, *cis*-astaxanthins accumulate preferentially in blood plasma compared with the *trans*-form due to apparent shorter chain lengths [19].

Xanthophyll esters seem to be of low bioavailability, but there is a scientific controversy [19]. Studies suggested that xanthophyll esters were hydrolyzed in the small intestine for absorption in humans [20]. Recently, Sugawara *et al.* [20] found the enzymatic esterification of xanthophylls such as astaxanthin in intestinal cells at a lower rate, and suggested that the esterification of xanthophylls was mediated by enzymatic activity after intestinal absorption. The esterified xanthophylls were likely to be incorporated into the lipid core in chylomicron and carried into a variety of tissues including the skin. In addition, by esterifying xanthophylls into highly nonpolar products, intestinal cells might be protected from the cytotoxic effects of xanthophylls. It was important to clarify that polar xanthophylls were suitable for esterification in intestinal cells in order to understand the absorption, metabolism, and biological function of carotenoids [20]. The presence of astaxanthin esters in *H. pluvialis* might be an added advantage to influence the higher bioavailability of astaxanthin [10].

Few data have been found on possible toxic or harmful effects of astaxanthin. A recent clinical study showed that a higher dose of astaxanthin (40 mg daily) from *H. pluvialis* during a 4-wk treatment period did not reveal any harmful effects [21].

3 Potential health-promoting effects of astaxanthin

Many earlier studies suggested that the bioactivities of carotenoids might be due to their prior conversion to vitamin A and focused on β-carotene. Subsequent studies showed that some carotenoids without provitamin A activity were as active and at times more active than β-carotene [22]. Astaxanthin, not possessing a pro-vitamin A activity, has attracted considerable interest because of its potent bioactivities including its antioxidative, anticancer, antidiabetic, and anti-inflammatory activities, gastro-, hepato-, neuro-, cardiovascular, ocular, and skin-protective effects, and other activities, which are distinctly different and, at least in some cases, more potent than that of other carotenoids [22, 23].

3.1 Antioxidant activity

Oxidative molecules with very high reactivity, such as free radicals and reactive oxygen species, are produced by normal aerobic metabolism in organisms for sustaining life processes; however, excess quantities of oxidative molecules may react with cellular components such as proteins, lipids, and DNA, through a chain reaction, to cause protein and lipid oxidation and DNA damage, which are associated with various diseases [7]. These injurious actions induced by oxidative stress can be restrained by endogenous antioxidases and exogenous antioxidants such as carotenoids. The common chemical feature of carotenoids is the polyene chain, a long-conjugated double-bond system, which is responsible for the antioxidant activities of carotenoids by quenching singlet oxygen and scavenging radicals to terminate chain reactions [24, 25]. The different chemical anti- and pro-oxidant behavior of carotenoids is caused by the different structure of the end groups, and the number and position of methyl groups [26]. The biological benefits of carotenoids may be due to their potent antioxidant properties attributed to specific physico-chemical interactions with membranes [25].

Naguib [27] compared the antioxidant activities of various carotenoids and showed that the relative reactivities of astaxanthin, lutein, lycopene, α-carotene, β-carotene, α-tocopherol, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid toward peroxyl radicals were 1.3, 0.4, 0.4, 0.5, 0.2, 0.9, and 1.0, respectively, indicating that astaxanthin had the highest antioxidant activity. The effects of astaxanthin, zeaxanthin, lutein, β-carotene, and lycopene, on lipid hydroperoxide generation in membranes enriched with polyunsaturated fatty acids were evaluated by McNulty *et al.* [25], who found that apolar carotenoids, such as lycopene and β-carotene, could disorder the membrane bilayer and showed a potent pro-oxidant effect with a 85% of increase in lipid hydroperoxide levels, whereas astaxanthin preserved membrane structure and exhibited significant antioxidant activity with a 40% of decrease in lipid hydroperoxide levels, indicating distinct effects of carotenoids on lipid peroxidation due to membrane structure changes. A recent study showed that when the micoalgal biomass (*H. pluvialis*, *S. platensis*, or *B. braunii*) was fed to rats, the antioxidases catalase, superoxide dismutase, peroxidase, and thiobarbituric acid reactive substances were significantly high in plasma at 2 h and in liver at 4 h, evidently offering protection from free radicals in living cells, especially for astaxanthin from *H. pluvialis* [10].

Guanosine is the most easily oxidized nucleoside and has the lowest of the nucleoside one-electron reduction potentials and hence internal electron transfer processes will lead to the accumulation of guanosine radicals as a key intermediate in nucleoside oxidation [28]. Edge *et al.* [28] recently reported that β-carotene, lycopene, zeaxanthin, and astaxanthin could reduce oxidized guanosine and minimize its formation, and the reaction of the carotenoid with the oxidized guanosine produced the radical cation of the carotenoid. It was suggested that carotenoids might offer additional protection against free radical-induced nucleoside damage in addition to the well-established protection afforded by direct quenching of oxidizing free radicals

themselves. The electron transfer rate constants for the efficient reduction of guanosine radicals are the fastest for astaxanthin ($k = 6.23 \times 10^7\ M^{-1}.s^{-1}$) compared with β-carotene, lycopene, and zeaxanthin ($k = 1.67$, 2.23, and $4.43 \times 10^7\ M^{-1}.s^{-1}$, respectively) [28].

