Astaxanthin as a Novel Mitochondrial Regulator

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Astaxanthin is a member of the carotenoid family that is found abundantly in marine organisms. It has been reported that astaxanthin functions both as a pigment, and as an antioxidant with superior free radical quenching capacity.

astaxanthin obesity mitochondria energy metabolisms natural antioxidant insulin resistance AMPK

1. Introduction

1.1. Hidden Bioactivity of Natural Pigments

1.1.1. Nature Is Full of Splendid Color!

When we look at the natural world around us, we can find a biodiversity of colors in both plants and animals. Colors can be formed when light is absorbed and reflected by pigments and dyes, or when light scatters from micro- and nanostructures to form structural colors. In nature, most colors are produced by pigments derived from both organic and mineral sources. Major organic pigment types include the following: porphyrins, such as green chlorophylls and red hemes; flavonoids, such as blue-purple anthocyanins of flowers and fruits; and carotenoids, a large group of yellow, orange, and red pigments found in plants, algae, bacteria, and fungi [1]. In addition to contributing color, pigments also have a great variety of documented physiological activities [2][3][4][5].

1.1.2. Carotenoids

Most carotenoids are strongly lipophilic, including β-carotene—found abundantly in carrots—and lycopene, which gives tomatoes and watermelons their red color [1] (**Figure 1**). In animals, many carotenoids, such as β-carotene, are known as provitamin A carotenoids, because they serve as precursors in the metabolic synthesis of vitamin A and its derivatives [1]. With few exceptions, such as some arthropods, animals cannot synthesize carotenoids de novo [6]. Therefore, animals depend on dietary sources for a supply of carotenoids.

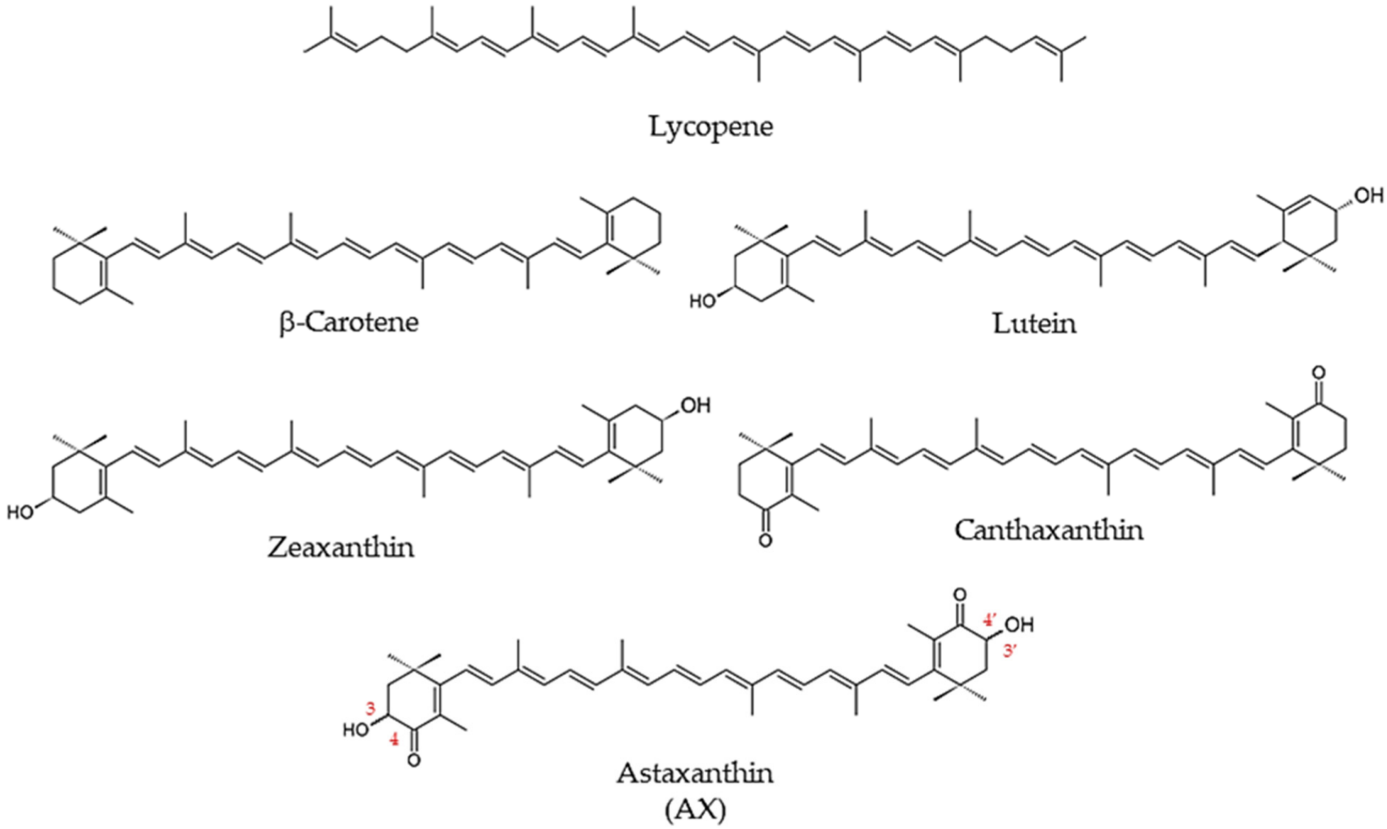

Figure 1. Structure of astaxanthin (AX) and related carotenoids.

1.1.3. What Is Astaxanthin?

AX is a carotenoid that is frequently found in aquatic organisms, where it contributes its bright orange-to-red color, as in the shells of shrimp and crab, and in the muscles of salmon and trout [7][8][9]. Although AX is best recognized as a pigment characteristic of aquatic organisms, it's extensive presence in prokaryotes and eukaryotes is less commonly known. AX is a derivative of β-carotene, bearing a similar structure that differs at its terminal β-ionone rings. In contrast to β-carotene, the β-ionone rings of AX have hydroxyl groups at the 3,3′-positions, and keto groups at the 4,4′-positions. The long central polyene chain consists of conjugated double bonds (**Figure 1**).

Unlike β-carotene, AX shows negligible pro-vitamin A activity, except under unusual conditions such as severe vitamin A deficiency [10]. The carbons attached to the hydroxyl groups at both ends are chiral, producing optical isomers that differ based on the orientations of the hydroxyl groups. The hydroxyl groups of AX can bind to fatty acids, sugars, or proteins. In addition, the central polyene chain often has an all-trans configuration, but there are also geometric isomers, in which portion(s) of AX may bear a *cis* configuration [9].

Based on its long-standing presence in the human diet, and an abundant number of published safety studies, AX is considered safe for food consumption, and has been used as a functional food additive for humans in recent years. The most common source of AX used in functional foods and supplements comes from a unicellular green alga called *Haematococcus*, with krill representing a more minor secondary source.

