Astaxanthin and Human Health

Literature Survey

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(1) Introduction

Oxidative stress in living cells arises both as a natural result of aerobic metabolism and as a result of environmental and pathologic conditions (Papas 1999). Reactive-oxygen species (ROS) are regularly produced during normal metabolic processes (Reviewed in Davies 1995, Dore 2005). These chemically unbalanced and harmful molecules contain reduced oxygen molecules as free radicals and reactive compounds. The strong tendency of ROS to react with neighboring molecules such as proteins, DNA, RNA, carbohydrates, and lipids puts these molecules at risk. The results of such “oxidative attack” may include protein and lipid peroxidation and structural changes in DNA and RNA, which in turn may lead to damage, mutations, and even loss of function. Environmental (air pollution, tobacco smoke, toxic chemicals or ultraviolet [UV] light), as well as physiological (stress, inflammation, diabetes) conditions can enhance the production of ROS. Indeed, oxidative damage has been linked to aging, atherosclerosis, ischemia-reperfusion injury, macular degeneration of the eye, carcinogenesis, neuro-degenerative diseases such as Alzheimer’s and Parkinson’s diseases, bacterial and viral meningitis, and many other biological processes and diseases. The human body has evolved a large array of endogenous antioxidant defenses against oxidative insult. These include antioxidant enzymes that neutralize ROS prior to their induced damage, (Matés 1999, Matés 2000, Limón-Pacheco 2009) and certain repair enzymes that can reverse the damage produced by ROS in DNA (Wells 2009). In addition, endogenous small molecules with antioxidant activity such as glutathione (Fahey 1991), the hormone melatonin (Reiter RJ 1998), and uric acid (Yu 1998) are being activated in response to oxidative stress. However, these endogenous antioxidants do not completely protect against the sum of oxidative stresses challenging the body, and thus there is net oxidative damage that in the long term contributes to aging and various diseases. In addition to the body's endogenous defenses against oxidative stress, diet-derived antioxidants including ascorbic acid (Vitamin C), alpha-tocopherol (Vitamin E), and the carotenoids may be important in protecting against disease and age-related phenomena (Ames 1993; Davies 1995, Halliwell 1996).
(1.1) The Carotenoids

The group of antioxidants mostly investigated for their potential health benefits, are the carotenoids (reviewed in Cooper 1999). Carotenoids are lipid soluble pigments produced in some plants, algae, fungi and bacterial species that account for their red, orange, or yellow hues. Although some crustaceans have a limited capacity to convert closely related dietary carotenoids, other animals are unable to synthesize carotenoids, and must acquire them from the diet.

Carotenoids are antioxidants due to their ability to quench singlet oxygen, be oxidized, and isomerized (Edge 1997, Mortensen 1997). In addition, carotenoids absorb light due to their special bonding structure, hence providing defense from photo-oxidative damage. The carbon backbone of a carotenoid (Figure 1) consists of a long and uniform chain which may contain terminal rings bearing various chemically active groups. Oxygen-free carotenoids are called carotenes, while oxygen-containing carotenoids are called xanthophylls. The ability of the various carotenoids to react with ROS differs greatly and is closely related to their chemical structures (Mortensen 1997). Among the various carotenoids, Astaxanthin is the most potent natural antioxidant. This was shown by comparative studies in many systems as further discussed below.
Figure 1: Structures of selected carotenoids.
(2) Astaxanthin chemistry

(2.1) Astaxanthin – chemical structure

Astaxanthin (3,3’-dihydroxy-β-β-carotene-4,4’-dione) is a xanthophyll carotenoid, commonly found in marine environments where it gives an orange-pink coloration to several sea-species. As seen in Fig. 1, Astaxanthin is closely related to other well-known carotenoids, such as β-carotene, zeaxanthin and lutein, with which it shares many of the metabolic and physiological functions attributed to carotenoids. The presence of the hydroxyl and keto endings on each ionone ring (Figure 1), explains some unique features of Astaxanthin, such as its ability to be esterified, a higher anti-oxidant activity and a more polar configuration than other carotenoids. Free Astaxanthin is particularly sensitive to oxidation. In nature, it is found either conjugated to proteins or esterified with one or two fatty acids, which stabilize the molecule. In the algea Haematococcus pluvialis (H. pluvialis), the major natural source for mass production of natural Astaxanthin, the esterified form predominates, mostly as a monoester (Lorenz 2000). Various Astaxanthin stereoisomers are found in nature, which differ in the configuration of the two hydroxyl groups on the molecule (Figure 1). The 3S, 3’S stereoisomer is the main form found in H. pluvialis H. pluvialis and in wild salmon, while synthetic Astaxanthin consists of the racemic mixture of the three enantiomers (Turujman 1997). Synthetic Astaxanthin consist of the “un-natural” 3R-3S stereoisomer (about 50%), which is rarely found in nature and thus, may be inactive. Astaxanthin consists of geometric isomers as well, all-trans isomer, which is the most abundant and the cis isomers (mainly as 9Z and 13Z) (Turujman 1997). The cis isomers, particularly the 9Z, display higher bioavailability and potency in humans (Coral-Hinostroza 2004). This isomer is abundant (up to 20%) in the natural Astaxanthin complex produced by the alga H. pluvialis.
(2.2) Sources of Astaxanthin and their properties

Astaxanthin can be commercially obtained from four major sources (reviewed in Higuera-Ciapara 2006):

* Synthetic Astaxanthin
* Astaxanthin from *Phaffia rhodozyma* yeast
* Astaxanthin from crustacean by-products
* Astaxanthin from the algae *Haematococcus pluvialis*

The various sources differ in their yields, cost and chemical characteristics yet, of the natural sources available, the richest source of Astaxanthin is the algae *H. pluvialis*, which can accumulate up to 50 gr (Algatech, unpublished results) of Astaxanthin per kilogram of dry biomass (Higuera-Ciapara 2006, Ernst 2002). Moreover, Astaxanthin produced in *H. pluvialis* is currently the only one approved for use in humans as a dietary supplement by U.S.-FDA, and EU-EFSA, and Japanese authorities. The majority of the safety and efficacy studies were conducted with the 3S 3’S isomer of *H. pluvialis*.

Astaxanthin from different sources may may be differentiated in terms of bioavailability and metabolism; however, there are a limited number of studies comparing efficacy based upon raw material source, and these are primarily in reference to fish coloring. One study did demonstrate that the 9-cis isomer has much higher antioxidant potency than that of the all-trans isomer in rat microsome and rabbit erythrocyte ghost membrane lipid peroxidation systems, as well as in human neuroblastoma SH-SY5Y cells (Liu 2007).
(3) Bioavailability and pharmacokinetics of Asthaxanthin.

(3.1) Bioavailability

Asthaxanthin is not soluble in water. Its level of esterification determines Asthaxanthin’s absorption in the digestive system. When it is esterified, i.e. bound to fat or taken with oil, Asthaxanthin is readily absorbed. Other factors influencing bioavailability include levels of oxidation, chemical structure (isomerization and stereoisomerization), processing methods (Mendes-Pinto 2001) and raw material sources (Bowen 1999). Free Asthaxanthin is extremely sensitive to oxidation. The esterified form of the *H. pluvialis* alga is stable and displays higher bioavailability and potency. Other research indicates that the bioavailability of Asthaxanthin is significantly enhanced when esterified, and in the presence of fats (Odberg 2003, Rufer 2008, Barbossa 1999, Okada 2009).

(3.2) Pharmacokinetics - ADME: Absorption, Distribution, Metabolism and Elimination

[Inasmuch as similar results are found in pre-clinical and clinical ADME studies of Asthaxanthin, this review refers only to results of clinical studies.]

(3.2.1) Absorption

Absorption of Asthaxanthin starts with hydrolysis of the ester moiety before the final absorption takes place, as evident from the absence of esterified Asthaxanthin in human plasma (Odeberg 2003, Coral-Hinostroza 2004). The hydrolysis of the ester seems to be a rate-limiting step, since esterified Asthaxanthin had a prolonged absorption rate, compared to the non-esterified form (Coral-Hinostroza 2004). Following hydrolysis, Asthaxanthin digestion and intestinal absorption are closely associated with fatty acids uptake, transport and delivery, since it is a fat-soluble compound. Asthaxanthin is incorporated into chylomicrons, and distributed between very low density lipoprotein
(VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) and transported to the tissues (Wang 1992).

(3.2.2) Distribution

Following intake, plasma levels reach maximal values within a few hours (Okada 2009, Odeberg 2003, Østerlie 2000, Coral-Hinostroza 2004) and both $t_{\text{max}}$ and $C_{\text{max}}$ are higher after meal or fat intake and are shorter following smoking as shown in Table 1 (Odeberg 2003, Okada 2009). Accumulation of Astaxanthin in plasma, following dose response, shows non-linear kinetics (Rufer 2008, Coral-Hinostroza 2004). This may be explained by prolonged absorption through the Peyer’s patch route into the lymphatic system.

Table 1: Pharmacokinetic parameters of Astaxanthin after administration of a single oral dose of 48 mg Astaxanthin before and after meal to smokers and non-smokers (mean+standard deviation). [Adapted from Okada 2009].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before meal administration (non-smokers)</th>
<th>After meal administration (non-smokers)</th>
<th>After meal administration (smoker)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (ng/ml)</td>
<td>83.4 ± 32.5</td>
<td>114.4 ± 39.0</td>
<td>149.7 ± 111.7</td>
</tr>
<tr>
<td>$t_{\text{max}}$ (h)</td>
<td>7.43 ± 0.98</td>
<td>21.33 ± 6.53</td>
<td>13.71 ± 9.62</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>23.7 ± 13.6</td>
<td>30.5 ± 12.9</td>
<td>18.5 ± 11.0</td>
</tr>
<tr>
<td>Elimination rate constant (1/h)</td>
<td>0.05 ± 0.03</td>
<td>0.03 ± 0.03</td>
<td>0.06 ± 0.04</td>
</tr>
<tr>
<td>AUC$_{0-168}$ (µg h/l)</td>
<td>2,968 ± 959</td>
<td>7,219 ± 3,118</td>
<td>6,462 ± 4,065</td>
</tr>
<tr>
<td>AUC$_{0-\infty}$ (µg h/l)</td>
<td>2,996 ± 969</td>
<td>7,526 ± 3,300</td>
<td>6,518 ± 4,125</td>
</tr>
<tr>
<td>Oral clearance (1/h)</td>
<td>0.30 ± 0.13</td>
<td>0.13 ± 0.07</td>
<td>0.15 ± 0.06</td>
</tr>
</tbody>
</table>

Astaxanthin is distributed to all tissues, and is able to pass the blood brain barrier, as evident from studies in rodents (Aoi 2003). Studies in humans suggest that there is a specific accumulation of Astaxanthin in VLDL chylomicrons (36-64% of total Astaxanthin, after 6.7 h, Osterlie 2000, Coral-Hinostroza 2004), as shown in Figure 2.
(3.2.3) Metabolism

Kistler et al. (2002) investigated the metabolism of Astaxanthin as well as its capacity to induce cytochrome P450 genes in primary cultures of human hepatocytes and in two human volunteers. Four main free metabolites were found; and their reduction products were also present as glucuronides. Based on experiments done in cultured hepatocytes, and on the highest level of Astaxanthin reached in a clinical trial (40 mg/day, 4 weeks) (Kupcinskas 2008), Astaxanthin is not expected to affect CYP enzymes or metabolism of drugs.