The above-mentioned comparison studies for various carotenoids indicate that astaxanthin has the higher antioxidant activity than other carotenoids. It has been generalized that astaxanthin has an antioxidant activity, as high as ten times more than other carotenoids such as zeaxanthin, lutein, canthaxantin, and β-carotene, and 100 times more than α-tocopherol, and thus has been dubbed a ''super vitamin E'' [7]. Astaxanthin has unique chemical properties based on its molecular structure. The presence of the hydroxyl and keto moieties on each ionone ring is responsible for its higher antioxidant activity [29]. The oxo function is capable to resonance-stabilize carbon-centered radicals, which may explain the powerful antioxidative properties of astaxanthin without pro-oxidative contributions [26]. Astaxanthin catches radicals not only at the conjugated polyene chain but also in the terminal ring moiety. Goto *et al.* [30] suggested that the hydrogen atom at the C3 methine in the terminal ring was a radical trapping site. Although the unsaturated polyene chain of astaxanthin trapped radicals only in the membrane, the terminal ring of astaxanthin could scavenge radicals both at the surface and in the interior of the phospholipid membrane. The unique properties of astaxanthin should be associated with its potent antiperoxidation activity [30]. Recently, it was reported that astaxanthin could inhibit lipid peroxide formation and enhance the antioxidant enzyme status in glycated protein/iron chelate-exposed endothelial cells by suppressing reactive oxygen species generation [31].

Interestingly, Liu and Osawa [32] found that *cis*-astaxanthins, especially the 9-*cis* isomer, might have a higher antioxidant activity than that of the all-*trans* isomer in inhibition of the generation of reactive oxygen species induced by 6-hydroxydopamine in human neuroblastoma SH-SY5Y cells as well as on the degradation of collagen type II induced by docosahexaenoic acid and linoleic acid hydroperoxides, indicating that astaxanthins, especially 9-*cis*-astaxanthin, may show potential neuroprotective effect for Parkinson's disease and possible prophylactic effect for arthritis.

3.2 Anti-inflammatory effects

It was reported that astaxanthin could inhibit the expression or production of inflammatory mediators and cytokines in both lipopolysaccharide-stimulated RAW264.7 cells and primary macrophages by suppressing the activation of nuclear factor-κB, which is a significant transcription factor for inducible nitric oxide synthase, probably as a result of scavenging intracellular reactive oxygen species [33]. Choi *et al.* [34] showed that astaxanthin could exert its anti-inflammatory actions by inhibiting the expression of inducible nitric oxide synthase and cyclooxygenase-2 and the production of nitric oxide in lipopolysaccharide-stimulated BV2 microglial cells. This inhibitory effect of astaxanthin on the production of nitric oxide has important implications for the development of anti-inflammatory drugs for chronic inflammatory diseases such as sepsis, rheumatoid arthritis, atherosclerosis, inflammatory bowel disease, and brain inflammatory diseases [33, 34].

Recently, Bolin *et al.* [35] found that astaxanthin displayed interesting anti-inflammatory effects by preserving redox-sensitive and essential structures of human lymphocytes, which could be mainly deduced by the increased nitric oxide formation, the observed reduced O_2^-/H_2O_2 production, and induced superoxide dismutase and catalase activities in parallel to lower indexes of oxidative injury in lipids and proteins. It was suggested that astaxanthin was potentially nutritional therapeutic agent for prevention/prophylaxy of immune-impaired diseases, such as type 2 diabetes, sepsis, and cardiovascular disorders [35]. Macedo *et al.* [36] showed that astaxanthin significantly reduced the production of pro-inflammatory cytokines, such as tumor necrosis factor-α and interleukin-6 in lipopolysaccharide-stimulated neutrophils. The results also showed that astaxanthin improved neutrophil phagocytic and microbicidal capacity and reduced superoxide anion and hydrogen peroxide production, which appeared to be mediated by calcium released from intracellular storages and nitric oxide production, indicating a beneficial effect of astaxanthin on human neutrophils function [36].

Immune cells are particularly sensitive to oxidative stress due to a high percentage of polyunsaturated fatty acids in their plasma membranes and generally produce more oxidative products [37]. Park *et al.* [37] studied the possible immune-enhancing, antioxidative, and anti-inflammatory activity of astaxanthin in young healthy adult female human subjects, and showed that astaxanthin could decrease a DNA oxidative damage biomarker and inflammation, and enhance immune response. The immunomodulatory, antioxidative, and anti-inflammatory activity of astaxanthin would likely influence the etiology of cancer and inflammatory diseases [37].

The anti-inflammatory activity of astaxanthin may also have a role in the prevention or treatment of asthma. It was reported that that ginkgolide B, astaxanthin, or their combination could suppress activation of T cells from asthma patients [38]. In the recent experiment, Haines *et al.* [39] showed that the asthmatic animals fed astaxanthin, *Ginkgo biloba* extract and vitamin C alone or in combination exhibited significantly lower bronchoalveolar lavage fluid inflammatory cell numbers and enhancement of lung tissue content of cAMP and cGMP, and the efficacy was equal to or better than ibuprofen, a widely used nonsteroidal anti-inflammatory drug.

Sakai *et al.* [40] showed that astaxanthin, fucoxanthin, zeaxanthin, and β-carotene significantly inhibited the

antigen-induced release of β-hexosaminidase, an index of mast cell degranulation, in rat basophilic leukemia RBL-2H3 cells and mouse bone marrow-derived mast cells, and antigen-induced aggregation of high-affinity IgE receptor which was the most upstream of the degranulating signals of mast cells.

3.3 Gastro-protective effect

3.3.1 Anti-*Helicobacter pylori* activity

Studies both *in vivo* and *in vitro* have shown that astaxanthin is not only a free radical scavenger but also shows antimicrobial activity against *H. pylori* [41]. It was reported that treatment with a cell extract of the microalgae *H. pluvialis* containing 2–3% of astaxanthins [42] or a *Chlorococcum* sp. algal extract [43] could significantly reduce bacterial load and gastric inflammation in *H. pylori*-infected mice. Wang *et al.* [44] demonstrated that mice treated with *H. pluvialis* algal meal showed significantly lower colonization levels and lower inflammation scores. Nishikawa *et al.* [45] compared physiologically and biochemically the effects of three kinds of astaxanthins, including two extracts from the microalga *H. pluvialis* and the red yeast *P. rhodozyma*, and a synthetic astaxanthin, on stressed rats. The results indicated that rats given astaxanthins prior to stressing were appreciably protected against the evolution of gastric ulcerations. In particular, ulcer indexes were smaller with the rat group fed astaxanthin from *H. pluvialis* than the other two groups, indicating that astaxanthin from *H. pluviali* is more efficacious in preventing gastric ulcer evolution caused by stress [45].