Haematococcus algae are green and motile cells during their active growing or vegetative state, until the growth environment becomes unfavorable due to nutrient starvation, high light conditions, or high osmotic pressure. In response to such adverse growth conditions, the algal cells transition into a resting state in which they accumulate high concentrations of AX; transforming into red-colored cyst cells, with increased longevity [11]. The unique ability of *Haematococcus* algae to accumulate high concentrations of natural AX is leveraged for industrial production.

1.2. Biological Activity of Astaxanthin

1.2.1. Function of Astaxanthin in Lipid Bilayers: Antioxidant Activity and Impact on Physical Properties

AX has antioxidant activity, a well-known characteristic of carotenoids. Aside from its ability to quench a number of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and other free radicals, AX stands out among carotenoids due to its particularly strong singlet oxygen quenching activity [12][13][14]. AX is also well-known for strongly inhibiting the accumulation of lipid peroxides resulting from lipid peroxidation chain reactions [15][16]. In biological environments, AX has been detected in lipid droplets [17], cell membranes [18], or bound to proteins [16][19][20][21], due to its highly lipophilic properties. In addition, the structure of AX, like several other xanthophylls, it is thought to span across phospholipid bilayers that form biological membranes [22][23][24][25][26]. This is based, in part, on the observation that AX was able to quench or scavenge ROS, RNS and free radicals both in the interior and surface layers of lipid membranes (**Figure 2**).

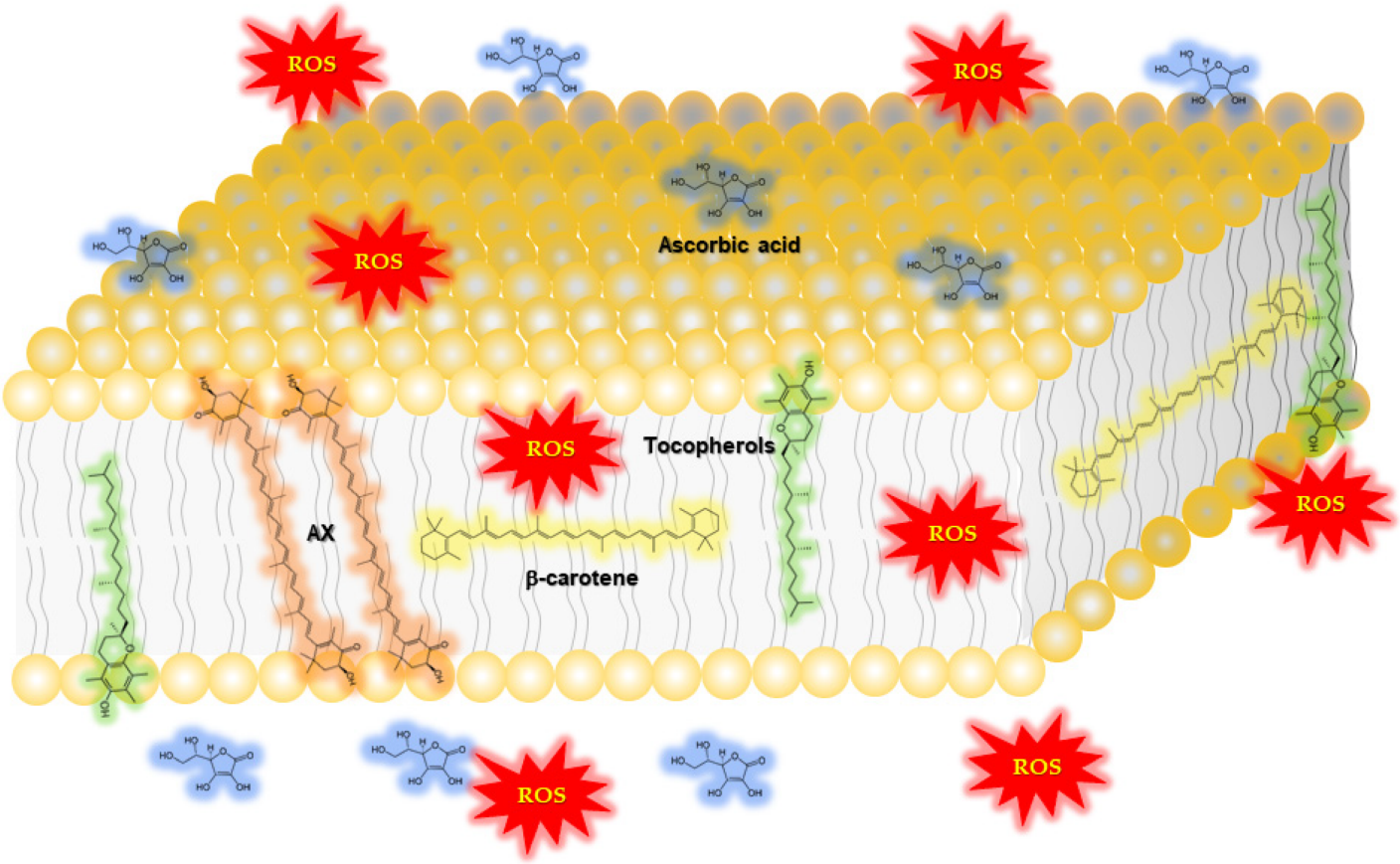

Figure 2. AX performs its antioxidant activity both inside and on the surface of the plasma membrane. Due to its strongly hydrophobic conjugated polyene structure and terminal polar groups, AX can exist both inside and on the surface of the phospholipid membrane. Therefore, AX is able to exert its effects against ROS both at the surface and inside of phospholipid membranes. On the other hand, β-carotene exerts its antioxidant activity only inside the phospholipid membrane. As for other antioxidants, ascorbic acid cannot exert its effect inside the phospholipid membrane, due to its high hydrophilicity, whereas tocopherols are relatively effective at the surface of the phospholipid membrane. This figure excludes the detailed structure of the cell membrane, including localization of different levels of lipids lipid rafts and proteins to avoid complications.

The antioxidant activity of some carotenoids can shift to pro-oxidant activity depending on carotenoid concentrations, under conditions of high oxygen tension, or based on interactions with other compounds [27]. Therefore, carotenoids are categorized into three classes: (A) those without significant antioxidant properties; (B) those with good antioxidant, but also pro-oxidant properties; and (C) those with strong antioxidant and without any pro-oxidant properties. AX was categorized as class (C), whereas β-carotene and lycopene were identified as class (B) [27]. Therefore, AX is often described as a “pure antioxidant”. In fact, it has been demonstrated that AX, in contrast to β-carotene and lycopene, exhibited significant antioxidant activity and reduced lipid peroxidation in a liposomal model membrane [23]. When applied to biological membranes, AX may allow *Haematococcus* cyst cells to resist oxidative stress resulting from adverse environmental conditions [11][28]. AX may also exert a protective role in muscle cell membranes during the extreme physical exertion experienced by salmon, during migration from the sea to their spawning ground. Based on this scenario in salmon, AX has also been investigated as an intervention for oxidative muscle damage during and after endurance exercise [29]. Although it is still unclear whether the observed effects of AX are a result of its direct and/or indirect antioxidant activity, several clinical reports have shown that AX reduced oxidative stress markers in humans (**Table 1**).