(3.2.4) Elimination

Elimination of Astaxanthin is relatively fast with elimination half life ranging between 15.9h (Odenberg 2003) to 52h (Coral-Hinostroza 2004). In addition, studies in rodents showed that the length of exposure to Astaxanthin is not related to plasma concentration (Stewart 2008), and that there is a rapid elimination or catabolism of Astaxanthin without long-term storage of astaxanthin in tissues (Petri 2007). Taken together, these results suggest that Astaxanthin is unlikely to accumulate in the body. In humans, a three-day follow-up of 5 volunteers who ate 1.5mg Astaxanthin in a single meal, detected no Astaxanthin in plasma while 64% of Astaxanthin was excreted in feces.
(Elmadfa 1999). This study suggests that majority of the ingested Astaxanthin is excreted in feces.

(4) Safety of Astaxanthin

Astaxanthin is naturally found in many animals and plants. In salmon flesh its concentration may range from 3 to 40 mg/kg (Turujman 1997). The main Astaxanthin isomer identified in salmon was the 3S, 3'S stereoisomer identical to that found in H. pluvialis. In addition to salmon, Astaxanthin is also found in crustacean species such as krill, shrimp, crab, lobster and crawfish. Thus, humans have ingested Astaxanthin for centuries, with amounts depending on the importance of seafood in the diet. Indeed, the European Food Safety Authority (EFSA, 2005) estimated daily intake of Astaxanthin for consumers with a high intake of fish and seafood, in the range of 1.6 to 4.1 mg/day. Astaxanthin was approved by the FDA as dietary supplement in aquaculture in 1987 and as a human dietary supplement in 2000 (Pashkew 2008). It has been marketed as a dietary supplement for approximately 10 years without any reported adverse effects. Furthermore, there is no evidence that consumption of Astaxanthin in foods or as a dietary supplement has any cumulative effect that would affect its safety. The published recommended daily intake varies among different regulatory authorities. It is 6 mg/day in Japan, 5 mg/day in the US and 4 mg/day in Europe. The safety of Astaxanthin either as an extract from natural sources, in its synthetic form, or as present in algal biomass has been documented in many pre-clinical and clinical studies. The evidences for its safety can be summarized as:

1) The long-term repeat dose exposure (1.5 and 3 months) of Astaxanthin did not result in its bioaccumulation or any adverse effects (Ono 1999, Nishikawa 1997).

2) Many animal studies support the safety of Astaxanthin. These include specially designed safety tests such as ADME studies, acute and subchronic (Takashi 2005) studies (LD50 > 2000 mg/kg body weight, NOEL = 50 mg /kg/day), repeated-dose toxicity and fertility study (Nishikawa 1997), embryotoxicity and teratology (Roche 1987), multi generation study (Roche 1987), micronucleus test (Scantox 1998) and many efficacy-related experiments in which histological and biochemical follow-up
was included. None of the above mentioned studies revealed any adverse effects of Astaxanthin.


4) In multiple human clinical studies (>25) (Table 2), the safety of Astaxanthin was confirmed at doses of up to 40 mg/day for 8 weeks or 4 mg/day for one year. Results from these trials provide a huge margin of safety above the currently recommended levels.
Table 2: Findings from Astaxanthin clinical trials

<table>
<thead>
<tr>
<th>Reference; Study design; Substance used</th>
<th>Dose (mg/day); Duration</th>
<th>Number Subjects</th>
<th>Nature of trial</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andersen et al. (2007); Placebo-controlled 1</td>
<td>40 mg/day for 8 wk</td>
<td>21</td>
<td>Dyspepsia treatment</td>
<td>No adverse effects reported</td>
</tr>
<tr>
<td>Bloomer et al. (2005); DB-PC 2</td>
<td>4 mg/day for 3 wk</td>
<td>10</td>
<td>Endurance</td>
<td>No adverse effects reported</td>
</tr>
<tr>
<td>Comhaire et al. (2005); DB-PC 1</td>
<td>16 mg/day for 12 wk</td>
<td>11</td>
<td>Infertility</td>
<td>No adverse effects reported</td>
</tr>
<tr>
<td>Cicero et al. (2007); Single-blind 4</td>
<td>6 mg/day for 4 wk</td>
<td>20</td>
<td>Dyslipidemias</td>
<td>No adverse events or impairments of liver transaminases or CKP</td>
</tr>
<tr>
<td>Iwamoto et al. (2000); Controlled 3</td>
<td>1.8, 3.6, 14.4, 21.6 mg/day for 2 wk</td>
<td>5, 5, 3, 5, respectively</td>
<td>LDL Oxidation</td>
<td>No adverse effects reported</td>
</tr>
<tr>
<td>Iwasaki and Tawara (2006); DB-PC, crossover 5</td>
<td>6 mg/day 2 wk</td>
<td>10</td>
<td>Eye function</td>
<td>No adverse effects reported</td>
</tr>
<tr>
<td>Karppi et al. (2007); DB-PC 1</td>
<td>8 mg/day for 12 wk</td>
<td>20</td>
<td>Safety</td>
<td>Well tolerated</td>
</tr>
<tr>
<td>Kupcinskas et al. (2008); DB-PC prospective 1</td>
<td>0, 16, 40 mg/day in algal meal for 4 wk</td>
<td>44, 43, 44, respectively</td>
<td>Dyspepsia treatment</td>
<td>No treatment-related adverse effects were noted</td>
</tr>
<tr>
<td>Kuge and Silver (1999); Placebo-controlled 2</td>
<td>3.85 or 19.25 mg/day for 4 wk</td>
<td>33</td>
<td>Safety</td>
<td>No adverse effects noted; blood chemistry analyses and urinalysis were conducted four times through the trial</td>
</tr>
<tr>
<td>Lignell et al. (1999); Open label 1</td>
<td>40 mg/day 3 wk</td>
<td>10</td>
<td>Dyspepsia; safety</td>
<td>No adverse effects reported</td>
</tr>
<tr>
<td>Malmsten and Lignell (2008); DB-PC 1</td>
<td>4 mg/day for 24 wk</td>
<td>20</td>
<td>Endurance; safety</td>
<td>No adverse effects reported. No changes in hemoglobin at 3 and 6 months</td>
</tr>
<tr>
<td>Miyawaki et al. (2005; 2008); single blind-PC 2</td>
<td>6 mg/day for 10 days</td>
<td>10</td>
<td>Safety; blood flow</td>
<td>No adverse effects reported</td>
</tr>
<tr>
<td>Nagaki et al. (2002); DB-PC 2</td>
<td>5 mg/day for 4 wk</td>
<td>13</td>
<td>Eye function</td>
<td>No adverse effects noted</td>
</tr>
<tr>
<td>Nagaki et al. (2005); DB-PC 2</td>
<td>6 mg/day for 4 wk</td>
<td>25</td>
<td>Retinal blood flow; safety</td>
<td>No treatment-related adverse effects noted. No treatment-related effects on hematology, serum chemistry</td>
</tr>
<tr>
<td>Nagaki et al. (2006); DB-PC 2</td>
<td>6 mg/day for 4 wk</td>
<td>31</td>
<td>Eye</td>
<td>No treatment-related adverse effects reported</td>
</tr>
<tr>
<td>Reference; Study design; Substance used</td>
<td>Dose (mg/day); Duration</td>
<td>Number Subjects</td>
<td>Nature of trial</td>
<td>Findings</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-------------------------</td>
<td>-----------------</td>
<td>----------------</td>
<td>----------</td>
</tr>
<tr>
<td>PC&lt;sup&gt;2&lt;/sup&gt;</td>
<td>5 mg/day for 2 wk</td>
<td>19</td>
<td>Exercise endurance</td>
<td>No adverse reaction noted.</td>
</tr>
<tr>
<td>Nagata et al. (2006); DB-PC-crossover&lt;sup&gt;2&lt;/sup&gt;</td>
<td>2, 4, 12 mg/day for 4 wk</td>
<td>39</td>
<td>Eye function; safety</td>
<td>No adverse effects noted.</td>
</tr>
<tr>
<td>Nitta et al. (2005); DB-PC&lt;sup&gt;2&lt;/sup&gt;</td>
<td>6, 12 mg/day for 4 wk</td>
<td>20</td>
<td>Eye function; safety</td>
<td>No adverse effects noted. No effects on hematology, serum chemistry</td>
</tr>
<tr>
<td>Ohgami et al. (2005); Open label&lt;sup&gt;1&lt;/sup&gt;</td>
<td>30 mg/day for 4 wk</td>
<td>10</td>
<td>Safety/Toxicity</td>
<td>No adverse effects noted. No effects on hematology, serum chemistry during treatment (2 and 4 wks) and after the treatment (2 and 4 wks)</td>
</tr>
<tr>
<td>Parisi et al. (2008); Randomized- controlled*</td>
<td>4 mg/day for 52 wk</td>
<td>15</td>
<td>Eye function</td>
<td>No adverse effects noted</td>
</tr>
<tr>
<td>Satoh et al., (2009); Open label&lt;sup&gt;1&lt;/sup&gt;</td>
<td>4, 8, 20 mg/day for 4 wk</td>
<td>73; 38; 16, respectively</td>
<td>Safety/Toxicity</td>
<td>No adverse effects noted. No effects on hematology, serum chemistry, urine analysis</td>
</tr>
<tr>
<td>Satoh et al., (2009); Open label&lt;sup&gt;1&lt;/sup&gt;</td>
<td>12 mg/day for 12 wk</td>
<td>10</td>
<td>Safety; Forgetfulness</td>
<td>No adverse effects reported</td>
</tr>
<tr>
<td>Sawaki et al. (2002); DB-PC&lt;sup&gt;2&lt;/sup&gt;</td>
<td>6 mg/day for 4 wk; 6 mg/day for 4 weeks</td>
<td>9; 8</td>
<td>Endurance; Eye function; safety</td>
<td>No adverse effects reported. No effects on hematology, serum chemistry</td>
</tr>
<tr>
<td>Shiratori et al. (2005); DB-PC&lt;sup&gt;2&lt;/sup&gt;</td>
<td>6 mg/day for 4 wk</td>
<td>20</td>
<td>Eye function</td>
<td>No adverse effects noted</td>
</tr>
<tr>
<td>Spiller and Dewell (2003); DB-FC&lt;sup&gt;2&lt;/sup&gt;</td>
<td>6 mg/day for 8 wk</td>
<td>19</td>
<td>Safety</td>
<td>No effects on clinical chemistry, blood pressure and blood counts; increased serum calcium, protein and eosinophil count, not considered clinically significant</td>
</tr>
<tr>
<td>Takahashi and Kajita (2005); Open label&lt;sup&gt;1&lt;/sup&gt;</td>
<td>6 mg/day for 2 wk</td>
<td>10</td>
<td>Eye function</td>
<td>No adverse effects reported</td>
</tr>
<tr>
<td>Tsukahara et al. (2005); Open label&lt;sup&gt;2&lt;/sup&gt;</td>
<td>6 mg/day for 12 wk</td>
<td>15</td>
<td>Safety</td>
<td>No adverse effects noted. No effects on hematology, serum chemistry, urine analysis</td>
</tr>
<tr>
<td>Tsukahara et al. (2008); Open label&lt;sup&gt;2&lt;/sup&gt;</td>
<td>6 mg/day for 4 wk</td>
<td>13</td>
<td>Blood flow; safety</td>
<td>No adverse effects reported</td>
</tr>
</tbody>
</table>

DB-PC = double-blind placebo-controlled; wks = weeks  
<sup>1</sup> H. pluvialis biomass,  
<sup>2</sup> H. pluvialis extract,  
<sup>3</sup> Krill oil/extract,  
<sup>4</sup> H. pluvialis oil,  
* Multi-vitamin formulation
(5) Anti-oxidation activity of Astaxanthin

Astaxanthin found inside cells protects against oxidative damage by three general mechanisms:
1) Quenching of singlet oxygen and dissipating the energy as heat.
2) Scavenging of radicals to prevent or terminate chain reactions.
3) Preservation of membrane structure thereby inhibiting membrane lipid peroxidation.