The anti-infective and anti-inflammatory effects of astaxanthin are associated with a change in the immune response to *H. pylori* by shifting the T-lymphocyte response from a predominant Th1 response dominated by interferon-γ to a Th1:Th2 response with interferon-γ and interleukin-4 [41, 42, 44], indicating that mice treated with astaxanthin showed a significant increase in interleukin-4 release, which was probably the result of the downregulation of Th1 cells and upregulation of Th2 cells by astaxanthin [41]. Another possible mechanism of action is that astaxanthin as antioxidant neutralizes reactive free oxygen metabolites in the mucosa and may have attenuated the inflammation [42]. It was suspected that the antioxidant properties of astaxanthin played an important role in the protection of the hydrophobic lining of the mucous membrane making colonization by *H. pylori* much more difficult [7].

In a clinical study, although no curative effect of astaxanthin was shown in functional dyspepsia patients, significantly greater reduction of reflux symptoms was found in patients treated with a higher dose of astaxanthin (40 mg daily), and the response was more pronounced in *H. pylori*-infected patients compared with non-*H. pylori*-infected patients, suggesting that suppression of *H. pylori* by astaxanthin led to amelioration of reflux symptoms within the spectrum of functional dyspepsia [21].

3.3.2 Protecting against ethanol and drug toxicities

Studies had revealed that the ethanol-induced gastric damage was mediated by the generation of free radicals [46]. Kim *et al.* [47] found that the oral administration of astaxanthin had significant protection against ethanol-induced gastric lesion in rats and could inhibit elevation of the lipid peroxide level in gastric mucosa. The histologic examination clearly indicated that the acute gastric mucosal lesion induced by ethanol nearly disappeared after pretreatment with astaxanthin [47]. Kamath *et al.* [46] compared the antioxidant and anti-ulcer potency of esterified astaxanthins, saponified astaxanthin, and total carotenoid from the microalga *H. pluvialis* in ethanol-induced gastric ulcers in rats, and showed that total carotenoids and astaxanthin esters, especially esterified astaxanthin exerted a dose-dependent gastroprotective effect on acute, ethanol-induced gastric lesions in rats. The anti-ulcerogenic potency of astaxanthin might be due to inhibition of H^+, K^+-ATPase, upregulation of mucin content, and increase of antioxidant status [46].

The production of oxygen-free radicals and lipid peroxidation plays a crucial role in the development of the gastric mucosal lesions induced by nonsteroidal anti-inflammatory drugs indomethacin or naproxen, which are used clinically as anti-inflammatory and analgesic agents [48, 49]. Studies showed that astaxanthin had the *in vivo* protective effect on indomethacin- or naproxen-induced gastric lesions in rats in a dose-dependent manner, indicating that astaxanthin may offer an attractive new treatment strategy for curing gastric lesions in humans [48, 49].

3.4 Hepatoprotective effect

Astaxanthin was transferred to the liver with lipid and accumulated in the microsomal and the mitochondrial fractions of the liver tissue [50]. Astaxanthin may protect the liver against chemicals such as CCl_4. A study showed that astaxanthin could obstruct the increase of glutamate-oxalacetate transaminase and glutamate-pyruvate transaminase activities and thiobarbituric acid reactive substances in response to CCl_4 while causing an increase in glutathione levels and superoxide dismutase activities in the CCl_4-treated rat liver [51]. A recent study also showed that astaxanthin could attenuate the adverse effect of CCl_4 and protect hepatocytes [52]. These studies suggested that astaxanthin protected liver damage induced by CCl_4 by inhibiting lipid peroxidation, stimulating the cellular antioxidant system [51, 52], and modulating the inflammatory process. Moreover, an early study investigated the preventive effects of β-carotene, β-apo-8′-carotenal, astaxanthin, canthaxanthin,

lycopene, and vitamin A on the initiation of liver carcinogenesis by aflatoxin B1 in male weanling rats, and showed that astaxanthin, β-carotene, β-apo-8′-carotenal, and canthaxanthin were very efficient in reducing the number and the size of liver preneoplastic foci [53]. In particular, astaxanthin, β-apo-8′-carotenal, and canthaxanthin exerted their protective effect through the deviation of aflatoxin B1 metabolism to aflatoxin M1, and thus protected aflatoxin B1 from genotoxicity and initiating actions [53].

Oval cells can differentiate into hepatocytes and biliary epithelial cells, leading to liver regeneration when mature hepatocytes are injured. However, oval cells can trigger hepatic cancer, especially when an irreversible block of the process of normal differentiation is disturbed [54]. Wójcik *et al.* [54] showed that both astaxanthin and β-carotene could inhibit the proliferative activity of oval cells and intensify the differentiation process of oval cells obtained especially from the neoplastic liver, indicating their hepatoprotective properties. In addition, liver ischemia-reperfusion injury is an important clinical problem in many clinical conditions such as liver transplantation, hepatic surgery for tumor excision, and trauma and hepatic failure after hemorrhagic shock [55]. Recently, Curek *et al.* [55] found that total histopathological scoring of cellular damage was significantly decreased in hepatic ischemia-reperfusion injury following astaxanthin treatment, and parenchymal cell damage, swelling of mitochondria, and disarrangement of rough endoplasmatic reticulum were also partially reduced. It was concluded that astaxanthin could offer protection in liver ischemia-reperfusion injury by reducing oxidant-induced protein carbonyl formation and conversion of xanthine dehygrogenase to xanthine oxidase [55].