Table 1. Human clinical studies with astaxanthin (AX) that examined oxidative stress markers.

Author/Year/Reference	Study Design	Subjects	Dose	Duration	Outcome
McAllister M.J. et al., 2021 [30]	Randomized, double-blind, placebo-controlled, crossover study	14 healthy subjects	0, 6 mg/day	4 weeks	Glutathione was ~7% higher following AX compared with placebo ($p < 0.05$). No effect on plasma hydrogen peroxide or malondialdehyde (MDA; $p > 0.05$). Advanced oxidation protein products (AOPP)

Author/Year/Reference	Study Design	Subjects	Dose	Duration	Outcome
					reduced by ~28% (N.S.; $p = 0.45$).
Petyaev I.M., et al., 2018 [31]	Randomized, blinded, four-arm, prospective study	32 subjects with oxidative stress, 8 subjects taking AX only	0, 7 mg/day *	4 weeks	Reduced serum oxidized LDL by 55.4% after 4 weeks ($p < 0.05$). Reduced MDA by 52.7% after 4 weeks ($p < 0.05$).
Chalyk, N. et al., 2017 [32]	Open-label, prospective study	31 subjects; 18 obese, 8 overweight, 5 healthy weight	4 mg/day	92 days	Plasma MDA decreased with AX by 11.2% on day 15 and by 21.7% on day 29 (N.S.)
Hashimoto H. et al., 2016 [33]	Open-label, prospective study	35 subjects during cataract surgery	6 mg/day	2 weeks	Superoxide anion scavenging activity (U/mL) 18.2 ± 4.1 at 0 weeks reduced to 19.9 ± 3.6 after 2 weeks of supplementation compared with baseline, $p < 0.05$. Total hydroperoxides (U CARR) from 1.16 ± 0.18 at 0 weeks reduced to 1.04 ± 0.31 after 2 weeks of supplementation compared with baseline, $p < 0.05$
Baralic, I. et al., 2015 [34]	Randomized, double-blind,	40 healthy subjects (soccer	0, 4 mg/day	90 days	Improved prooxidant-antioxidant balance (PAB;

Author/Year/Reference	Study Design	Subjects	Dose	Duration	Outcome
	placebo-controlled, prospective study	players)			$p < 0.05$)
Baralic I. et al., 2013 [35]	Randomized, double-blind, prospective study	40 healthy subjects (soccer players)	0, 4 mg/day	90 days	Protected thiol groups against oxidative modification (increase in -SH groups, $p < 0.05$; improved PON1 activity towards paraoxon and diazoxon, $p < 0.05$ and $p < 0.01$, respectively)
Hashimoto, H. et al., 2013 [36]	Open-label, prospective study	35 cataract patients	6 mg/day	2 weeks	Reduced total hydroperoxides (hydrogen peroxides, lipid peroxides, and peroxides of protein in aqueous humor; $p < 0.05$), increased superoxide scavenging activity ($p< 0.05$)
Choi H.D. et al., 2011 [37]	Randomized, two-arm, prospective study	23 obese and overweight subjects	5 and 20 mg/day	3 weeks	5 mg/day: MDA decreased by 34.6%, isoprostane (ISP) decreased by 64.9%, superoxide dismutase (SOD) increased by 193%, and total antioxidant capacity (TAC) increased by 121% after 3 weeks compared with baseline ($p < 0.01$).

Author/Year/Reference	Study Design	Subjects	Dose	Duration	Outcome
					20 mg/day: MDA decreased by 35.2%, ISP decreased by 64.7%, SOD increased by 194%, and TAC increased by 125% after 3 weeks compared with baseline ($p < 0.01$).
Choi, H.D. et al., 2011 [38]	Randomized, double-blind, placebo-controlled, prospective study	27 overweight subjects	0, 20 mg/day	12 weeks	MDA reduced by 17.3% and 29% after 8 and 12 weeks compared with placebo ($p < 0.01$), isoprostane (ISP) reduced by 40.2% and 52.9% after 8 and 12 weeks compared with placebo ($p < 0.01$), superoxide dismutase (SOD) increased by 124.8% after 12 weeks compared with placebo ($p < 0.01$), and total antioxidant capacity (TAC) increased by 130.1% after 12 weeks compared with placebo ($p < 0.05$) (See Table 3 for other outcomes.)
Hashimoto H. et al., 2011 [39]	Open-label, prospective study	35 cataract patients	6 mg/day	2 weeks	Reduced total hydroperoxides (hydrogen peroxides, lipid peroxides, and peroxides of protein

Author/Year/Reference	Study Design	Subjects	Dose	Duration	Outcome
					in aqueous humor; $p < 0.05$)
Kim, J.H. et al., 2011 [40]	Randomized, Repeated, measured, prospective study	39 heavy smokers, 39 non-smokers	0, 5, 20, or 40 mg/day	3 weeks	5 mg/day: MDA and ISP significantly lower after 2 and 3 weeks compared with baseline in smokers ($p < 0.05$). SOD and TAC significant increase after 1, 2, and 3 weeks compared with baseline in smokers ($p < 0.05$) 20 mg/day: MDA and ISP significantly lower after 1, 2, and 3 weeks compared with baseline in smokers ($p < 0.05$). SOD and TAC significant increase after 1, 2, and 3 weeks compared with baseline in smokers ($p < 0.05$). 40 mg/day: MDA and ISP significantly lower after 1, 2, and 3 weeks compared with baseline in smokers ($p < 0.05$). SOD and TAC significant increase after 2 and 3 weeks compared with baseline in smokers ($p < 0.05$)
Nakagawa K. et al., 2011 [41]	Randomized, double-blind,	30 healthy subjects	0, 6, 12 mg/day	12 weeks	6 mg/day: reduction in total phospholipid hydroperoxides (PLOOH) after 12 weeks compared with baseline ($p < 0.01$)

Author/Year/Reference	Study Design	Subjects	Dose	Duration	Outcome
	placebo-controlled, prospective study				and compared with placebo ($p < 0.05$). Reduced phosphatidyl-ethanolamine hydroperoxide (PEOOH) after 12 weeks compared with baseline ($p < 0.05$) and compared with placebo ($p < 0.05$). 12 mg/day: 48% reduction in total PLOOH after 12 weeks compared with baseline ($p < 0.01$) and 35% less total PLOOH at 12 weeks compared with the control group ($p < 0.05$). The 12 mg/day group had 46% less phosphatidylcholine hydroperoxide (PCOOH) at 12 weeks compared with baseline ($p < 0.01$).
Peng L. et al., 2011 [42]	Randomized, placebo-controlled study	115 healthy subjects	0, 40 mg/day	90 days	Comparing with the control group, MDA contents in the test group decreased significantly ($p < 0.01$), and SOD and GSH-Px activities increased significantly ($p < 0.01$).
Park J.S. et al., 2010 [43]	Randomized, double-blind,	42 healthy subjects	2 or 8 mg/day	8 weeks	2 mg/day: Concentrations of plasma 8-hydroxy-2′-deoxyguanosine reduced