Since the antioxidative effect of Astaxanthin lies in the basis of most (if not all) of its observed positive influence in many biological systems, numerous studies have been conducted in an attempt to evaluate its effects and to understand their underling mechanism(s). Astaxanthin’s protective affect was studied in many experimental systems including *in vitro* solutions, membranal models (liposomes, microsomes), cell cultures, and animal models, and in recent years, humans.

(5.1) Evidences from *in vitro* studies

Astaxantin’s superior potency as an anti-oxidant, compared to other carotenoides, was demonstrated in many studies, conducted under various conditions. Representative evidences from these *in vitro* studies are summarized in Tables 3 and 4. Since carotenoids are lipophilic (oil soluble), *in vivo* they are expected to exert most of their antioxidant effects in lipid environments. Such environments are found in cell membranes and mitochondria, and are studied *in vitro* using the model system of liposomes (single-bilayer phospholipid vesicles). In this respect, it is important to note that the popular method of quantifying antioxidant capacity of a given substance (known as ORAC – Oxygen Radical Absorbance Capacity; developed by Brunswick Labs, Norton, Massachusetts, USA) can not be applied for Astaxanthin (nor for other carotenoids), as it is inadequate in measuring oil soluble compounds. Comparative studies, done under lipophlic conditions, showed that Astaxanthin has the highest singlet oxygen quenching activity (Table 3) and free radical scavenging activity (Table 4) both in liposomes and in cell culture. Part of this
enhanced anti-oxidative activity can be attributed to its remarkable resistance to photo irradiation as shown in liposomes by Oshima, et al (1993) (Figure 3).

![Graph showing loss of antioxidant in unilamellar liposomes following photo irradiation.](image)

**Figure 3**: Loss of antioxidant in unilamellar liposomes following photo irradiation. [Adapted from Oshima (1993)]

Thus, as demonstrated in Tables 3 and 4, although the fold of anti-oxidative activity of Astaxanthin compared to other carotenoids changes dramatically, depending on the experimental conditions (e.g. ranges between 2 to up to 550 times higher than vitamin E, Table 3), it is **consistently the highest of the carotenoids**.
### Table 3: Quenching of singlet oxygen potency of Astaxanthin and other carotenoids.

<table>
<thead>
<tr>
<th>Experimental system</th>
<th>Other carotenoids</th>
<th>Astaxanthin activity rate</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical solution (ORAC)</td>
<td>Vitamin E</td>
<td>x80 more active</td>
<td>Di Mascio 1991</td>
</tr>
<tr>
<td></td>
<td>Lutein</td>
<td>x3 more active</td>
<td></td>
</tr>
<tr>
<td></td>
<td>β-caroten</td>
<td>x2 more active</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Canthaxanthin</td>
<td>comparable</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vitamin E</td>
<td>x100 more active</td>
<td>Miki 1991</td>
</tr>
<tr>
<td></td>
<td>Zeaxanthin</td>
<td>x10 more active</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lutein Tunaxanthin</td>
<td>x10 more active</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Canthaxanthin</td>
<td>x10 more active</td>
<td></td>
</tr>
<tr>
<td></td>
<td>β-carotene</td>
<td>x10 more active</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vitamin E</td>
<td>x550 more active</td>
<td>Shimidzu 1996</td>
</tr>
<tr>
<td></td>
<td>Lutein</td>
<td>x3 more active</td>
<td></td>
</tr>
<tr>
<td></td>
<td>β-carotene</td>
<td>comparable</td>
<td></td>
</tr>
<tr>
<td>Protection against lipid oxidation in Liposoms</td>
<td>Vitamin E</td>
<td>x2 more active</td>
<td>Oshima 1993</td>
</tr>
<tr>
<td></td>
<td>β-carotene</td>
<td>x2 more active</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Canthaxanthin</td>
<td>More stable</td>
<td>Christopherson 1991</td>
</tr>
<tr>
<td>Protection of human lymphoid cells against oxidation</td>
<td>β-carotene</td>
<td>1.5 more active</td>
<td>Tinkler 1994</td>
</tr>
<tr>
<td></td>
<td>Canthaxanthin</td>
<td>1.5 more active</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lycopene</td>
<td>comparable</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4: Scavenging of free radicals potency of Astaxanthin and other carotenoids in vitro.

<table>
<thead>
<tr>
<th>Experimental system</th>
<th>Other carotenoids</th>
<th>Astaxanthin activity rate</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical solution</td>
<td>β-caroten</td>
<td>x 1.5</td>
<td>Terao 1989</td>
</tr>
<tr>
<td></td>
<td>Zeaxanthin</td>
<td>x 1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Canthaxanthin</td>
<td>comparable</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zeaxanthin</td>
<td>x 2</td>
<td>Miki 1991</td>
</tr>
<tr>
<td></td>
<td>Lutein</td>
<td>x 3.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Canthaxanthin</td>
<td>x2.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>β-carotene</td>
<td>x 4.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vitamin E</td>
<td>x100</td>
<td></td>
</tr>
<tr>
<td>Protection against lipid oxidation in Liposoms</td>
<td>Lycopene</td>
<td>x2</td>
<td>Barros 2001</td>
</tr>
<tr>
<td></td>
<td>Vitamin E</td>
<td>x 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Canthaxanthin</td>
<td>x3-10</td>
<td>Rengel 2000 (+Cu)</td>
</tr>
<tr>
<td></td>
<td>β-carotene</td>
<td>x 2</td>
<td>Goto 2001</td>
</tr>
<tr>
<td></td>
<td>β-carotene</td>
<td>x 6</td>
<td>Naguib 2000</td>
</tr>
<tr>
<td></td>
<td>α-carotene</td>
<td>x 2.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lycopene</td>
<td>x 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lutein</td>
<td>x 3</td>
<td></td>
</tr>
</tbody>
</table>
Protection against lipid oxidation in rat microsoms

<table>
<thead>
<tr>
<th>Protection against lipid oxidation in rat microsoms</th>
<th>β-carotene</th>
<th>comparable</th>
<th>Nakagawa 1997 (+Fe)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-carotene x 4 comparable</td>
<td>Canthaxanthin</td>
<td>Vitamine E</td>
<td>Palozza 1992</td>
</tr>
</tbody>
</table>

Protection against lipid oxidation in rat microsoms and organs

| Protection against lipid oxidation in rat microsoms and organs | Vitamine E | More potent | Nishigaki 1994 |

(5.2) Evidences from animal models (*in vivo*)

Astaxanthin was tested *in vivo* in various animal models. Early studies done in vitamin E-deficient rats demonstrated that Astaxanthin protects their mitochondria from damage by Fe$_2$(+)-catalyzed lipid peroxidation, both *in vivo* and *in vitro*. This protective effect was stronger than that of vitamin E itself (Nishigaki 1994, Kurashige 1990). That Astaxanthin has a general protective effect against oxidative damage was demonstrated by a study in rats that followed Nitric Oxide (NO) generation, as a marker of oxidation. Quantification of NO end products in plasma showed a decrease in Astaxanthin-fed rats (Hussein 2006) (Figure 4).

![Figure 4: Effect of oral administration of olive oil (control) or Astaxanthin for 7 weeks on the level of plasma oxidation markers NO2/NO3 in spontaneous hypertensive rats. [Adapted from Hussein (2006)]](image)


(5.3) Evidences from human trials

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Oxidative damage is related to many diseases in humans, and the ability of dietary Astaxanthin supplementation to improve or even prevent these pathologic situations is constantly being tested in humans. Two clinical studies directly addressed the effect of Astaxanthin on overall oxidation level: Karppi, et al. (2007) examined in a randomized double-blind study the effect of three-month Astaxanthin supplementation (4mg daily, as capsules) on lipid peroxidation of 20 healthy non-smoking Finnish men. It was observed that levels of plasma 12- and 15-hydroxy fatty acids were reduced in the Astaxanthin group only. The study indicated that intestinal absorption of Astaxanthin delivered as capsules is adequate. The second study tested the efficacy of eight-week treatment with Astaxanthin in 35 postmenopausal women, 21 of which suffered from high oxidative stress (Iwabayashi 2009). Astaxanthin-treated women showed enhanced antioxidant capacity, reduced lower limb vascular resistance, decreased blood pressure, and improved physical and mental symptoms.

(5.4) Preservation of membrane structure

The ability of Astaxanthin to inhibit lipid peroxidation via stabilization of the membranal structure was demonstrated in a recent comparative study that followed lipid peroxidation in the presence of several carotenoids (McNulty 2008). Interestingly, it was found that the nonpolar carotenoids, lycopene and β-carotene, disordered the membrane bilayer and stimulated membrane lipid peroxidation, whereas Astaxanthin (a polar carotenoid) preserved membrane structure and exhibited significant antioxidant activity (>40% decrease in lipid hydroperoxide levels) (Figure 5).
These results suggest that the antioxidant potential of carotenoids is dependent on their distinct interactions with the membrane lipids.