Current recommended therapy for previously untreated and relapsed hepatitis C patients is a combination of pegylated interferon and ribavirin. Moreover, a large number of supplements are used by patients universally to maintain their health condition. Resveratrol and astaxanthin might be good candidates for an antioxidative as well as an anti-hepatitis C virus agent [56]. Nakamura *et al.* [56] investigated the effect of the two antioxidants on hepatitis C virus replication, and found that pegylated interferon and ribavirin significantly reduced hepatitis C virus RNA replication, but these effects were dose dependently hampered and attenuated by the addition of resveratrol, which significantly enhanced hepatitis C virus RNA replication. On the contrary, astaxanthin did not affect antiviral effects of pegylated interferon or ribavirin, and was suitable as an antioxidant therapy for chronic hepatitis C.

The effects of astaxanthin supplementation in obese mice fed a high-fat diet had been investigated by Ikeuchi *et al.* [57], who found that astaxanthin could inhibit the increases in body weight and weight of adipose tissue with a high-fat diet and reduce liver weight, liver triglyceride, plasma triglyceride, and total cholesterol. One of the mechanisms may be through ameliorating impaired lipid metabolism by increasing adiponectin level and improving insulin sensitivity [58]. Recently, Bhuvaneswari *et al.* [59] also evaluated the effects of astaxanthin in obese mice fed a high fat plus high fructose diet, and showed that astaxanthin restricted weight gain, promoted insulin sensitivity, and prevented liver injury by decreasing cytochrome P 4502E1, myeloperoxidase, and nitro-oxidative stress, and improving the antioxidant status. In addition, lipid deposition and increased transforming growth factor-β expression induced by the high calorie diet were also abolished by astaxanthin [59]. These studies indicated that astaxanthin might be of value in preventing obesity, metabolic syndrome, and liver disease arising from insulin resistance/obesity in affluent societies [57, 59].

3.5 Antidiabetic activity

3.5.1 Diabetes

Diabetes mellitus is strongly associated with oxidative stress, which can be a consequence of increased free radical production, reduced antioxidant defenses, or both [60]. Oxidative stress induced by hyperglycemia possibly causes the dysfunction of pancreatic β-cells and various forms of tissue damage in patients with diabetes mellitus [61]. It was found that astaxanthin could diminish the oxidative stress caused by hyperglycemia in the pancreatic β cells, significantly improve glucose tolerance, increase serum insulin levels, and decrease blood glucose levels, indicating that astaxanthin might exert beneficial effects on pancreatic β-cell function and could protect pancreatic β-cells against glucose toxicity by preventing the progressive destruction of these cells [61].

Based on the strong correlation between oxidative stress and immune dysfunction in diabetic patients, Otton *et al.* [62] recently studied the antioxidant effects of astaxanthin in the reactive oxygen/nitrogen species metabolism of lymphocytes isolated from alloxan-induced diabetic rats. The results showed that astaxanthin could be a good adjuvant in prophylaxis or recovery of lymphocyte dysfunctions associated with diabetic patients, especially when focusing on the re-establishment of the redox balance and a hypothetical antiapoptotic effect in lymphocytes [62].

Nakano *et al.* [63] compared the effect of astaxanthin in combination with other antioxidants such as ascorbic acid and α-tocopherol against oxidative damage in streptozotocin-induced diabetic rats, and indicated that astaxanthin in combination with α-tocopherol could ameliorate oxidative injury through the suppression of oxidative stress induced by diabetes. On the contrary, a high dose of ascorbic acid intake was found to increase lipid peroxidation in diabetic rats [63]. Nishigaki *et al.* [31] recently found that astaxanthin could inhibit the nonenzymatic glycation and glycated protein/iron chelate-induced cytotoxicity in human umbilical-vein endothelial cells by preventing lipid and protein oxidation and increasing the activity of antioxidant enzymes *in vitro*.

In addition, Hussein *et al.* [58] investigated the effects of astaxanthin in a metabolic syndrome animal model of spontaneously hypertensive corpulent rat, and found that astaxanthin significantly lowered the levels of blood glucose, nonesterified fatty acids and triglycerides, and significantly increased the levels of high-density lipoprotein cholesterol and adiponectin, indicating that astaxanthin ameliorates insulin resistance and improve insulin sensitivity by mechanisms involving the increase of glucose uptake, and by modulating the levels of circulating adiponectin and blood lipids [58]. Recently, Bhuvaneswari *et al.* [59] showed that significant elevation in both glucose and insulin levels induced by a high fat plus high fructose diet in mice was abolished by astaxanthin supplementation, also indicating that astaxanthin could substantially improve insulin sensitivity.

3.5.2 Diabetic nephropathy

Uchiyama *et al.* [61] evaluated the renal damage by measuring urinary albumin level, and found that this parameter was significantly lower in astaxanthin-treated db/db mice than in untreated mice. However, it was uncertain whether the antioxidant activity of astaxanthin was directly responsible for the lessened glomerular damage because the blood glucose level of astaxanthin-treated mice was also significantly lower [61]. Naito *et al.* [64] examined whether chronic administration of astaxanthin could prevent the progression of diabetic nephropathy induced by oxidative stress in mice, and showed that astaxanthin could exert beneficial effects on renal mesangial cells and ameliorate the progression of diabetic nephropathy in the rodent model of type 2 diabetes. Kim *et al.* [65] examined the protective action of astaxanthin against high-glucose-induced oxidative stress, inflammation, and apoptosis in proximal tubular epithelial cells. The results demonstrated that astaxanthin had a protective efficacy against several deleterious effects caused by high glucose exposure in proximal tubular epithelial cells. Manabe *et al.* [66] investigated the protective mechanism of astaxanthin on the progression of diabetic nephropathy using an *in vitro* model of hyperglycemia, focusing on normal human mesangial cells, and found that astaxanthin significantly suppressed high glucose-induced reactive oxygen species production, the activation of transcription factors, and cytokine expression or production by mesangial cells. These studies suggested that astaxanthin might prevent the progression of diabetic nephropathy mainly through the reduction of the oxidative stress on the kidneys and the prevention of renal cell damage [64], the modulation of oxidative stress, inflammation, and apoptosis in high-glucose-treated proximal tubular epithelial cells [65], or reactive oxygen species scavenging effect in mitochondria of mesangial cells [66].