Author/Year/Reference	Study Design	Subjects	Dose	Duration	Outcome
	placebo-controlled, prospective study				after 4 weeks and 8 weeks compared with placebo ($p < 0.05$). 8 mg/day: Concentrations of plasma 8-hydroxy-2′-deoxyguanosine reduced after 4 weeks and 8 weeks compared with placebo ($p < 0.05$)
Iwabayashi M. et al., 2009 [44]	Open-label, prospective study	35 healthy subjects (with high oxidative stress)	12 mg/day	8 weeks	Increased blood biological antioxidant potential (BAP; +4.6%, $p < 0.05$)
Yamada T. et al., 2010 [45]	Open-label,prospective study	6 healthy subjects and 6 Sjoegren's syndrome subjects	12 mg/day	2 weeks	Reduced protein oxidation (−10%, $p < 0.05$)
Fassett, R.G. et al., 2008 [46]	Randomized, double-blind, placebo-controlled, prospective study	58 renal transplant recipients	0, 12 mg/day [23][25]	12 months	Total plasma F2-isoprostanes reduced by 23.0% in placebo and 29.7% in AX groups (N.S.)
Karppi, J. et al., 2007 [47]	Randomized, double-blind,	39 healthy subjects	0, 8 mg/day	3 months	Decreased oxidation of fatty acids in healthy men ($p < 0.05$)

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phospholipid membrane packing, and decrease alkyl-chain motion in the fluid phase. These effects are strongest for dipolar xanthophylls (i.e., AX), significantly weaker for monopolar xanthophylls (i.e., β-cryptoxanthin), and

Author/Year/Reference	Study Design	Subjects [49]	Dose	Duration	Outcome
	placebo-controlled, prospective study				
Kim Y.K. et al., 2004 [48] [53]	Open-label, prospective study [52] [53]	15 healthy postmenopausal women	0, 2, 8 mg/day	8 weeks [49]	Decreased plasma TBARS levels: 2 mg group from 1.42 ± 0.18 to 1.13 ± 0.18 nM/mg ($p < 0.05$). 8 mg AX group from 1.62 ± 0.14 nM/mg to 1.13 ± 0.12 nM/mg after 8 weeks [50][51] ($p < 0.05$). Increased TAS from 0.85 ± 0.42 mM/L to 1.90 ± 0.58 mM/L in the 8 mg group. Urinary 8-isoprostanes excretion did not decrease significantly. (See Table 3 for other outcomes.)

One of the most important physiological activities of AX, which is strongly associated with its antioxidant activity, is its anti-inflammatory activity in response to inflammation triggered by ROS-induced oxidative damage. Numerous studies have shown that AX inhibits canonical nuclear factor-kappa B (NFκB) signaling in response to oxidative stress via the inhibition of IKK oxidation, regardless of the source of ROS, cell types, or organ [29][54][55][56][57][58][59][60][61][62][63][64][65]. As a result, AX suppressed NFκB-mediated gene expression of pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, iNOS or TNFα, thereby inhibiting the development of inflammation. AX is reported to inhibit the phosphorylation and nuclear translocation of STAT3 in the 7,12-dimethyl benz[a]anthracene (DMBA)-induced hamster buccal pouch (HBP) carcinogenesis model [66]. Therefore, it is likely that AX can act in an inhibitory manner on the JAK/STAT pathway, which is an inflammatory signaling pathway of cytokines such as IL-6, although there is little evidence that it works in the same way in all cells (**Figure 3**).