**Figure 5**: various carotenoids (10 mol/L) were incorporated into dilinoleoylphosphatidylcholine membranes and underwent lipid peroxidation at 37°C for 48 hours. [Adapted from McNulty (2008)]

Goto et al. (Goto 2001) showed that Astaxanthin was about 2-fold more effective than β-carotene in inhibiting the production of lipid peroxides in liposomes. The analysis of the reaction mechanisms of β-carotene and Astaxanthin suggested that Astaxanthin trapped radicals both in the membrane and on its surface (model depicted in Figure 10), whereas β carotene could do so only near the surface at the interior of the membrane. The investigators suggested that the efficient antioxidant activity of Astaxanthin is due to the unique structure of the terminal ring moiety. This structure-function relation may explain the differences in biologic activities of the various carotenoids, with important therapeutic implications.
Figure 6: Transmembrane orientation of polar carotenoids facilitates electron shuttling. Specific physicochemical interactions of Astaxanthin with membranes is likely responsible for its antioxidant properties and its biologic benefits. The transmembranous alignment provides exposure of the polar (hydrophilic) ends of the molecule to the internal cytoplasm and to the aqueous environment external to the cell (or the mitochondrial matrix and the intermembrane space of mitochondria), potentially facilitating electron transfer via the double bonds of the carbon scaffold of the compound. The intramembranous alignment of the molecule also likely provides proximity to vitamin C, which serves as a “sink” for accepting the radical cations generated effectively “recharging” the capacity of the degraded carotenoid. RNS- reactive nitrogen species; ROS- reactive oxygen species.
(6) Astaxanthin and skin health

Our skin is often exposed to sunlight, which contains hazardous UV light. Excessive exposure of the skin to sunlight results in erythema, sunburn and can lead to photo-induced inflammation, immunosuppression, aging and even carcinogenesis of skin cells (melanoma) (Figure 7). Pre-clinical studies illustrated that dietary antioxidants, such as vitamin E, vitamin C, or β-carotene could reduce such damage. The characteristics of Astaxanthin, as a superior antioxidant, motivated the investigation of its potential in skin health and cosmetics.

Figure 7: Effects of UVA, UVB and Ozone on skin.
UV rays induce the production of in situ ROS and matrix metalloproteinases (MMP). These factors are the root of wrinkle formation as they destroy the collagen matrix in the dermis. The skin’s repair mechanism will rebuild the damaged collagen, however, the hindrance of skin renewal by repeated exposure to ROS and MMP leads to formation of wrinkles. The presence of Astaxanthin attenuates the effects of ROS and MMP and therefore allows proper regeneration of the skin.
(6.1) Evidences from cell cultures

The ability of Astaxanthin to protect from UV-induced oxidative stress was first documented in cell cultures (O’Connor 1998, Camera 2009, Lyons 2002, Santocono 2006). UV-induced damage usually expresses itself in a battery of cellular reactions, including apoptosis (programed cell death), which are being followed in the presence or absence of the tested carotenoids.

In a recent study (Camera 2009) performed in human dermal fibroblasts, it was found that Astaxanthin exhibited a pronounced photoprotective effect and counteracted all of the UVA-induced alterations. β-Carotene only partially prevented few of the UVA-induced reactions, but it increased membrane damage and stimulated HO-1 expression. In addition, uptake of Astaxanthin by fibroblasts was higher than that of β-Carotene. The photostability of Astaxanthin in fibroblasts was much higher than that of β-Carotene. The data indicate that Astaxanthin has a superior preventive effect towards photo-oxidative changes in human dermal fibroblasts (Figure 8).

![Figure 8: Effect of different carotenoids on irradiation-induced apoptotic cell death in human dermal fibroblasts. While Astaxanthin (Ax) and canthaxanthin (CX) inhibited irradiation induced cell death, β-caroten (βC) enhanced it, compared to control. Adapted from Camera (2009).](image-url)
Earlier comparative studies showed that Astaxanthin could be more effective than β-carotene and lutein at preventing UV-light photo-oxidation of lipids in rat kidney fibroblasts by a factor of up to 200 and 1000 folds, respectively (O’Connor 1998) [Table 5]. The protective effect of Astaxanthin from UV-induced DNA damage was also shown in human skin fibroblast [Figure 9] (Lyons 2002) and in rat epithelial cells (Santocono 2006).

Table 5: Carotenoids protection from UVA radiation in rat kidney fibroblasts.
[Adapted from O’Connor (1998)]

<table>
<thead>
<tr>
<th>Carotenoid</th>
<th>CAT (u/mg protein)</th>
<th>SOD (u/mg protein)</th>
<th>TBARS (nmol MDA/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>β-carotene</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (no irradiation)</td>
<td>8.59</td>
<td>10.28</td>
<td>3.68</td>
</tr>
<tr>
<td>Irradiated (no carotenoid)</td>
<td>4.78</td>
<td>3.89</td>
<td>7.04</td>
</tr>
<tr>
<td>1000 nm</td>
<td>7.88</td>
<td>9.80</td>
<td>4.22</td>
</tr>
<tr>
<td><strong>Lutein</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (no irradiation)</td>
<td>7.35</td>
<td>9.78</td>
<td>2.34</td>
</tr>
<tr>
<td>Irradiated (no carotenoid)</td>
<td>4.05</td>
<td>3.83</td>
<td>5.34</td>
</tr>
<tr>
<td>1000 nm</td>
<td>8.63</td>
<td>10.34</td>
<td>2.60</td>
</tr>
<tr>
<td><strong>Astaxanthin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (no irradiation)</td>
<td>7.58</td>
<td>9.78</td>
<td>4.88</td>
</tr>
<tr>
<td>Irradiated (no carotenoid)</td>
<td>3.84</td>
<td>3.81</td>
<td>9.30</td>
</tr>
<tr>
<td>10 nm</td>
<td>7.67</td>
<td>9.73</td>
<td>3.56</td>
</tr>
</tbody>
</table>
Astaxanthin was also found to inhibit the over-production of melanin in melanoma cells, an important step in their carcinogenesis (Arakane, 2002). This phenomenon is known as hyper-pigmentation of the skin, in the form of suntan, stains or freckles. Mouse melanoma cells cultured with Astaxanthin for 3 days had a 60% lower amount of melanin than control cells, and the inhibitory effect was dose-dependent.

Repeated exposure of the skin to UVA irradiation elicits sagging which is mainly attributed to its biochemical mechanism to up-regulate the expression of matrix-metalloproteinase (MMP)-1 and skin fibroblast elastase (SFE). A recent study in cultured human dermal fibroblast showed that Astaxanthin attenuated the UVA-induced up-regulation of these two enzymes (Suganuma 2010) [Figure 10]

Figure 9: Ability of Astaxanthin to protect against UVA induced damage in human melanocytes. [Adapted from Lyons (2002)]
(6.2) Studies in animal models

Studies in animal models were restricted to hairless UV-irradiated mice while Astaxanthin is applied either topically (Arakane, 2002) or orally (Savoure 1995). Astaxanthin was shown to accumulate in rat skin when given orally (Petri 2007) or topically (Ying-Shu 2006). The first group (Arakane 2002) followed wrinkles formation, and showed decreased irreversible wrinkling in Astaxanthin- treated mice, as compared with control mice. Scanning electron micrographs of the ultra structure of dermal collagen fiber bundles indicated that their structure was maintained by the application of Astaxanthin. This paper demonstrated the potential of post-irradiation topical treatment with Astaxanthin to reduce skin wrinkling, one of the most important cosmetic targets. The second group (Savoure 1995), searched for UV-induced modifications in epidermal concentrations of free polyamines, as markers for skin photo damage. UV-irradiated mice had a higher level of the polyamine putrescine. Astaxanthin supplementation inhibited putrescine accumulation better than retinol, and decreased the levels of two additional
polyamines, spermidine and spermine. These results support the role of Astaxanthin as skin photo-protector when given orally.

(6.3) Studies in humans

Studies in humans focused on two directions: the reddening (erythema) of the skin after exposure to UV irradiation, and prevention and smoothening of wrinkles and dry skin for cosmetic purposes.

Erythema is a leading cause for hyper-pigmentation and was found to be reduced by both synthetic and natural forms of Astaxanthin in healthy subjects (Yamashita, 1995) (Figure 11). Compared to natural Astaxanthin, synthetic Astaxanthin showed lower suppression. Furthermore, natural Astaxanthin demonstrated faster recovery times of the erythema index, suggesting anti-inflammatory properties that together with inhibition of melanin formation may contribute to the reduction of hyper-pigmentation. Since Astaxanthin does not absorb UVB light, the mechanism of erythema suppression might be related directly with oxidation of melanin that is produced in response to UV radiation.

![Figure 11: Inhibitory effect of topically applied Astaxanthin (Ax) on Erythema in human. Melanine index was measured 1 week following UVB irradiation. Each color corresponds to a participant in the trial. [Adapted from Yamashita (1995)]](image)
Three additional studies (Yamashita 2002, Yamashita 2007, Seki 2001) were performed in order to evaluate the effect of Astaxanthin dietary supplementation with or without topical application on skin elasticity and moisture. The results of the studies showed, that supplementation with Astaxanthin improves moisture and elasticity of the skin and thereby reduces formation of fine wrinkles (Figure 12, Table 6). The mechanism behind the results is most likely the strong antioxidant effect of Astaxanthin in combination with the high deposition of Astaxanthin in the skin. Astaxanthin present in the skin quenches free radicals generated by UV-light and other inducers and thereby protects the collagen fibrils from increased breakdown.

![Figure 12](image_url)

**Figure 12**: Subject response after 6 weeks of Astaxanthin supplementation. Adapted from Yamashita (2007).
Table 6: Cosmetic benefits of Astaxanthin and vitamin E on Human skin.
Adapted from Yamashita (2002)

<table>
<thead>
<tr>
<th>Study Method</th>
<th>Double blind test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test samples</td>
<td>Capsule contains 2mg astaxanthin+40mg Vit E.</td>
</tr>
<tr>
<td></td>
<td>Placebo capsules (for control) with canola oil</td>
</tr>
<tr>
<td>Subjects</td>
<td>16 healthy woman with dry skin around 40 years old</td>
</tr>
<tr>
<td>Dosage</td>
<td>One capsule a day</td>
</tr>
<tr>
<td>Duration</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Self tested</td>
<td>After 2 and 4 weeks according to a questionnaire (FCG model)</td>
</tr>
<tr>
<td>Skin specialist’s inspection</td>
<td>After 2 and 4 weeks</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Results (specialist)</th>
<th>Significantly increased moisture levels, consistent natural oils, a reduction of fine wrinkles and a reduction of pimples.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results (self tested)</td>
<td>Less swelling under the eyes, improved elasticity and better skin feeling</td>
</tr>
<tr>
<td>Results (placebo group)</td>
<td>No improvements</td>
</tr>
</tbody>
</table>

Table 7 summarises the benefits of Astaxanthin in skin health and its mechanisms of action.

Table 7: Astaxanthin effects on skin health – a summary.
(7) Astaxanthin and modulation of the immune system

In the normal human body the activities of various types of the immune system cells, are strictly balanced. An excess of activity of one type of cells (for example, Th1 type) may result from an autoimmune disease, or from an ongoing infection. Normally, the cell activity diminishes when the physiological need thereof is reduced. An excess activity is thus seen when the normal reduction of cell activity is not achieved even though the inducer has diminished.

Immune modulation aims at restoring the balance between different subsets of T cells, so that damaging responses are suppressed. The drugs currently used to suppress the immune system have very broad action and inhibit protective functions of the immune system as well as pathological responses. Opportunistic infection is therefore a common complication of immune suppressive drugs.

Upon sensitization, lymphocytes undergo proliferation by oxyradical-based mechanisms. Through continuous resting-stimulation cycles, lymphocytes accumulate auto-induced oxidative lesions which lead to cell dysfunction and limit their viability and proper function of the immune response. In light of its known anti-oxidative activity, the effect of Astaxanthin as a booster and modulator of the immunological system was investigated in cell cultures and in rodent models.