In addition, the importance of the transforming growth factor-β signaling in the pathophysiology of diabetic nephropathy was confirmed by Naito *et al.* [67], who determined the gene expression patterns in the glomerular cells of the diabetic mouse kidney and investigated the effects of astaxanthin on the expression of these genes, and found that long-term treatment with astaxanthin significantly decreased the expression of upregulated probes, including those genes associated with oxidative phosphorylation, oxidative stress, and the transforming growth factor-β-collagen synthesis system.

3.5.3 Dental pulp and salivary gland

Leite *et al.* [60] evaluated the effect of astaxanthin on antioxidant enzymes of dental pulp from alloxan-induced diabetic rats. The results showed that although having no effect on superoxide dismutase and catalase activities, astaxanthin could stimulate glutathione peroxidase in control and diabetic rats and partially improved the diabetic complications [60]. In addition, Leite *et al.* [68] also evaluated the effect of astaxanthin on the antioxidant enzymes of salivary gland from alloxan-induced diabetic rats, and showed that astaxanthin restored the enzymatic activities in the salivary gland.

3.6 Cardiovascular protective effect

Carotenoids are believed to have therapeutic benefit in treating cardiovascular disease because of their antioxidant properties [24]. However, clinical trials with several well-known agents such as β-carotene have been disappointing [69], and fail to demonstrate a consistent benefit in patients at risk for cardiovascular disease. This may be attributed to the distinct antioxidant properties of various carotenoids resulting from their structure-dependent physicochemical interactions with biologic membranes [24].

The antioxidant activity of several carotenoids has been investigated by Palozza *et al.* [70] during spontaneous and peroxyl radical-induced cholesterol oxidation. The results showed that these carotenoids exhibited significant antioxidant activity by inhibiting spontaneous and free radical-induced formation of 7-keto-cholesterol and the overall order of efficacy of these carotenoids was astaxanthin > canthaxanthin > lutein = β-carotene. The finding might have important beneficial effects on human health by limiting the formation of atheroma [70]. Iwamoto *et al.* [71] found a dose–response relationship between astaxanthin and low-density lipoprotein oxidation time both *in vitro* and *in vivo*, indicating that astaxanthin could inhibit low-density lipoprotein oxidation and possibly therefore contributed to the prevention of atherosclerosis.

Lipid and macrophage infiltration is closely associated with early plaque development [72]. It was found that astaxanthin significantly reduced the macrophage infiltration in the lesions, and lowered the occurrence of

macrophage apoptosis and plaque ruptures, indicating that astaxanthin might improve plaque stability in the atherosclerotic setting [72] by increasing adiponectin. Another study showed that astaxanthin could suppress the scavenger receptors upregulation, matrix metalloproteinases activation, and pro-inflammatory cytokines expression in macrophages, indicating that astaxanthin is effective to regulate the macrophage atherogenesis-related functions [29].

Hussein *et al.* [73] found that oral administration of astaxanthin for 14 days significantly lowered the arterial blood pressure in spontaneously hypertensive rats but not in normotensive Wistar Kyoto strain, and the long-term administration of astaxanthin for 5 wk could also delay the incidence of stroke in the stroke prone spontaneously hypertensive rats. Subsequently, Hussein *et al.* [74] showed that astaxanthin might modulate the blood fluidity in hypertension, and the antihypertensive effects of astaxanthin might be exerted through mechanisms including normalization of the sensitivity of the adrenoceptor sympathetic pathway, particularly α-adrenoceptors, and by restoration of the vascular tone through attenuation of the angiotensin II- and reactive oxygen species-induced vasoconstriction. In the succedent experiment, Hussein *et al.* [8] further found that astaxanthin significantly reduced the plasma level of NO_2^- and NO_3^-, an indicator of the endogenous formation of NO, and definitive structural alterations in the coronary artery and aorta of spontaneously hypertensive rats were ameliorated by astaxanthin, suggesting that astaxanthin could modulate the oxidative condition and might improve vascular elastin and arterial wall thickness in hypertension. These results indicated that astaxanthin could exert beneficial effects in protection against hypertension and stroke [8, 73, 74]. In addition, it was shown that astaxanthin lowered blood pressure and lessened the activity of the renin-angiotensin system in Zucker Fatty Rats, indicating that the renin-angiotensin system was involved in the ability of astaxanthin to lower blood pressure [75].

A recent study showed that astaxanthin could increase heart mitochondrial membrane potential and contractility index dose dependently and tend to decrease plasma interleukin-1α, tumor necrosis factor-α, and serum amyloid A concentrations in BALB/c mice, supporting the possible effect of astaxanthin for cardiac protection [76]. Pashkow *et al.* [69] suggested that there might be a potential therapeutic role for astaxanthin in the management of myocardial injury, oxidized low-density lipoprotein, and rethrombosis after thrombolysis, as well as other cardiac diseases such as atrial fibrillation. Augusti *et al.* [77] indicated that although astaxanthin did not prevent hypercholesterolemia or atherosclerotic lesions caused by the atherogenic diet in rabbits, it could play a beneficial role by preventing lipid peroxidation and changes in antioxidant enzyme activities.

Moreover, Lauver *et al.* [78] reported that disodium disuccinate astaxanthin, a water-dispersible synthetic astaxanthin derivative, reduced myocardial damage in a rabbit model of ischemia/reperfusion and suggested that the mechanisms of action might include both antioxidant and anticomplement components.

In a human study, Yoshida *et al.* [79] recently indicated that astaxanthin increased adiponectin and ameliorated triglyceride and high-density lipoprotein cholesterol in humans, and the changes of adiponectin correlated positively with high-density lipoprotein cholesterol changes independent of age and body mass index. In addition, Miyawaki *et al.* [80] studied healthy adult male volunteers with a blood transit time of 45–70 s to evaluate the effect on blood rheology from continuous ingestion of astaxanthin 6 mg/day for a 10-day period, and showed a shortening of blood transit time from 52.8 ± 4.9 to 47.6 ± 4.2 s, indicating an improvement of human blood rheology by astaxanthin.