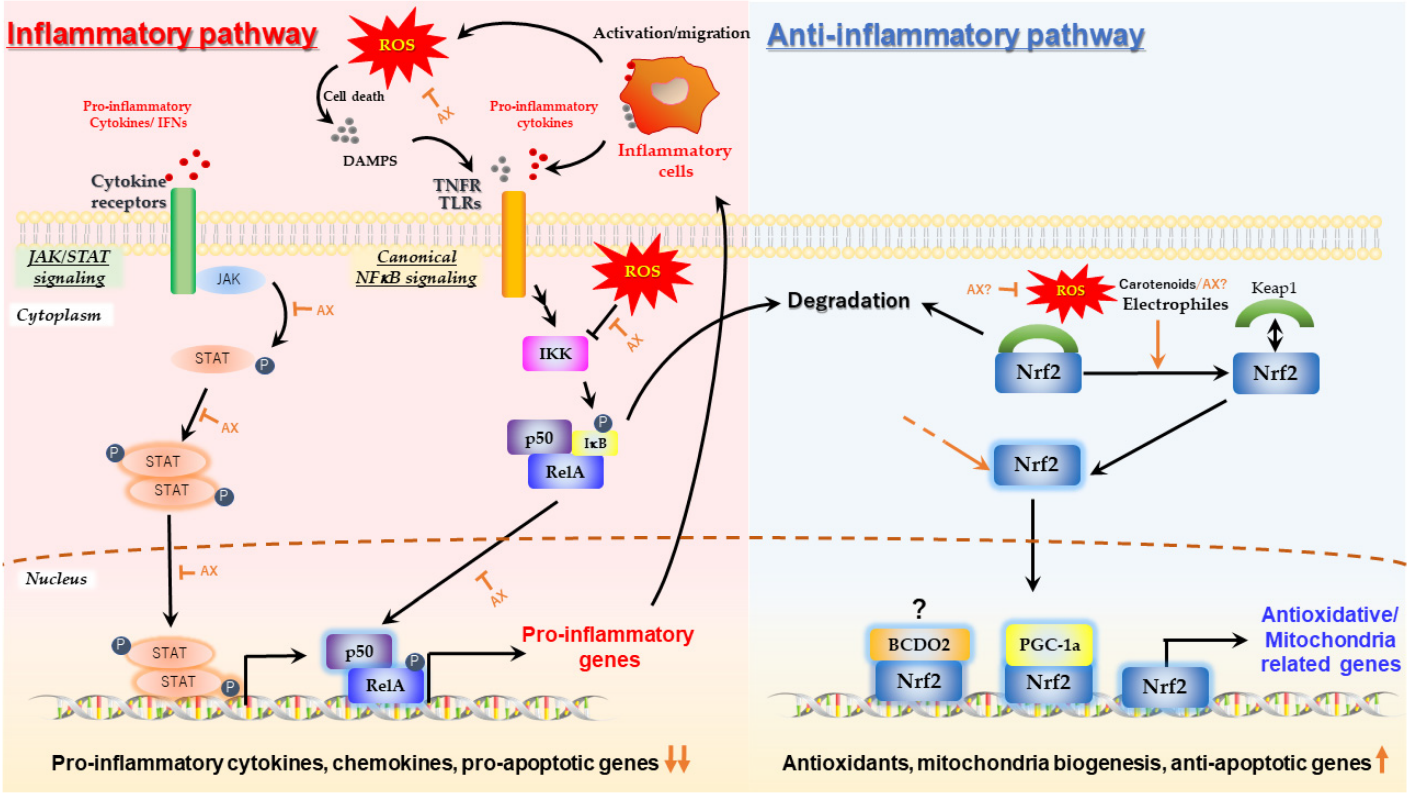

Figure 3. AX partially induces the antioxidant defense system while inhibiting the ROS-mediated inflammatory signaling pathway. AX inhibits ROS-mediated activation of canonical NFκB signaling and related signals such as JAK/STAT3. Consequently, the induction of pro-inflammatory cytokine gene expression is suppressed, resulting in attenuation of inflammatory signals. On the other hand, AX produces partial activation of Nrf2 via dissociation of Nrf2/Keap-1 by electrophiles, and/or other pathways. Consequently, antioxidant enzymes are induced and act in an anti-inflammatory function in vivo. Thus, AX suppresses the exacerbation cycle of chronic inflammation and shifts the cycle toward improvement. The regulation of these inflammation-related signaling pathways by AX involve a mixture of acute-phase responses to AX that result from ROS scavenging, modulation of phosphorylation and protein modifications related to the regulation of intracellular Redox balance, modulation of adaptor protein association with receptors, and the more chronic induction of gene expression mediated by these results. In this figure, lipid rafts and precise and detailed signal pathways are not shown to avoid complications. In particular, it has been reported that AX affects the points indicated by the orange arrows. This figure was adapted from the reference [67][68].

In conclusion, the antioxidant activity of AX exhibits potent antioxidant activity, and is able to inhibit ROS-induced damage, particularly in lipid membranes.

2. Mechanism by Which Astaxanthin Enhances Mitochondrial Energy Metabolism

2.1. Protective Effect of Astaxanthin on Mitochondria; Astaxanthin as a Mitochondrial Antioxidant

Many studies have observed a variety of cellular and molecular changes in response to AX treatment. Consequently, it can be difficult to determine which of these effects may be attributed to the direct mechanisms of action of AX, such as its direct antioxidant activity, or indirect downstream effects in response to chronic AX treatment. To address this, we focus below on the early changes resulting from acute exposure to AX.

The mitochondrion is an organelle that produces energy by electron transport chain (ETC)/oxidative phosphorylation, and oxygen is consumed in this process. Most of the oxygen molecules entering the ETC are reduced to water, but a significant amount escapes in the form of ROS byproducts [69]. AX can significantly inhibit the lipid peroxidation of biological membranes. It has also been reported that AX added to cultured cells was transported to the mitochondria [47]. Since most of the important components of the mitochondrial ETC are located within the inner membrane of mitochondria, AX is expected to protect mitochondrial membranes against oxidative damage caused by ROS. This is particularly relevant under conditions where ROS are overproduced, such as during conditions of metabolic stress caused by metabolic diseases and senescence [70][71][72]. For example, AX was reported to be nephroprotective in a mouse model of diabetes mellitus [73], and inhibit the generation of mitochondrial-derived ROS in human renal mesangial cells induced by hyperglycemic insults in vitro [65].

AX inhibited the damaging effects of mitochondrial overload, including resulting in reduced muscle damage in rodents after heavy exercise [29], as well as reduced oxidative modification of skeletal muscle proteins, and reduced inflammatory markers after treadmill exercise in mildly obese mice given a high-fat diet [74]. These results suggest that AX may protect mitochondria from oxidative damage caused by ROS production when mitochondria are overloaded under conditions of physiological stress.

To investigate the antioxidant effect of AX on mitochondria, Wolf et al., examined PC12 cells, which are highly responsive to oxidative stress. This report challenged PC12 cells with antimycin A (AnA), which inhibit Complex III triggering ROS overproduction, resulting in cytotoxicity. AX pre-treatment showed a time- and dose-dependent protective effect of AnA-treated PC12 cells, using sub-nanomolar amounts of AX [75]. This treatment did not cause cell death in HeLa or Jurkat cells, which have the ability to utilize the glycolytic pathway, bypassing the mitochondrial ETC. These results suggest that the addition of sub-nanomolar AX has a protective effect against oxidative damage caused by mitochondrial dysfunction in these cells. Interestingly, when organelle-localized redox-sensitive fluorescent proteins (roGFPs) were expressed in the cells, AX treatment did not change the level of cytoplasmic-reduced state under basal conditions or hydrogen peroxide (H_2O_2) treatment, but AX maintained a mitochondrial-reduced state under oxidative stress. In addition, when evaluated by the fluorescence of MitoSOX, a dihydroethidium (DHE)-derived mitochondrial-selective superoxide probe, there was no decrease in the production of mitochondrial-derived superoxide in the presence of AnA. The lack of evidence for the direct scavenging of AnA-mediated superoxide by AX in this in vitro experimental model may be due to superoxide being diffused into the aqueous space, while AX remains in the mitochondrial inner membrane. Despite not being able to observe the direct antioxidant activity of AX in this model, AX has exhibited physiological antioxidant activity or other physiological activities in a number of other studies, as will be discussed in later sections. In relation to that consideration, although the addition of AX did not increase the membrane potential of basal cells, it was useful in maintaining the membrane potential, which gradually decreased with incubation. Taken together, these results

suggest that although AX does not inhibit ROS formation, it could be effective in improving mitochondrial function by neutralizing ROS to curtail the downstream effect on mitochondrial membranes.

In a recent report from another group, skeletal muscle cells (Sol8 myotubes) derived from mouse soleus muscle were challenged [76] by the addition of succinate, a substrate of Complex II and AnA that triggers the accumulation of ROS. ROS generated in the cells were observed using a fluorescent whole-cell superoxide probe (DHE), following the addition of AnA. Ax decreased the ROS-induced fluorescence in a concentration-dependent manner. Mitochondrial membrane potential was evaluated using JC-1 dye, which accumulates in mitochondria in the presence of mitochondrial membrane potential. Using JC-1 as an indicator of mitochondrial health and membrane integrity showed that the addition of AX alone did not change the basal mitochondrial membrane potential, but did inhibit the decrease in membrane potential resulting from AnA-induced ROS accumulation. Additional studies examined the ability of AX to protect mitochondrial membranes under various conditions triggering oxidative stress. Another study reported that AX helped protect mitochondrial respiratory chain activity against Fe^{2+}-induced lipid peroxidation in mitochondria that were isolated from vitamin E-deficient rats [77]. AX also had a protective effect against ROS-mediated angiotensin II (Ang II)-induced mitochondrial dysfunction in vascular smooth muscle cells (VSMCs), and normalized mitochondrial respiratory parameters in the presence of ROS [77].

In response to oxidative stress, mitochondria can initiate programmed cell death, also known as apoptosis. Oxidative stress disturbs intracellular Ca^{2+} homeostasis, resulting in excessive Ca^{2+} efflux from the endoplasmic reticulum and an influx into mitochondria, which subsequently triggers mitochondrial membrane permeabilization, loss of mitochondrial membrane potential, and the release of mitochondrial pro-apoptotic factors [78]. It has been widely reported that AX prevents the ROS-induced Ca^{2+} influx into mitochondria, protects against mitochondrial dysfunction, and inhibits apoptosis [79][80][81][82][83][84][85].