In cultured mouse thymocytes and spleen cells, Astaxanthin stimulated cell proliferation and immunoglobulin production of murine spleen cells, and enhanced the release of interleukin-1α and tumor necrosis factor-α from murine peritoneal adherent cells (Okai 1996). In similar experiments, Astaxanthin increased the production of T-helper cell antibody and increased the number of antibody secretory cells from primed spleen cells (Jyonouchi 1996) (Figure 13). These authors also studied the effect of Astaxanthin in the production of immunoglobulins in vitro by human blood cells and found that it increased the production of IgA, IgG, and IgM in response to T-dependent stimuli (Jyonouchi 1995). Other studies done in vivo in mice have demonstrated the immunomodulating action of Astaxanthin and other carotenoids for humoral responses to T-dependent antigens, and suggested that the supplementation with carotenoids may be useful to restore immune responses (Jyonouchi 1994). Moreover, comparative studies
done in mice showed that Astaxanthin was consistently more active than β-carotene, lutein and canthaxanthin (Jyonouchi 1994, Bennedsen 1999, Chew 1999)(Figure 13).

![Figure 13: Effect of carotenoids on number of IgM antibodies (Ab)-secreting cells formed in cultures of Th2 and spleen cells. [Adapted from Jyonouchi (1996)]](image)

Due to its immunomodulating action, Astaxanthin has also been suggested as a medication for the treatment of autoimmune diseases such as multiple sclerosis, rheumatoid arthritis and Crohn’s disease (Lignell 2001). When tested in autoimmune-prone mice (Tomita 1993), Astaxanthin was able to delay symptoms of proteinuria and lymphadenopathy.

Recently, a first study in humans attempted to investigate the action of dietary Astaxanthin in modulating immune response, oxidative status and inflammation in young healthy adult female subjects, receiving 0-8 mg/day Astaxanthin for 8 weeks in a randomized double-blind, placebo-controlled study (Park 2010). The results showed that Astaxanthin stimulated mitogen-induced lymphocytes proliferation, increased natural killer cell cytotoxic activity, and increased total T and B cell subpopulations.
(8) Astaxanthin and inflammation

One of the ways in which an immune response becomes a pathological condition is inflammation. Severe or chronic inflammation, such as in Crohn’s disease and ulcer disease, involve the action of many ROS. These toxic molecules not only induce oxidative stress, but also stimulate the expression of inflammatory genes in endothelial cells, which in turn aggravate the inflammation. In addition, the production of ROS by the immune cells themselves, as part of the normal immune response, along with their high membranal content of polyunsaturated fatty acids render them especially sensitive to oxidative damage (Chew 2009). Therefore, molecular effectors with anti-inflammatory properties are highly valuable. Astaxanthin exhibited such properties in several studies reviewed below.

(8.1) Studies in cell cultures

Attempts to understand the mechanism of action of Astaxanthin in inflammation were performed in cell lines including macrophages (Lee 2003), microglia (Choi 2008), nerve PC12 cells (Chan 2009) and human lymphocytes (Bolin 2010). Two representative experiments are detailed below. In the first study (Lee 2003) (Figure 14), Astaxanthin was shown to inhibit inflammation-induced nitric oxide production and inflammatory gene expression in both stimulated cells and primary macrophages. In addition, Astaxanthin suppressed the serum levels of NO, prostaglandin E2, tumor necrosis factor-α, and interleukin-1 β in lipopolysaccharide-administrated mice. The importance of the Astaxanthin-mediated suppression of the NF-kappa-B pathways stems from the seminal role of the latter in immunity, as it activates pro-inflammatory genes encoding for NO synthase, tumor necrosis factor-α, and several interleukins.
Figure 14: Effect of Astaxanthin on ROS generation in mouse macrophage cells with and without Inflammation induction by lipopolysaccharide (LPS). [Adapted from Lee (2003)]

Similar results were found recently by Kishimoto et. al (2010)(Figure 15), showing that Astaxanthin has inhibitory effects on macrophage activation, such as scavenger receptors up-regulation, MMPs activation, and pro-inflammatory cytokines secretion. Activated macrophages are located in atherosclerotic lesions, where they are responsible for the clearance of pathogenic lipoproteins and for promotion of the inflammatory process which is a crucial factor in cardiovascular diseases.

Figure 15: Effect of Astaxanthin on expression levels of in lipopolysacaride-stimulated MMP-I in activated macrophages. [Adapted from Kishimoto (2010)]
In the second study (Choi 2008), Astaxanthin was tested in lipopolysaccharide-stimulated microglia cells. Microglia cells are the resident macrophages and immune surveillance cells of the central nervous system. The activation of microglia is associated with release of NO and subsequent release of pro-inflammatory cytokines (Gonzalez-Scarano 1999), two processes that are implicated in the development of inflammation and neurodegenerative disorders. Specifically, the high level of NO, produced by NO synthase (NOS), has been defined as an indicator of cytotoxicity in inflammation and endotoxemia (Schmidt 1994). Choi, et al showed that Astaxanthin inhibited the production of inflammatory mediators by blocking NOS activation and by the suppression NOS protein levels (Figure 16).

Figure 16: Modulation of LPS-induced iNOS expression by astaxanthin in BV-2 microglial cells. BV-2 cells were treated with astaxanthin for 1 h, and then treated with LPS for 6 h. Adapted from Choi (2008).

(8.2) Studies in rodents

Kurashige et. al. (1990) showed that carrageenan-induced inflammation of a rat’s paw was significantly inhibited by intraperitoneal administration of Astaxanthin [Figure 16].
More recent studies investigated the efficacy of Astaxanthin in lipopolysaccharide- (Ohgami 2003) and endotoxin- (Suzuki 2006) induced inflammation (uveitis) of rat’s eye, or following additional inflammation inducers (Izumi-Nagai 2008). Rats injected with Astaxanthin showed a significant decrease in the number of infiltrating cells in the anterior chamber. Additionally, there was a significantly lower concentration of protein, NO, TNF-alpha and PGE2, all markers of inflammation, in the aqueous humour. Moreover, even early stages of EIU were suppressed by injection of Astaxanthin. These results suggest that Astaxanthin reduces ocular inflammation in eyes with EIU by downregulating proinflammatory factors and by inhibiting the NF-kappaB-dependent signaling pathway.

![Figure 17: Effect of Astaxanthin and Vitamine E on induced inflammation of rat paw. Adapted from Kurashige (1990).](image)
Additional insight to the cellular target of Astaxanthin in inhibition of inflammation was found in mice injected with inflammation inducers following by treatment with Disodium Disuccinate Astaxanthin – a water soluble derivative of Astaxanthin (Lockwood 2006). A generalized antioxidant effect was identified at the time point of maximal neutrophilic recruitment. Moreover, Astaxanthin specifically inhibited both 5-lipoxgenase and cyclooxygenase (COX-2) at the time point of maximal monocyte/macrophage recruitment and activation. This interaction of Astaxanthin with 5-lipoxgenase and the subsequent inhibition of NF-kappaB, provide the foundation for further evaluation of its therapeutic effects on these signaling pathways which are important in inflammation.

(8.3) Studies in humans

Currently, only one study directly checked the anti-inflammatory effect of Astaxanthin in humans (Park 2010). Fourteen young and healthy women received 0, 2, or 8 mg Astaxanthin daily for 8 week in a randomized double-blind, placebo-controlled study. The results showed a decrease in DNA damage biomarker after 4 week but no affect on lipid peroxidation. Plasma C-reactive protein (an inflammation marker) concentration was lower in subjects given 2 mg Astaxanthin, while some markers of the immune response were enhanced (see chapter 7 above). The authors suggested that the lack of efficacy of Astaxanthin in certain response measured may result from the fact that in many cases greater effect of antioxidants is seen in excess amounts of oxidative stress, in immuno-compromised individuals, while the subjects in the current study were healthy ones.
(9) Astaxanthin and gastric ulcer

Gastric ulcer may be caused by several factors. Seventy to ninety percent of gastric ulcers are estimated to be due to chronic inflammation caused by the bacteria Helicobacter pylori (H. pylori) that colonize the antral mucosa, while the rest of the cases are caused by drugs (e.g. NSAIDs), smoking, diet and stress. The effects of Astaxanthin on H. pylori- and chemically-induced gastric ulcers were determined in rodents and humans, as described below.

(9.1) Helicobacter pylori-induced gastric ulcer

In mice infected with *H. pylori* which are fed an Astaxanthin-rich diet, Astaxanthin had an anti-bacterial action manifested as reduced gastric mucous, lower load and colonization by the bacterium and shift in the immune response (Bennedsen 1999, Wang 2000) (Figure 18). The mechanism of anti-bacterial action of Astaxanthin is not known, but it is suspected that its antioxidant properties play an important role in the protection of the hydrophobic lining of the mucous membrane making colonization by H. pylori much more difficult (Wadstron 2001).
Figure 18: Effect of Astaxanthin on H. pylori colonization of gastric mucosa and inflammation score of gastritis in mice treated with Astaxanthin at 3.5 weeks post inoculation. [Adapted from Bennedsen (1990)]

The single study of *H. pylori*-infected humans suffering from functional dyspepsia was however, less definitive (results published in two papers by Kupcinskas 2008 and by Andersen 2007). In this study, the ability of Astaxanthin to ameliorate gastrointestinal discomfort (GD), along with its effect on gastric inflammatory markers and interleukins were tested in 44 patients with functional dyspepsia. It was found that after 4 weeks of treatment no difference between the treatment groups was observed regarding mean GD scores of abdominal pain, indigestion and reflux syndromes. However, reduction of reflux syndrome was achieved in the higher (40mg) dose. There were no significant changes in the density of *H. pylori* or in any of the interleukins during or after treatment. The authors suggested that the differences between positive results obtained in mice compared to the lack of influence in humans, may be due to different dietary conditions: the mice had a controlled diet lacking any antioxidants, while humans had a varied diet. Hence, it is possible that when the host has access to antioxidants in his diet, the effects of Astaxanthin is not pronounced. As these are only preliminary results, the effect of Astaxanthin on functional dyspepsia should be investigated in additional clinical experiments.

(9.2) Stress- and chemically-induced gastric ulcer

The effects of Astaxanthin on chemically - induced gastric ulcers were tested in rats following treatments with various chemicals including alcohol (Kim 2005a, Kamath 2008) (Figure 19), naproxen (Kim 2005b) and acetic acid (Yang 2009). In all the studies positive effects of Astaxanthin were demonstrated in various measurements and markers such as decreased or complete prevention of evolution of gastric ulcerations, lower ulcer indexes, inhibited elevation of the lipid peroxide level in gastric mucosa and increase in the activities of radical scavenging enzymes and antioxidants following pretreatment with Astaxanthin.
In a comparative study (Nishiwaka 2005), β-carotene and Astaxanthin prepared from three different sources (alga Haematococcus, the yeast Phaffia, and synthetic Astaxanthin), were tested for their effect on stress-induced gastric ulcer in rats. It was found that rats given Astaxanthins or β-carotene prior to stressing were appreciably protected against the evolution of gastric ulcerations compared to control rats. Ulcer indexes were particularly lower in the rats fed Haematococcus-extracted Astaxanthin than those of the other groups (Figure 20).