3.7 Anticancer activity

A case-control study with a large cohort involving ten countries showed that higher plasma concentrations of some individual carotenoids, retinol, and α-tocopherol were associated with reduced risk of gastric cancer [81]. There are two different classes of chemopreventive agents, retinoids/provitamin A carotenoids and the nonprovitamin carotenoids, which may act through separate mechanisms [82]. Increasing evidence has shown that carotenoids possess potent cancer chemopreventive properties independent of their antioxidant activity or their potential for conversion to retinoids [23]. Some of carotenoids showed more potent anticarcinogenic activity than β-carotene and might be more useful for cancer prevention [83]. It had been reported that in individuals at high risk for developing lung cancer as a consequence of smoking and/or asbestos exposure, β-carotene failed to demonstrate protection, and even was found to induce lung pathology [84], suggesting that the use of carotenoids without pro-vitamin A activity such as astaxanthin might provide protection and avoid the toxicity associated with retinoids [82]. Chew and Park [22] had suggested that although astaxanthin, canthaxanthin, and β-carotene inhibited tumor growth, astaxanthin showed the highest anti-tumor activity. Growth-inhibitory effects of astaxanthin have been reported in different tumor cells, including colon, oral fibrosarcoma, breast, prostate cancer cells, and embryonic fibroblasts [23].

Daubrawa *et al.* [85] compared the effects of canthaxanthin and astaxanthin on gap junctional intercellular communication, which is important for homeostasis, growth control, and development of cells, in primary human skin fibroblasts, and found that astaxanthin was a strong suppressor of gap junctional intercellular communication and affected channel function by changing the phosphorylation pattern of connexin43. However, in contrast to astaxanthin, canthaxanthin and other carotenoids could stimulate gap junctional intercellular communication and

enhance connexin43 expression in cell culture. Briviba *et al.* [86] compared the subcellular localization of astaxanthin and β-carotene in cultured HT29 human colon adenocarcinoma cells. The results showed that astaxanthin was effectively taken up by the cells and localized mostly in the cytoplasm, and cells incubated with β-carotene showed about a 50-fold lower cellular amount of β-carotene. The difference of astaxanthin and β-carotene distribution in cells of intestinal origin suggested that the possible defense against reactive molecules by carotenoids in these cells might also be different [86].

Astaxanthin was found to have considerable preventive activities on azoxymethane-induced large bowel carcinogenesis and 4-nitroquinoline-1-oxide-induced tongue carcinogenesis in rats [87]. Tanaka *et al.* [88, 89] found that astaxanthin was possible chemopreventive agent for *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine-induced bladder carcinogenesis in male ICR mice and 4-nitroquinoline-1-oxide-induced oral carcinogenesis in male F344 rats, partly due to suppression of cell proliferation. Jyonouchi *et al.* [90] showed that astaxanthin could suppress fibrosarcoma cell growth and stimulated immunity against tumor antigen, suggesting that astaxanthin might exert antitumor activity through the enhancement of immune responses.

Kozuki *et al.* [91] found that astaxanthin could inhibit the invasion of rat ascites hepatoma AH109A cells in a coculture system with rat mesentery-derived mesothelial cells in a dose-dependent manner. AH109A cells cultured with hypoxanthine and xanthine oxidase showed a highly invasive activity and astaxanthin could suppress this reactive oxygen species-potentiated invasive capacity [91]. Kurihara *et al.* [92] showed that astaxanthin could inhibit stress-induced impairment to the antitumor activity of natural killer cells *via* its antioxidative property, and thus inhibit the stress-induced promotion of hepatic metastasis in mice. Moreover, astaxanthin could improve stress-induced immune dysfunction better potently than α-tocopherol and β-carotene [92]. Tripathi and Jena [93] found that astaxanthin could attenuate oxidative stress, DNA damage, cell death as well as induction of early hepatocarcinogenesis in rat induced by cyclophosphamide. It was suggested that the protective effect of astaxanthin was mediated through the upregulation of nuclear factor E_2-related factor 2 – antioxidant-response element pathway [93].

It has been reported that astaxanthin could inhibit the growth of mammary tumors in female BALB/c mice [94]. Recently, Nakao *et al.* [95] reported that astaxanthin fed prior to tumor initiation could suppress mammary tumor growth, and increase the natural killer cell populations and plasma interferon-γ concentration in BALB/c mice injected with a mammary tumor cell line. However, astaxanthin supplementation after tumor initiation might be contraindicated and would result in more rapid tumor growth and elevate palsma inflammatory cytokines interleukin-6 and tumor necrosis factor-α, emphasizing the importance of antioxidant status prior to disease initiation [95].

The inhibitory effect of astaxanthin against chemically induced colonic pre-neoplastic progression was found by Prabhu *et al.* [96], who showed that the decreased levels of colon enzymic and nonenzymic antioxidants and increased levels of lipid peroxidation marker levels in a dimethylhydrazine-induced rat colon carcinogenesis model were significantly reversed on astaxanthin administration. Palozza *et al.* [23] demonstrated that *H. pluvialis* extract could inhibit the growth of HCT-116, HT-29, LS-174, WiDr, and SW-480 human colon cancer cells by arresting cell-cycle progression and promoting apoptosis. Moreover, it was also found that the effects of *H. pluvialis* extract on cell growth and apoptosis were more pronounced than those of purified astaxanthin at the same astaxanthin concentration [23].

Adult T-cell leukemia is a fatal malignancy of T lymphocytes caused by human T-cell leukemia virus type 1 infection and remains incurable [97]. Ishikawa *et al.* [97] found that β-carotene and astaxanthin had mild inhibitory effects on human T-cell leukemia virus type 1-infected T-cell lines, and the inhibitory activities of fucoxanthin and its deacetylated metabolite fucoxanthinol were stronger than those of β-carotene and astaxanthin.