Although the effects of AX differ slightly depending on the cell type, detection system, and mitochondrial substrate and condition, all reports have indicated that AX has a protective effect on mitochondria, especially on membrane components. Thus, the antioxidant effects of AX on membranes are not isolated to a single cell strain.

Summarizing these reports, it was suggested that AX could somehow act to maintain and protect the integrity of the mitochondrial ETC and oxidative phosphorylation against oxidative stress. However, the cells used in these studies underwent relatively long-term AX treatments, possibly to overcome the slow intracellular uptake of AX. Thus, it is unclear whether the observed mitochondrial protective effects were due to the direct antioxidant action of AX, induction of antioxidant enzymes via the Nrf2-Keap1 pathway, or remodeling of mitochondria-related genes. Therefore, the presence of AX-mediated regulation of mitochondria-related gene expression and its putative mechanisms are presented in the following sections.

2.2. Aggressive Enhancement of Mitochondrial Activity and Metabolism via Gene Expression by Astaxanthin

We, among others, have shown that AX improves glucose and lipid metabolism and muscle strength [74][81][86][87][88][89], mainly by correcting abnormal gene expression or protein modification in the mitochondria, which is altered

during oxidative injury [74][90]. These effects are mainly attributed to the antioxidant effects of AX.

In fact, ROS production due to decreased activity of the mitochondrial ETC is thought to be involved in energy overload and metabolic disturbances [70][91]. Paradoxically, it is widely recognized that at physiological levels, ROS generated from mitochondria are also beneficial in improving metabolism in response to exercise [92][93][94]. Unfortunately, it is practically difficult to distinguish between the physiological levels of ROS and levels resulting in oxidative stress. Furthermore, the pharmacological effects of AX were considered too complicated to be explained by only its antioxidant effects as a single compound.

3. Prospect of Astaxanthin for Human Health Promotion

In rodents such as mice and rats, effective concentrations of AX were probably achieved at the doses used in the study in the targeted organs, and the medications were considered to be effective. Importantly, the doses of AX given to animals in the pharmacological studies presented in this work were quite high. The concentration of AX in the blood of humans and rodents deviates greatly, with the former reaching considerably higher concentrations than the latter [47][95][96][97][98][99]. In humans, although differences in absorption were observed in each clinical trial, this was thought to be due to dietary conditions, formulation, and individual differences. Therefore, it can be confidently expected that the benefits of AX for human subjects can be demonstrated by designing the formulation and administration method. Although they still remain to be improved, we summarized the human clinical studies reported to date on the antioxidant effects of AX (**Table 1**), as well as its impact on physical activity (**Table 2**) and cardiovascular, endocrine, and metabolic effects (**Table 3**). Based on the outcomes presented in **Table 1**, **Table 2** and **Table 3**, AX can be expected to be especially useful in the prevention of metabolic diseases associated with obesity, T2DM, and sarcopenia, based on the mechanisms described in this work. The effects of AX are only mild, based on the results of clinical studies, and are additive to exercise, so it should be used in combination with standard therapeutic interventions and exercise therapy. Therefore, further research studies are warranted to elucidate the exact mechanism of action in more detail and consider the interaction with the mechanism of medication.

Table 2. Human clinical studies of AX on physical performance, endurance and fatigue.

Author/Year/Reference	Study Design	Subjects	Dose	Duration	Outcome
<Subjects: healthy athletes, high daily physical activity>					
Brown, R.D. et al., 2021 [100]	Randomized, double-blind,	12 recreationally trained male cyclists 27.5 ± 5.7 years,	0, 12 mg/day	7 days	Completion time of the 40-km cycling time trial improved by 1.2 ± 1.7% with AX supplementation, from 70.76 ± 3.93 min in the

Author/Year/Reference	Study Design	Subjects	Dose	Duration	Outcome
	placebo-controlled, crossover study	VO_{2peak}: 56.5 ± 5.5 $mL·kg^{-1}·min^{-1}$, W_{max}: 346.8 ± 38.4 W			placebo condition to 69.90 ± 3.78 min in the AX condition (mean improvement time = 51 ± 71 s, p = 0.029, g = 0.21). Whole body fat oxidation rate was also greater in the AX group between 39–40 km (+0.09 ± 0.13 $g·min^{-1}$, p = 0.044, g = 0.52) and respiratory exchange ratio was lower (−0.03 ± 0.04, p = 0.024, g = 0.60).
Talbott I. et al., 2018 [101]	Randomized, double-blind, placebo-controlled, prospective study	28 recreational runners (42 ± 8 years)	0, 12 mg/day	8 weeks	Reduced average heart rate at submaximal endurance intensities (aerobic threshold, AeT and anaerobic threshold, AT), but not at higher “peak” intensities.
Klinkenberg L.J. et al., 2013 [102]	Randomized, double-blind, placebo-controlled prospective study	32 well-trained male cyclists 25 ± 5 years, $V\dot{O}_2^{peak}$ = 60 ± 5 $mL·kg^{-1}·min^{-1}$, W_{max} = 5.4 ± 0.5 $W·kg^{-1}$	0, 20 mg/day *	4 weeks	N.S; effect on exercise-induced cardiac troponin T release (p = 0.24), changes in antioxidant capacity markers (trolox equivalent antioxidant capacity, uric acid, and malondialdehyde). Markers of inflammation (high-sensitivity C-reactive protein) and exercise-

Author/Year/Reference	Study Design	Subjects	Dose	Duration	Outcome
					induced skeletal muscle damage (creatine kinase).
Res T. et al., 2013 [103]	Randomized, double-blind, placebo-controlled, prospective study	32 trained male cyclists or triathletes 25 ± 1 years, $V^{\cdot}O_2^{peak}$ = 60 ± 1 $mL \cdot kg^{-1} \cdot min^{-1}$, W_{max} = 395 ± 7 W	0, 20 mg/day	4 weeks	N.S; total plasma antioxidant capacity (p = 0.90) or attenuated malondialdehyde levels (p = 0.63). Whole-body fat oxidation rates during submaximal exercise (from 0.71 +/− 0.04 to 0.68 ± 0.03 $g \cdot min^{-1}$ and from 0.66 ± 0.04 to 0.61 ± 0.05 $g \cdot min^{-1}$ in the placebo and AX groups, respectively; p = 0.73), time trial performance (from 236 ± 9 to 239 ± 7 and from 238 ± 6 to 244 ± 6 W in the placebo and AX groups, respectively; p = 0.63).
Djordjevic B. et al., 2011 [104]	Randomized, Double-blind, placebo-controlled, prospective study	32 male elite soccer players	0, 4 mg/day	90 days	Changes in elevated $O2^{-\cdot}$ concentrations after soccer exercise were statistically significant only in the placebo group (exercise × supplementation effect, p < 0.05); TAS values decreased significantly only in the placebo group after exercise (p < 0.01). After intervention, total SH group content increased (21% and 9%, respectively),