Figure 19: Effect of Astaxanthin on the formation of gastric mucosal lesions after oral administration of 80% Ethanol to rats. [Adapted from Kim (2005a)]
Figure 20: Effect of Astaxanthin from Hematococcus (Ax(H)), synthetic Astaxanthin (Ax(S)) or β-carotene (β-C) on ulcer development in stressed rats. HD – High dose of Astaxanthin (40mg/rat), ND – Normal dose of Astaxanthin (8mg/rat). [Adapted from Nishiwaka (2005)]
(10) Astaxanthin and the cardiovascular system

Cardiovascular diseases (CVD) include heart diseases, stroke, blood hypertension, hardening of the arteries, and additional diseases of the circulatory system. Oxidative stress (increased ROS and NOS) and inflammation play an important role in a number of aspects of CVD such as endothelial dysfunction, lipid disorders, myocardial damage and arterial fibrillation. Nutritional antioxidants, including Astaxanthin, can decrease lipid and protein oxidation and can modulate the inflammation reaction, hence potentially protecting against atherosclerosis and arterial stiffening (Carpenter 2003, Ellingsen 2009). Indeed, several epidemiological studies showed an association between nutritional antioxidant intake and reduced adverse cardiovascular disease outcomes (Wilcox 2008, Fasset 2009 and references therein).

Astaxanthin was tested regarding its effects on various cardiovascular pathologies (reviewed in Pashkew 2008, Fasset 2009). With this regard, it should be mentioned, that all the ischemia-reperfusion studies were done with a synthetic water-soluble derivative of Astaxanthin – Disodium Disuccinate Astaxanthin (DDA, Cardax) that enables intravenous and oral administration (Lockwood 2005).

(10.1) Astaxanthin in prevention of atherosclerosis and dyslipidemia

Arteriosclerosis is a process in which plaque builds up in the artery walls, reducing blood flow. Total blockage of the narrowed artery results in a heart attack, stroke and peripheral circulation problems; leading to many dysfunctions including loss of limb functions and impaired sexual potency. Susceptibility to arteriosclerosis is determined by a combination of genetic and environmental factors, including diet, especially a diet high in cholesterol. High blood levels of LDL (Low Density Lipoprotein)-cholesterol, are associated with an increased risk of arteriosclerosis and the opposite is true for HDL (High Density Lipoprotein)-cholesterol. The combination of high LDL and triglycerides (TG) levels and low HDL level is termed dyslipidemia and is considered a risk factor for cardiovascular problems. LDL-cholesterol aggravates endothelial dysfunction, mainly via reduction of blood vessels flexibility and rupture of
plaques accumulating in the atherosclerotic lesions, thus increasing the risk for heart attack and stroke (reviewed in Pashkew 2008). In addition, plasma, LDL is usually not oxidized, and when it does occur, it is believed to contribute to the development of arteriosclerosis. Therefore, a number of pre-clinical and clinical studies tested the efficacy of Astaxanthin in reduction of dyslipidemia and LDL oxidation.

Early studies in rodents showed that Astaxanthin (but not β-caroten) led to an increase in blood levels of HDL (Murillo, 1992). Similarly, Astaxanthin has been reported to decrease serum TG and increase HDL-cholesterol and adiponectin in insulin resistant rats and in obese mice fed a high fat diet (Hussein 2007, Ikeuchi 2007)(Figures 21, 22).

![Figure 21: Effect of Astaxanthin (18 weeks) on HDL (A) and triglycerides (TG) in normal Wistar rats or hypertensive, insulin-resistant rats (SHRcp). OL-olive oil, ASX – Astaxanthin. [Adapted from Hussein (2007)]](image-url)
A recent randomized double-blind trial in 61 mildly hyperlipidemic subjects receiving doses of 0, 6, 12, 18 mg/day for 12 weeks demonstrated that Astaxanthin consumption ameliorates triglyceride and HDL-cholesterol levels in correlation with increased adiponectin in humans (Yoshida 2010). Adiponectin has been reported to correlate positively with HDL-cholesterol, and inversely with triglyceride (TG) and very low density lipoprotein (VLDL)-cholesterol (Okamoto 2006, Yoshida 2005).

Atheriosclerosis is not limited to the heart, but rather influences the entire body blood circulation. As a result, it is a risk factor for cerebral ischemic injury as well as an important factor in the normal function of skeletal muscles. Astaxanthin was shown to reduce cerebral infraction and to improve locomotive activity in treated vs. control animals (Shen 2009). In addition, Astaxanthin reduced exercise-induced oxidation markers in treated rats muscles (Aoi 2003, Aoi 2008) and exercise-induced fatigue in both rats (Ikeuchi 2006) and humans (Nagata 2006), indicating a better blood and oxygen supply to the muscles.
(10.2) Astaxanthin and prevention of lipid oxidation

The inhibitory effects of Astaxanthin on lipid oxidation were demonstrated both in vitro and in vivo. Mason, et al (2006) showed that Astaxanthin eliminated the lipid peroxidation caused by the drug rofecoxib in cellular membrane models. The ability of Astaxanthin to protect from lipid oxidation was further supported in an ex-vivo analysis of LDL particles from humans fed Astaxanthin (Iwamoto 2000) [Figure 23]. In this study, Astaxanthin conferred resistance to copper-induced oxidation seen as increased LDL oxidation lag times. Astaxanthin reduced LDL oxidation in contrast to other antioxidants lacking this effect, such as vitamin E, and lutein.

Figure 23: Effect of Astaxanthin on the lag phase of conjugated dienes formation, as a result of oxidation, in LDL. [Adapted from Iwamoto (2000)]

An in vivo study done in rabbits (Setnikar 2005), demonstrated the inhibitory effect of Astaxanthin not only on plasma lipid peroxidation but more important on plaque formation on the aorta wall. However, in a recent study the latter result could not be repeated (Augusti 2009). Augusti, et al observed that high-fat atherogenic diet given to
rabbits increased the serum cholesterol levels and the ratio of the intima/media area in the aortic arch. However, these changes were not prevented by Astaxanthin. Astaxanthin only attenuated plasma lipid peroxidation and certain antioxidant enzyme activities.

(10.3) Astaxanthin and hypertension

One of the risk factors for cardiovascular disorders is high blood pressure. Blood pressure is highly regulated and is being determined by the relaxation level of the blood vessels (vasodilatation), with NO being one of the major regulators. In a series of experiments (reviewed in Yanai 2008), conducted in spontaneously hypertensive rats (SHR), which have been widely used as a model for hypertension, Astaxanthin was shown to exert the following anti-hypertensive effects: significant reduction in blood pressure and delayed incidence of stroke (Figure 24), significant reduction of NO end products and the contractile responses of the aorta to α-adrenergic receptor agonist and angiotensin II, and finally, decreased coronary artery wall thickness compared with control, indicating the possibility that Astaxanthin ameliorates hypertension induced vascular remodeling.

Figure 24: Effect of chronic oral administration of Astaxanthin (Asta) on the incidence of stroke in rats after cessation of the treatment. [Adapted from Hussein (2005)]
(10.4) Stroke and thrombosis damages

Failure to supply blood to the heart can lead to myocardial infarction (MI), one of the major causes of stroke. Following MI and the accompanied ischemia, an additional damage to tissue is caused when blood supply returns to the tissue (reperfusion). The absence of oxygen and nutrients during the ischemic period creates a condition in which the restoration of circulation results in inflammation and oxidative damage through the induction of oxidative stress rather than restoration of normal function. This situation is referred to as ischemia-reperfusion damage. The water-soluble derivative of Astaxanthin, DDA, was assessed for its efficacy in several ischemia-reperfusion studies in rats, rabbits and dogs (reviewed in Fasset 2009, Pashkew 2008).

In the heart muscle (myocardium) of rats it has been demonstrated that prior treatment for 4 days with intravenous DDA (Cardax), significantly reduced the subsequent myocardial infarct size and hence improved myocardial recovery (Gross 2004)(Figure 25). There was a correlation between this effect and the dose administered.
Figure 25: Mean myocardial salvage as a percentage of infarct size/area at risk. Control set at 0% salvage. Control animals received vehicle injection alone; Cardax - treated animals received the appropriate dose once daily I.V. for 4 days prior to experimental infarction and IS/AAR determinations on day 5. Adapted from Gross (2004).

Lauver et al. (2005) used prior treatment for 4 days of intravenous DDA in a rabbit myocardial ischemia reperfusion model. Again, there was a significant reduction in myocardial infarct size and improved myocardial salvage in the DDA-treated animals. In addition, as an assessment of inflammatory activity, complement activation was measured. This marker was found to be attenuated in DDA treated animals suggesting a DDA-associated reduction in tissue inflammation. High levels of free Astaxanthin and reduced levels of lipid peroxidation were detected in myocardial tissues of treated rats (Gross 2006). When extended to a dog model, Gross, et al (2005), using intravenous DDA at either 2 h or 4 days, prior to coronary artery occlusion, observed a significant reduction in myocardial infarct size in DDA treated dogs after both treatments (Figure 26). Moreover, two out of three dogs in the 4-day treatment group had 100% cardiac protection. These findings were recently repeated in ischemic injury study in rat liver (Gulten 2010).
Figure 26: Effect of Astaxanthin (Cardax) on mean infract size (IS) as percent of area at risk (AAR) following acute infract of dogs myocardium. 2hr, 4 days – treatment with Cardax 2 hours or 4 days prior to infract study, respectively. Adapted from Gross (2005).

DDA has been efficient in a dog model of an additional complication of atherosclerosis, namely, rethrombosis and platelets aggregation which are thought to be related to ROS generation (Krötz 2004). A dose-dependent reduction in the incidence of carotid artery rethrombosis and inhibition of ex-vivo platelets aggregation and thrombus weight were observed.

The results of the aforementioned pre-clinical experiments raise the possibility that Astaxanthin may have a therapeutic potential in humans.
(11) Astaxanthin and muscle endurance

Exercise may be accompanied by muscle damage and aches as well as fatigue, symptoms that are attributed to several factors: 1) Increased generation of free radicals as a result of increase in mitochondrial oxygen consumption and electron transport flux, leading to lipid peroxidation; 2) Myoglobin and coenzymes leak out into the blood from cells and tissues damaged by exercise, and destruction of red blood cells; 3) Consumption and depletion of energy sources such as glycogen and 4) Production and accumulation of metabolic products such as lactic acid. Therefore, recovery from exercise requires re-synthesis of the leaked cell and tissue components and consumed energy sources, as well as decomposition and removal of accumulated byproducts of metabolism. In addition, intracellular redox imbalance may affect nutrient metabolism during action in skeletal muscle. Due to its antioxidant activity, Astaxanthin was therefore checked for its effects on muscle performance and exercise-induced fatigue.