3.8 Neuroprotective effect

Ikeda *et al.* [98] found that astaxanthin markedly suppressed 6-hydroxydopamine-induced apoptosis in human neuroblastoma SH-SY5Y cells by inhibiting intracellular reactive oxygen species generation, thereby attenuating p38 MAPK activation and mitochondrial dysfunction. Liu *et al.* [99] demonstrated that astaxanthin could prevent docosahexaenoic acid hydroperoxide- or 6-hydroxydopamine-induced neuronal apoptosis, mitochondrial abnormalities, and intracellular reactive oxygen species generation in SH-SY5Y cells. Chan *et al.* [100] also showed that astaxanthin likely enhanced cell and mitochondrial membrane stability. These studies suggested that astaxanthin had the protective effects on a neurodegenerative disease, dependent on its antioxidant potential and mitochondria protection, and might be a promising neuroprotective therapeutic agent for oxidative stress-associated neurodegeneration such as Parkinson's disease [98–100].

Chang *et al.* [101] recently found that astaxanthin showed an amazingly potent protective effect against the damaging effects elicited by β-amyloid peptide 25–35 in PC12 cells, and might be used as a very potential neuron protectant and a potent anti-Alzheimer's disease adjuvant therapy, particularly in its early stage.

It was found that astaxanthin could reduce ischemia-induced free radical damage, apoptosis, neurodegeneration, and cerebral infarction in brain tissue through the inhibition of oxidative stress, reduction of glutamate release, and antiapoptosis, and might be clinically useful for patients vulnerable or prone to ischemic events [102]. Recently, Lin *et al.* [103] used isolated nerve terminals (synaptosomes)

purified from the rat cerebral cortex to investigate the effect of astaxanthin on endogenous glutamate release, and showed that astaxanthin exhibited a dose-dependent inhibition of 4-aminopyridine-elicited release of glutamate, presenting an additional explanation for the neuroprotective effect of astaxanthin besides antioxidant and anti-inflammatory properties. Kim *et al.* [104] found that astaxanthin could inhibit H_2O_2-mediated apoptotic death *via* modulation of p38 and MEK signaling pathways. These results highlighted the therapeutic potential of astaxanthin in the prevention and treatment of a wide range of neurological and neurodegenerative disorders [102, 104].

Recently, Kim *et al.* [105] demonstrated that astaxanthin as an extracellular factor enhanced stem cell potency *via* an increase of the proliferative capacity in neural stem cells, and improved the osteogenic and adipogenic differentiation potential of neural stem cells. In addition, Abadie-Guedes *et al.* [106] demonstrated that astaxanthin could antagonize the ethanol-induced facilitation of cortical spreading depression propagation in the young adult rat brain and its antioxidant properties might be involved in such effects.

3.9 Ocular protective effect

It had been reported that, after ingestion of astaxanthin for consecutive 28 days, the uncorrected far visual acuity was significantly improved and the accommodation time was significantly shortened in healthy volunteers over 40 years of age receiving 4 or 12 mg once a day, and there was no change in refraction, flicker fusion frequency, or pupillary reflex [107]. In another experiment, it was found that astaxanthin extracted from the microalga *H. pluvialis* significantly improved the deep vision and the critical flicker fusion of healthy adult male volunteers, and no effects on static and kinetic visual acuity were observed [108]. Nagaki *et al.* [109] found that 6 mg of astaxanthin from *H. pluvialis* *per* day could improve eye fatigue in visual display terminal workers. It was shown that astaxanthin might increase retinal capillary blood flow in both eyes in normal volunteers and intraocular pressures remained unchanged during the supplementation period [110]. In addition, Izumi-Nagai *et al.* [111] concluded that astaxanthin treatment, together with inflammatory processes including NF-κB activation, subsequent upregulation of inflammatory molecules, and macrophage infiltration, significantly suppressed the development of choroidal neovascularization capable of leading to severe vision loss and blindness.

Ohgami *et al.* [112] indicated that astaxanthin had a dose-dependent ocular anti-inflammatory effect on endotoxin-induced uveitis through suppressing the production of nitric oxide, prostaglandin E2, and tumor necrosis factor-α by directly blocking nitric oxide synthase activity. In their succedent study, Suzuki *et al.* [113] showed that astaxanthin could reduce ocular inflammation in eyes with endotoxin-induced uveitis by downregulating proinflammatory factors and inhibiting the nuclear factor-κB-dependent signaling pathway, suggesting that astaxanthin might be a promising agent for the treatment of ocular inflammation [112, 113].

Astaxanthin was found to be capable of providing appreciable protection for vulnerable tryptophan residues and β_{high}-crystallin against oxidative stress, and thus capable of protecting porcine lens crystallins against oxidative damage and degradation by calcium-induced calpain [114]. Liao *et al.* [115] reported that astaxanthin could interact with selenite, whose accumulation in the lens might cause cataract formation directly, and thus could delay selenite-induced lens crystalline precipitation and attenuate selenite-induced cataractogenesis in rats. Nakajima *et al.* [116] found that astaxanthin had neuroprotective effects against retinal ganglion cell damage. Recently, Cort *et al.* [117] showed that astaxanthin significantly decreased the percent of apoptotic cells on the retina in rats with elevated intraocular pressure. This study confirmed the role of oxidative injury in elevated intraocular pressure and highlighted the protective effect of astaxanthin in ocular hypertension [117].