Author/Year/Reference	Study Design	Subjects	Dose	Duration	Outcome
					and the effect of AX was marginally significant (p = 0.08). Basal SOD activity was significantly reduced in both the placebo and AX groups at the end of the study (main training effect, $p < 0.01$). Post-exercise CK and AST levels were significantly lower in the AX group than in the placebo group ($p <$ 0.05)
Earnest C.P. et al., 2011 [105]	Randomized, double-blind, placebo-controlled, prospective study	14 amateur endurance-trained subjects 18–39 years, $V^{\cdot}O_2^{peak}$ = 52.84 ± 3.5 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$, W_{max} = 330 ± 26 W	0, 4 mg/day	28 days	Improved performance in the 20-km cycling time trial in the AX group (n = 7, −121 s; 95% CI, −185, −53), but not in the placebo group (n = 7, −19 s; 95% CI, −84, 45). AX group significantly increased power output (20 W; 95% CI, 1, 38), whereas the placebo group did not (1.6 W; 95% CI, −17, 20). N.S; carbohydrate, fat oxidation and blood indices indicative of fuel mobilization.
Bloomer, R.J. et al., 2005 [106]	Randomized, placebo-controlled,	20 resistance trained male	0, 4 mg/day *	3 months	N.S; Muscle soreness, creatine kinase (CK), and muscle performance were

Author/Year/Reference	Study Design	Subjects	Dose	Duration	Outcome
	prospective study	subjects (25.1 ± 1.6 years)			measured before and through 96-h post-eccentric exercise
Sawaki K. et al., 2002 [107]	Randomized double-blind placebo-controlled, prospective study	16 healthy adult male subjects	0, 6 mg/day	4 weeks	In the AX group, the serum lactate concentration after 2 min of activity (1200 m run) was significantly lower than that in the control group.
<Subjects: healthy subjects>					
Kawamura A. et al., 2021 [108]	Randomized controlled open-label, prospective study	26 healthy male subjects	N/A (1 mg AX/100 g salmon) *	10 weeks	The skeletal muscle mass was higher after training than before training in both control and intervention groups ($p < 0.05$). Increased maximal voluntary contraction after training in the intervention group ($p < 0.05$), but not significantly increased in the control group. (See Table 3 for other outcomes.)
Fleischmann C. et al., 2019 [109]	Randomized, double-blind, placebo-controlled,	22 healthy subjects	0, 12 mg/day	30 days	Decreased raise in blood lactate caused by the $VO_{2\ Max}$ test; AX group (9.4 ± 3.1 and 13.0 ± 3.1 mmole·L^{-1} in the AX and placebo groups, respectively $p < 0.02$).

Author/Year/Reference	Study Design	Subjects	Dose	Duration	Outcome
	prospective study				Change in oxygen uptake during recovery (−2.02 ± 0.64 and 0.83 ± 0.79% of $VO2_{Max}$ in the AX and placebo group, respectively, $p = 0.001$). N.S; anaerobic threshold or VO2 Max. physiological or biochemical differences in the heat tolerance test (HTT) (2 h walk at 40 °C, 40% relative humidity.
Takami M. et al., 2019 [110]	Open-label, prospective study	20 healthy young male subjects	c.a, 4.5 mg/day * from salmon	4 weeks	Increased maximum workload by training in both groups ($p = 0.009$), and increased oxygen consumption during exercise in the antioxidant group only ($p = 0.014$). There were positive correlations between maximum workload and fat ($r = 0.575$, $p = 0.042$) and carbohydrate oxidations ($r = 0.520$, $p = 0.059$) in the antioxidant group. (See Table 3 for other outcomes.)
Imai A. et al., 2018 [111]	Randomized, double-blind,	42 healthy subjects	0, 6 mg/day *	4 weeks	Elevated PCOOH levels during mental and physical tasks were attenuated by AX supplementation. Improved

Author/Year/Reference	Study Design	Subjects	Dose	Duration	Outcome
	placebo-controlled, crossover study				recovery from mental fatigue compared with the placebo. No differences were found between AX and the placebo in other secondary outcomes, such as subjective feelings, work efficiency, and autonomic activity.
Hongo N. et al., 2017 [112]	Randomized, double-blind placebo-controlled, prospective study	39 healthy subjects	0, 12 mg/day *	12 weeks	Intent-to-treat (ITT) analysis; fatigue after physical and mental stress was significantly lower in the AX group than in the placebo at week 8; the change in POMS Friendliness was significantly higher in the AX group than in the control group at week 8; the rate of change in BAP values at week 12 was not significantly different between the AX and control groups. The rate of change in BAP values at week 12 was not significantly different between the AX group and the control.
Malmstena C.L.L. et al., 2008 [113]	Randomized, double-blind, placebo-controlled,	40 young healthy subjects (17–19 years)	0, 4 mg/day	3 months	Increased average number of knee bending (squats) increased by 27.05 (from 49.32 to 76.37, AX group) vs. 9.0 (from 46.06 to 55.06,

Author/Year/Reference	Study Design	Subjects	Dose	Duration	Outcome
	prospective study				placebo subjects), p = 0.047.
Tajima T. et al., 2004 [114]	Randomized, double-blind, placebo-controlled, crossover study	18 healthy subjects (35.7 ± 4 years)	0, 5 mg/day	2 weeks	Increased in CV_{RR} and HF/TF (Heart rate variability) were significant during exercise at 70% maximum heart rate (HRmax) intensity ($p < 0.05$). Also, after the AX supplementation, decreased minute ventilation (V_E) during exercise at 70% HRmax ($p < 0.05$). Decreased LDL cholesterol (chol) ($p < 0.05$) and respiratory quotient after exercise.
<Subjects: elderly subjects>					
Liu S.Z. et al., 2021 [115]	Randomized, double-blind, placebo-controlled, prospective study	42 elderly subjects (65–82 years)	0, 12 mg/day *	12 weeks	In endurance training (ET), specific muscular endurance was improved only in the AX group (Pre 353 ± 26 vs. Post 472 ± 41) and submaximal graded exercise test duration was improved in both groups (placebo 40.8 ± 9.1% vs. AX 41.1 ± 6.3%). The increase in fat oxidation
Author/Year/Reference	Study Design	Subjects	Dose	Duration	Outcome
Shokri-Mashhadi, N. et al., 2021 [118]	Randomized, double-blind, placebo-controlled,	44 patients with type 2 diabetes	0, 8 mg/day	8 weeks	Decrease plasma levels of MDA and IL-6 ($p < 0.05$) and decrease the expression level of miR-146a, associated with