(11.1) Studies in rodents

Studies in mice investigated the effect of Astaxanthin on muscle lipid metabolism in exercise (Aoi 2008), on oxidative damage induced by strenuous exercise in heart and skeletal muscles (Aoi 2003), and on exercise fatigue (Ikeuchi 2006). In the first study, Astaxanthin improved muscle lipid metabolism in exercise. In addition, it accelerated the decrease of body fat accumulation with exercise training, suggesting that Astaxanthin promoted lipid metabolism rather than glucose utilization during exercise leading to improvement of endurance and efficient reduction of adipose tissue with training [Figure 27].
Figure 27: Running time to exhaustion. Exercise groups performed treadmill running at 30 m/min after 4 week of treatment. [Adapted from Aoi (2008)]

In the second study (Aoi 2003), exercise-increased biochemical markers in skeletal muscle were reduced only in the Astaxanthin group. In addition, Astaxanthin lowered exercise-induced activity of creatine kinase and myeloperoxidase in the skeletal muscle. In the third study (Ikeuchi 2006) [Figure 28], the Astaxanthin-supplemented group showed a significant increase in swimming time to exhaustion as compared to the control group. This was reflected also by improved metabolic markers such as lower blood lactate, higher plasma non-esterfied fatty acid and glucose and decreased fat accumulation, compared to controls. These results suggest that improvement in swimming endurance by the administration of Astaxanthin is caused by an increase in utilization of fatty acids as an energy source.
Figure 28: Effect of Astaxanthin on swimming exercise in mice. The mice were given either vehicle (●), or an Astaxanthin dose of 1.2 (□), 6 (△), or 30 (○) mg/kg body weight. The mice were made to perform swimming exercise with weights attached to their tails corresponding to 10% of their body weight. [Adapted from Ikeuchi (2006)]

(11.2) Studies in humans [Research in humans is summarized in Table 8 below]

Table 8: Effect of Astaxanthin on muscle endurance. Summary of clinical studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Men** (no. +training)</th>
<th>Astaxanthin dose+time</th>
<th>Effect checked</th>
<th>Main outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignel 2001</td>
<td>20 NT</td>
<td>4 mg 6 months</td>
<td>Strength/explosiveness and Strength/endurance tests</td>
<td>No effect</td>
</tr>
<tr>
<td>(P)</td>
<td></td>
<td></td>
<td></td>
<td>Positive effect</td>
</tr>
<tr>
<td>Sawaki 2002</td>
<td>16 NT*</td>
<td>6 mg 28 days</td>
<td>Lactic acid accumulation after 1,200 m run</td>
<td>Reduced lactic acid.</td>
</tr>
<tr>
<td>(NA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fry 2004</td>
<td>9 RT</td>
<td>8 mg 21 days</td>
<td>Histology of muscle fibers following weight lifting, muscle sourness</td>
<td>No immediate effect. Reduced sourness after 48h + histological changes in fibers.</td>
</tr>
<tr>
<td>(P)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bloomer 2007</td>
<td>20 RT</td>
<td>4 mg 21 days</td>
<td>Markers of skeletal muscle injury</td>
<td>No effect</td>
</tr>
<tr>
<td>(P)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nagata 2006</td>
<td>19 NT</td>
<td>5 mg 14 days</td>
<td>Respiratory-circulation ability and activities of sympathetic nervous system</td>
<td>Positive effect</td>
</tr>
<tr>
<td>(P/B)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*NA-not available, P-placebo, B-blind, NT-not trained, RT- resistance trained
** All studies were performed in healthy men.

As evident from Table 8, research of Astaxanthin effects on muscle endurance is limited, with small numbers of participants in each study and overall mixed results. Further studies will have to evaluate the influence of additional factors that most probably affect the results such as physical fitness of the subject, his diet, the type of exercise followed etc.

(12) Astaxanthin and anti-cancer activity

Human epidemiological studies have revealed certain protective effect of vegetable and fruit consumption for certain types of cancer (Block 1992, Giovannucci 1999, Vainio 2006). Varieties of compounds, found in these foods, have known bioactive mechanisms and are suspected as anticancer agents. In a landmark paper, Peto, et. al (1981) suggested an inverse correlation between dietary intake of β carotene and cancer incidence, and recommended further larger evaluation of this effect. However, when tested in large human intervention trials the β-carotene hypothesis proved to be disappointing. Not only did β-carotene supplementation offer no significant protection from lung and other cancers, it actually increased lung cancer risk among smokers in two of the trials, maybe due to its ability to act as a pro-oxidant under certain conditions (ATBC 1994, Hennekens 1996, Omenn 1996, Gallicchio 2008). Similar disappointing results were obtained for additional carotenoids for lung cancer (Gallicchio 2008).

Because it is not a significant dietary carotenoid, epidemiological data on Astaxanthin in disease prevention is lacking. However, it has exhibited potential anticancer properties in vitro and in animal models.

(12.1) Cell culture Studies

Sun, et al (Sun 1998) showed that mouse tumor cells grown in the presence of Astaxanthin showed reduced DNA synthesis rate and lower overall cell number in compared to control cultures. Similarly, Astaxanthin inhibited murine mammary tumor
cell proliferation by up to 40%, in a dose-dependent fashion, when included in the culture medium (Kim 2001), and it was effective at inhibiting the invasion of rat ascites hepatoma cells in culture (Kozuki 2000)(Figure 29).

![Graph showing the effect of Astaxanthin on cell proliferation.](image)

**Figure 29:** Effect of Astaxanthin on the invasion of rat ascites hepatoma (AH109A) cells in culture. Adapted from Kozuki (2000).

The growth of human cancer cell lines has also been inhibited by Astaxanthin *in vitro*. Astaxanthin-rich alga *Haematococcus pluvialis* extract showed significant growth-inhibitory effects in several human colon cancer cells, accompanied by up-regulation of apoptotic enzymes and specific enzymes phosphorylation (Palozza 2009) [Figure 30]. In addition, a 98% inhibition of androgen-induced proliferation of human prostate cancer cells was demonstrated in the presence of Astaxanthin (Anderson 2005).
Figure 30: Effects of Haematococcus pluvialis (H.P.) extract on the growth of HCT-116 cells. [Adapted from Palozza (2009)]
(12.2) Studies in rodents

In studies with BALB/c mice, dietary Astaxanthin inhibited the growth of transplanted tumor cells in a dose-dependent fashion (Sun 1998). In a related study, tumor cells growth was inhibited when dietary Astaxanthin supplementation was started at one and three weeks prior to tumor inoculation, but not when supplementation was begun at the same time as tumor inoculation (Jyonouchi 2000)(Figures 31,32). These results suggest that Astaxanthin may inhibit tumor development in the early stages but not in the later stages of progression. In other studies with mice, Astaxanthin supplementation reduced transplanted mammary tumor growth (Chew 1999) (Figure 33) and suppressed spontaneous liver carcinogenesis (Nishimo 1999). Dietary consumption of egg yolks containing Astaxanthin inhibited chemically-induced tumors in mice (Lee 1997, Lee 1998).

![Figure 31: Tumor weight and size after inoculation of fibrosarcoma cells in mice fed control or Astaxanthin-supplemented diet. [Adapted from Jyonouchi (2000)]](image-url)
Figure 32: Cytotoxic T lymphocyte activity against tumor cells after inoculation in mice fed control or Astaxanthin-supplemented diet. [Adapted from Jyonouchi (2000)]

Figure 43: Effect of feeding with carotenoids on average mammary tumor volume on day 31 to 45 post-inoculation in mice. [Adapted from Chew (1999)]
A series of studies on cancer chemoprevention by natural and synthetic substances in mice and rats revealed several carotenoids, including Astaxanthin, as effective antitumor agents (Mori 1997). Thus, Astaxanthin was found to significantly reduce both the incidence and proliferation of chemically-induced urinary bladder cancer in mice (Tanaka 1994). In two related studies, the incidence and proliferation of chemically-induced cancers of the oral cavity and colon (Prabhu 2009, Nagendra Prabhu 2009) were significantly reduced in Astaxanthin-supplemented rats, relative to control rats [Figure 34].

![Figure 34: Effect of Astaxanthin (ASX) on 1,2-dimethyl hydrazine (DMH)-induced experimental colon carcinogenesis, expressed as no. of aberrant crypt foci in rats colon. Pre, Post – treatment with Astaxanthin 1 week before or 1 week after induction of carcinogenesis, respectively. [Adapted from Prabhu (2009)]](image)

Astaxanthin has shown effectiveness against the initiation of liver carcinogenesis in aflatoxin B1- (Gardelet 1998) and in cyclophosphamide-treated rats, in which it also decreased oxidative stress, and DNA damage (Tripathi 2010) (Figure 35). In the later study it was suggested that Astaxanthin may serve as a chemoprotective agent against the toxicity of the anticancer drug cyclophosphamide. Finally, Astaxanthin also reduced metastatic nodules and lipid peroxidation in livers of rats subjected to restraint stress (Yang 1997, Kurihara 2002).
Figure 35: Effect of post-treatment with Astaxanthin (Asta) on cyclophosphamide-induced (CP 50) carcinogenic foci in rat liver. [Adapted from Tripathi (2010)]

The possible mechanisms of action of the cancer-preventive effect of Astaxanthin are currently not known. However, in light of its activities detailed in previous chapters, Astaxanthin is expected to act via several pathways: as an antioxidant, as an enhancer of immune system, as a regulator of gene expression (especially of gap junction proteins) and in detoxification of carcinogenic compounds. Since all these pathways and their correspondence products have been shown to be involved in cancer development and fighting, Astaxanthin may prove to be a beneficial modulator of these reactions.
(13) Astaxanthin and the central nervous system

Intense metabolic activity, high levels of unsaturated fats and iron, and rich irrigation with blood vessels make the nervous system (brain, spinal cord, and peripheral nerves) very susceptible to oxidative damage (Halliwell 1992, Fachinetti 1998). Oxidative stress is also implied in the pathogenesis of major neurodegenerative diseases. These include Parkinson’s disease, Alzheimer’s disease, and amyotrophic lateral sclerosis (ALS, “Lou Gehrig’s disease”), as well as in cases of stroke, trauma, and seizures. Hence, a number of in vitro, in vivo and clinical studies have provided evidence that dietary supplementation with lipid-soluble antioxidants may inhibit the onset and progression of neurological diseases (Grant 1997, de Rijk 1997, Hajieva 2006, Zhao 2009). As previously mentioned, Astaxanthin is able to cross the Blood Brain Barrier in mammals (Tso 1996), and is therefore a promising candidate for research in neurological diseases.

Studies of Astaxanthin’s effect on the CNS focused on ischemic brain injury and on degenerative pathologies, including effect on memory.