3.10 Skin-protective effect

It was reported that preincubation with synthetic astaxanthin or an algal extract containing 14% of astaxanthin could prevent ultraviolet A-induced alterations in cellular superoxide dismutase activity and decrease in cellular glutathione content [118]. Camera *et al.* [119] compared the modulation of ultraviolet A-related injury by astaxanthin, canthaxanthin, and β-carotene for systemic photoprotection in human dermal fibroblasts, and found that astaxanthin exhibited a pronounced photoprotective effect and counteracted ultraviolet A-induced alterations to a significant extent, and uptake of astaxanthin by fibroblasts was higher than that of canthaxanthin and β-carotene, indicating that astaxanthin had a superior preventive effect toward photooxidative changes. Recently, Suganuma *et al.* [120] examined the effects of astaxanthin on the induction of matrix-metalloproteinase-1 and skin fibroblast elastase by ultraviolet A treatment of cultured human dermal fibroblasts, and showed that astaxanthin could interfere with ultraviolet A-induced matrix-metalloproteinase-1 and skin fibroblast elastase/neutral endopeptidase expression. These studies suggest that topical or oral administration of astaxanthin might prevent or minimize the effects of ultraviolet A radiation such as skin sagging or wrinkling [118, 120].

3.11 Effect on exercise endurance

It has been shown that astaxanthin from *H. pluvialis* could significantly lower serum lactic acid concentration in adult male volunteers at 2 min after 1200 m running and no other effects were observed, suggesting that astaxanthin is effective for the improvement of muscle fatigue that might lead

to sports performance benefits [108]. Another study showed that astaxanthin might preferentially attenuate sensations of delayed-onset muscular soreness, which is one of the symptoms of exercise induced muscle damage, in weight trained individuals with a high percentage area for fiber types IIA and IIAB/B [121].

Aoi *et al.* [122] found that astaxanthin could attenuate exercise-induced damage in mouse skeletal muscle and heart, including an associated neutrophil infiltration that induced further damage. In the succedent experiments, Aoi *et al.* [123] showed that astaxanthin promoted lipid metabolism rather than glucose utilization during exercise *via* carnitine palmitoyltransferase I activation, which led to the improvement of endurance and efficient reduction of adipose tissue with training. In another study, Ikeuchi *et al.* [124] also showed that astaxanthin could cause a decrease in glucose utilization and an increase in fatty acid utilization as an energy source during exercise. The glycogen thus saved could become an available energy source for the later stages of exercise, and thus slower utilization of glycogen resulted in improved endurance exercise performance and delaying the onset of fatigue [124].

3.12 Effect on fertility

Eskenazi *et al.* [125] suggested that a healthy diet with high intake of antioxidants might be an inexpensive and safe way to improve semen quality and fertility. Comhaire *et al.* [126] evaluated the effects of astaxanthin as complementary treatment to improve the outcome of the World Health Organization male infertility treatment guidelines in a pilot double-blind randomized trial. Sixteen milligrams *per* day of astaxanthin was given to the male partners of 20 infertile couples, whose semen characteristics were below the World Health Organization recommended reference values. The results showed that astaxanthin significantly decreased reactive oxygen species and the secretion of inhibin B by the Sertoli cells, indicating a positive effect of astaxanthin on sperm parameters and fertility [126]. In addition, Tripathi and Jena [127] showed that astaxanthin treatment significantly improved the testes weight, sperm count, and sperm head morphology as compared with only cyclophosphamide-treated animals, indicating the chemoprotective potential of astaxanthin against cyclophosphamide induced germ cell toxicity in mice.

3.13 Effect on kidney function impairment

Inorganic mercury is accumulated mainly in kidneys after absorption and causes acute renal failure. Reactive oxygen species are implicated as mediators of tissue damage in the acute renal failure induced by inorganic mercury [128]. Augusti *et al.* [128] investigated the possible protective effect of astaxanthin against nephrotoxicity induced by mercuric chloride, and indicated that astaxanthin could have a beneficial role against $HgCl_2$ toxicity by preventing lipid and protein oxidation, changes in the activity of antioxidant enzymes, and histopathological changes.

4 Concluding remarks

Growing evidence from tissue culture, animal, and clinical studies (Supporting Information Tables S1, S2, and S3) suggests that astaxanthin has potential health-promoting effects in the prevention and treatment of various diseases, such as cancers (gastric, colon, breast, prostate, oral, tongue, bladder, liver cancers, fibrosarcoma, and leukemia), chronic inflammatory diseases (asthma, sepsis, rheumatoid arthritis, atherosclerosis, inflammatory bowel disease, and brain inflammatory diseases), metabolic syndrome (obesity, dyslipidemia, hypertension, and insulin resistance), diabetes, diabetic nephropathy, cardiovascular disease (hypertension, atherosclerosis, stroke, atrial fibrillation, rethrombosis after thrombolysis, and myocardial injury), gastrointestinal diseases (gastritis, gastric ulcer, duodenal ulcer, and ethanol- or drug-induced gastric lesions), liver disease (fatty liver, hepatitis, liver ischemia-reperfusion injury, and chemicals-induced liver damages), neurodegenerative diseases (ischemia/reperfusion-induced neurodegeneration, Parkinson's disease, Alzheimer's disease, and other neurodegenerative disorders), eye disease (cataract, glaucoma, ocular inflammatory such as uveitis, choroidal neovascularization, and eye fatigue from visual display terminals), skin diseases (ultraviolet A-induced skin damage, skin cancer, and skin sagging or wrinkling), exercise-induced fatigue (muscle fatigue, delayed-onset muscular soreness), male infertility, and $HgCl_2$-induced acute renal failure.

These protections against various diseases by astaxanthin are likely to involve antioxidant mechanisms including prevention of oxidative damage and cellular necrosis or apoptosis induced by oxidative stress; other potential mechanisms include decreased expression or production of inflammatory mediators and cytokines by suppressing the activation of nuclear factor-κB, decreased expression or production of transforming growth factor-β1, increased levels of circulating adiponectin and insulin sensitivity, decreased activity of the renin-angiotensin system, and antimicrobial activity against *H. pylori*, *etc.* Although the currently available data and recent findings are very encouraging, more extensive, well-controlled clinical trials, especially for 9-*cis*-astaxanthin, are suggested for each of these categories.

This work is supported by Science and Technology Planning Project of Guangdong Province, China (2007B020708003).

The authors have declared no conflict of interest.

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