Author/Year/Reference	Study Design	Subjects	Dose	Duration	Outcome
	prospective study				inflammatory markers (fold change: −1/388) (p < 0.05).
Kawamura A. et al., 2021 [108]	Randomized controlled Open-label, prospective study	26 healthy male subjects	*N/A* (1 mg AX/100 g salmon) *	10 weeks	Higher resting oxygen consumption after training in the intervention group only (p < 0.05). Serum carbonylated protein level as an oxidative stress marker tended to be lower immediately after exercise than before exercise in the intervention group only (p = 0.056). (See **Table 2**. for other outcomes.)
Kato T. et al., 2020 [119]	Open-label, prospective study	16 patients with systolic heart failure	12 mg/day *	3 months	Increased left ventricular ejection fraction (LVEF) from 34.1 ± 8.6% to 38.0 ± 10.0% (p = 0.031) and 6-min walk distance increased from 393.4 ± 95.9 m to 432.8 ± 93.3 m (p = 0.023). Significant relationships were observed between percent changes in dROM level and those in LVEF.
Chan K. et al., 2019 [120]	Randomized controlled	54 patients with type 2 diabetes	0, 6, 12 mg/day	8 weeks	Increased plasma AX levels and decreased

Author/Year/Reference	Study Design	Subjects	Dose	Duration	Outcome
	Open-label, prospective study				fasting plasma glucose and HbA1c levels. In 12 mg AX group, reduced in plasma triglyceride, total chol and LDL levels. Lowered changes in plasma IL-6 and TNF-α levels and plasma vWF level and higher changes in AT-III level. In 12 mg AX group, decreased changes in plasma FVII and PAI-1 levels.
Takami M. et al., 2019 [121]	Open-label, prospective study	20 healthy young male subjects	c.a, 4.5 mg/day * from salmon	4 weeks	Higher carbohydrate oxidation during rest in the post-training than that in the pre-training only in the antioxidant group. More decreased levels of serum insulin and HOMA-IR after training were observed in the antioxidant group than in the control group. (See **Table 2**. for other outcomes.)
Mashhadi N.S. et al., 2018 [122]	Randomized, double-blind, placebo-controlled,	44 participants with type 2 diabetes	0, 8 mg/day	8 weeks	Increased the serum adiponectin concentration, reduced visceral body fat mass ($p < 0.01$), serum triglyceride and VLDL chol concentrations, systolic

Author/Year/Reference	Study Design	Subjects	Dose	Duration	Outcome
	prospective study				blood pressure, fructosamine concentration ($p < 0.05$) and marginally reduced the plasma glucose concentration ($p = 0.057$).
Canas J. A. et al., 2017 [121]	Randomized, double-blind, placebo-controlled, prospective study	20 children with simple obesity (BMI > 90%)	500 µg/day * (MCS)	6 months	Mixed-carotenoid supplementation (MCS) increased β-carotene, total adiponectin, and high-molecular-weight adiponectin in plasma compared with placebo; MCS decreased BMI z-score, waist-to-height ratio, and subcutaneous adipose tissue compared with placebo. AX was used as a part of MCS.
Takemoto M. et al., 2015 [123]	Case report	1 Werner syndrome patient	12 mg/day *	6 months	Improved blood transaminase concentrations before AX intervention and 3 and 6 months after initiation were: AST 40 IU/L, 41 IU/L, and 20 IU/L; ALT 69 IU/L, 62 IU/L, and 34 IU/L; GGT 38 IU/L, 41 IU/L, and 35 IU/L; and cholinesterase 360 IU/L, 366 IU/L, and 331 IU/L, respectively.

Author/Year/Reference	Study Design	Subjects	Dose	Duration	Outcome
					Liver-to-spleen Hounsfield units on CT were 0.41 before AX initiation, 0.71 at 3 months, and 0.94 at 6 months. No significant changes after AX intervention in hyaluronic acid, a marker of liver fibrosis; high-sensitivity C-reactive protein, a marker of inflammation; and MDA-modified LDL.
Ni Y. et al., 2015[95]	Randomized, single-blind, placebo-controlled, prospective study	12 NASH patients	12 mg/day	24 weeks	Improved steatosis ($p < 0.05$), marginally improved lobular inflammation ($p = 0.15$) and NAFLD activity score ($p = 0.08$)
Choi H.D. et al., 2011 [38]	Randomized, double-blind, placebo-controlled, prospective study	27 overweight subjects (BMI >25.0 kg/m^2)	0, 20 mg/day	12 weeks	Decreased LDL chol and ApoB. (See **Table 1**. For other outcomes.)
Yoshida H. et al., 2010 [124]	Randomized, ouble-blind,	61 non-obese subjects with fasting serum	0, 6, 12, 18 mg/day	12 weeks	Multiple comparison: triglycerides were significantly decreased by

Author/Year/Reference	Study Design	Subjects	Dose	Duration	Outcome
	placebo-controlled, prospective study	triglyceride of 120–200 mg/dL and without diabetes and hypertension			12 and 18 mg/day and HDL-cholesterol was significantly increased by 6 and 12 mg. Serum adiponectin was increased by AX (12 and 18 mg/day), and changes in adiponectin were positively correlated with changes in HDL-chol.
Satoh A. et al., 2009 [125]	Open-label, prospective study	20 subjects at risk for developing metabolic syndrome (from 127 healthy subjects)	4, (8, 20) mg/day	4 weeks.	When subjects who met the diagnostic criteria for metabolic syndrome in Japan (SBP ≥ 130 mmHg, DBP ≥ 85 mmHg, TG ≥ 150 mg/dL, FG ≥ 100 mg/dL) at the start of the study were selected from 4 mg group, significant decreased in SBP(p < 0.01). On the other hand, there was no significant decrease in DBP. Reduced TG after treatment (218 mg/dL) than the baseline value (292 mg/dL), marginally reduced fasting glucose after the intervention (p < 0.1).
Uchiyama A. et al., 2008 [126]	Open-label, prospective study	17 subjects at risk for developing metabolic syndrome	8 mg twice day	3 months	Significant decreases plasma HbAlc (p = 0.0433) and TNF-α levels (p = 0.0022) and increase

References

Author/Year/Reference	Study Design	Subjects	Dose	Duration	Outcome
					adiponectin concentration (p = 0.0053). N.S: body weight, BMI and waist circumference.
Fukamauchi M. et al., 2007 [127]	Randomized, double-blind, placebo-controlled, prospective study	32 healthy subjects	0, 6 mg/day	6 weeks	Synergistic effects of AX intake (12 mg/day, 6 weeks) and aerobic exercise (walking) were studied. AX contributed to reduction of body fat and suppressed the increase in blood lactate level after exercise.
Kim Y.K. et al., 2004 [48]	Open-label, prospective study	15 healthy postmenopausal female subjects	0, 2, 8 mg/day	8 weeks	Increase HDL-chol levels in 2 mg and 8 mg group increased significantly after 8 weeks from 50.6 ± 5.8 to 60.4 ± 7.1 mg/dL, 44.4 ± 10.7 to 49.4 ± 2.7 mg/dL respectively (p < 0.05). In the 2 mg group, triglyceride decreased significantly from 171.6 ± 67.4 mg/dL to 145.8 ± 5.1 mg/dL (p < 0.05). (See **Table 1**. For other outcomes.)

* In addition to AX, other nutrients such as antioxidants were used in the study.

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