(13.1) Studies in cell cultures and in rodents

Astaxanthin has a protective effect in neuronal cells exposed to oxidative damage. This effect was expressed in increased and dose-dependant viability of dopaminergic SH-SY5Y cells treated with chemical oxidative agents and with Astaxanthin and was reflected also in inhibited intracellular ROS generation and mitochondrial abnormalities (Liu 2009). Similar results were obtained in PC12 neuronal cells treated with Astaxanthin and oxidazing agents (Chan 2009) [Figure 36].
When tested in rodents, Astaxanthin showed an impressive protecting effect in mice subjected to transient cerebral ischemia, which were then followed for their learning and memory skills in Morris water maze (Hussein 2005a). Astaxanthin-fed mice presented better learning and memory skills. Similarly, when Astaxanthin was cerebraly-injected to rats prior to the ischemic induction, it increased locomotor activity and reduced cerebral infarction at 2 days after the injury, compared to vehicle (Shen 2009) [Figure 37]. To evaluate the protective mechanisms of Astaxanthin against stroke, brain tissues were assayed for free radical damage, apoptosis, and excitotoxicity. It was shown that Astaxanthin can reduce ischemia-related injury in brain tissue through the inhibition of oxidative stress, reduction of glutamate release, and an anti-apoptosis effect. In addition, Astaxanthin showed a protective effect in rats chronically fed with alcohol, in which it antagonized an alcohol-induced neural marker (Abadie-Guedes 2008).

Figure 36: Effect of Astaxanthin (AX) (10, 20 µM) or Canthaxanthin (CX) (10, 20 µM) on viability of PC12 cells, treated with H$_2$O$_2$ to induce cell death. [Adapted from Chan (2009)]
Finally, the impact of Astaxanthin-enriched *H. pluvialis* powder on auxiliary memory improvement was assessed in normal BALB/c mice, pre-supplemented with different powder dosages for 30 days (Zhang 2007). The results indicated that *H. pluvialis* powder was associated with dose-dependent memory improvement with the low dosage of algal powder being the best. These results suggest that Astaxanthin may have beneficial effects in improving memory in vascular dementia, ischemic injury and in healthy brain.

**(13.2) Studies in humans**

Currently, only a single clinical study was conducted, which addressed the effect of Astaxanthin on cognitive function (Satoh 2008). In this open-label study, 10 healthy male subjects (50–69 years of age), who complained of age-related forgetfulness, were given 12 mg Astaxanthin for 12 weeks. Compared to base level, Astaxanthin improved response time and accuracy of several tasks [Table 9], suggesting that it might improve higher brain function including cognition, attention, memory, information processing, and resultant behaviors in older persons.
Table 9: Mean response times and accuracies (±SD) on CogHealth tasks at baseline, and after 6 and 12 weeks of Ax treatment. [Adapted from Satoh (2008)]

<table>
<thead>
<tr>
<th>Tasks</th>
<th>Baseline</th>
<th>6 weeks</th>
<th>12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Response time (ms)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simple Reaction</td>
<td>341.68 ± 94.41</td>
<td>303.31 ± 33.80</td>
<td>281.76 ± 33.56**</td>
</tr>
<tr>
<td>Choice Reaction</td>
<td>504.53 ± 56.84</td>
<td>480.63 ± 39.87</td>
<td>463.63 ± 26.49**</td>
</tr>
<tr>
<td>Divided Attention</td>
<td>494.13 ± 135.57</td>
<td>419.52 ± 59.32**</td>
<td>412.07 ± 51.97**</td>
</tr>
<tr>
<td>Working Memory</td>
<td>762.94 ± 141.65</td>
<td>732.95 ± 174.83</td>
<td>654.83 ± 128.42**</td>
</tr>
<tr>
<td>Delayed Recall</td>
<td>1008.19 ± 153.37</td>
<td>975.40 ± 190.75</td>
<td>916.77 ± 151.04**</td>
</tr>
<tr>
<td><strong>Accuracy (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Working Memory</td>
<td>90.46 ± 7.18</td>
<td>95.22 ± 5.37</td>
<td>96.30 ± 3.94**</td>
</tr>
<tr>
<td>Delayed Recall</td>
<td>70.95 ± 6.42</td>
<td>71.19 ± 5.98</td>
<td>70.71 ± 8.91</td>
</tr>
</tbody>
</table>

** p<0.05 vs baseline.
(14) Astaxanthin and eye health

A primary source for oxidative stress is incoming sunlight, especially UV light, that can lead to photo-oxidative damage to lipids and tissues. Any tissue exposed to light is prone to undergo this photo-oxidative damage, and hence our skin and eyes are the most vulnerable tissues and carotenoids play an essential role in their maintenance. Furthermore, the high concentration of polyunsaturated fatty acids in the center of the retina renders the eye a sensitive target for lipid peroxidation. Oxidative damage to the eye and skin by UV light has been widely documented (Pappas 1999) and thus the unique UV protection properties of Astaxanthin is believed to be very important to eye and skin health. In order to accumulate in the retina, Astaxanthin must first penetrate the blood-brain barrier, as was shown to occur in mammals, as well as in birds (Maher 2000, Tso 1996).

Two of the main causes of visual impairment and blindness are Age-Related Macular Degeneration (AMD) and Age-Related Nuclear Cataracts (Guerin 2003). Both diseases appear to be related to light-induced oxidative processes within the eye, whereas reduced risk for AMD and nuclear cataracts is associated with a high dietary intake of carotenoids (Lyle 1999). The protective effects of Astaxanthin against photo-oxidative damage were demonstrated in cell cultures, in animal models and in humans (discussed in chapter 6 above). In addition, Astaxanthin was investigated for its effect on vision and eye function in general, as discussed below.

(14.1) Studies in animal models

Following intraperitoneal administration of Astaxanthin to rats, an Astaxanthin content of 0.17µg/mg was measured in the rats’ retinas, indicating that Astaxanthin can cross the Blood-Retina Barrier (Tso 1996). Upon Astaxanthin administration, the rats were exposed to visible light for 24 hours. While control rats lost ~35% of the thickness of the outer nuclear layer of the retina, those treated with Astaxanthin had only a 6% decrease. In addition, Astaxanthin was able to prevent the depletion of rhodopsin from...
the retinas of rats treated with similar photo-damaging conditions. The effect of Astaxanthin on endotoxin-induced uveitis (EIU, inflammatory processes of the middle layers of the eye) was studied in rats (Ohgami 2003). Astaxanthin administrated intravenously had a dose-dependent anti-inflammatory effect on EIU, having a possible mechanism of suppressing the production of nitric oxide (NO). Similar results were obtained when the inhibitory effect of Astaxanthin on EIU was demonstrated by measuring expression of inflammatory cytokines and chemokines in the aqueous humour (Suzuki 2005), indicating that Astaxanthin not only protects the eyes, but also blocks the biologic pathway leading to inflammation (Figure 38).

![Figure 38: Number of inflammatory cells in aqueous humour of rats with uveitis.](image)

The anti-inflammatory effect of Astaxanthin plays an important role in an often seen complication of AMD called choroidal neovascularization (CNV), leading to severe vision loss and blindness (Izumi-Nagai 2008 and ref. therein). CNV seen in AMD develops with oxidative stress and chronic inflammation and is associated with the influx
of inflammatory cells including macrophages. Pharmacologic depletion of macrophages significantly suppress murine CNV. Indeed, when mice were pretreated with intraperitoneal injections of Astaxanthin and then experimental CNV was induced, the index of CNV volume was significantly suppressed by the treatment compared with control animals (Figure 39). Astaxanthin treatment led to significant inhibition of macrophage infiltration into CNV and inhibited in vivo and in vitro expression of inflammation-related molecules.

![Figure 39: Suppression of CNV in mice receiving Astaxanthin. Flatmounted choroids from vehicle- and Astaxanthin (1, 10, and 100 mg/kg body weight (BW))-treated mice. Arrowheads indicate lectin-stained CNV tissues. [Adapted from Izumi-Nagai (2008)]](image)

Astaxanthin was demonstrated to have positive effects in additional pathologies of the retina using animal models. These include suppressive effect of Astaxanthin on retinal injury induced by elevated intraocular pressure in rats (Cort 2010), protection of mice retinal cells against oxidative stress in-vitro and in-vivo (Nakajima 2008), interaction with selenite and attenuation of Selenite-induced cataractogenesis in rats (Liao 2009) and reduction of cataract in Atlantic salmon (Waagbo 2003).

(14.2) Studies in humans

The studies done in humans focused mainly on improving vision and function, with two studies looking directly for anti-oxidative effects. In the first research (Hashimoto 2009), the effect of Astaxanthin consumption on superoxide scavenging activity in aqueous humor was examined in 35 subjects, classified into non-diabetic and diabetic groups. Superoxide scavenging activity was measured before and after Astaxanthin...
consumption (2 weeks, 6 mg/day). Results showed that superoxide scavenging activity was increased by Astaxanthin consumption in the DM group. Although the experimental group was small, this study points to the possible use of Astaxanthin in the restriction of humoral oxidative stress.

In the second study, 15 subjects with nonadvanced AMD were given daily Astaxanthin (4 mg) along with vitamin C, vitamin E, zinc, copper, lutein and zeaxanthin for 12 months (Parisi 2008). The investigators concluded that in nonadvanced AMD, supplementation with carotenoids and antioxidants improves the function of the central retina.

The effect of Astaxanthin on vision and eye function was followed in several populations including healthy young subjects (Sawaki 2002, Nakamura 2004, Iwasaki 2006), healthy subjects who complained of asthenopia (eye fatigue) (Kenji 2005), middle-aged and old subjects complaining of presbyopia (difficulties to focus on nearby objects with increasing age) (Kajita 2009) and visual display terminal (VDT) workers (Nagaki 2002, Nagaki 2006). Table 10 summarizes the results of these studies.

Table 10: Effect of Astaxanthin on vision and eye function. Summary of clinical studies.

<table>
<thead>
<tr>
<th>Study (P/B)</th>
<th>Population</th>
<th>Dose (mg)/Time (days)</th>
<th>Main outcome in Astaxanthin group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sawaki 2002 (P)</td>
<td>18 healthy</td>
<td>6/28</td>
<td>Deep vision and critical flicker fusion improved. No effects on static and kinetic visual acuity.</td>
</tr>
<tr>
<td>Nakamura 2004 (NA)</td>
<td>49 healthy, ≥40 years</td>
<td>0-12/28</td>
<td>Improved uncorrected far visual acuity. Accommodation time shortened. No change in refraction, flicker fusion frequency, or pupillary reflex.</td>
</tr>
<tr>
<td>Iwasaki 2006 (P/B)</td>
<td>10 healthy</td>
<td>6/14</td>
<td>Accommodative contraction and relaxation times shortened. Blurred vision and eye dryness decreased.</td>
</tr>
<tr>
<td>Kenji 2005 (B)</td>
<td>40 asthenopia</td>
<td>6/28</td>
<td>Improved accommodation power and subjective symptoms of asthenopia</td>
</tr>
<tr>
<td>Kajita 2009 (O/NP)</td>
<td>22 45-65 years Presbyopia</td>
<td>6/28</td>
<td>Improved accommodation function and some subjective symptoms related to presbyopia.</td>
</tr>
<tr>
<td>Nagaki 2002 (P/B)</td>
<td>39 DVT</td>
<td>5/28</td>
<td>Accommodation amplitude improved. No change in critical flicker fusion.</td>
</tr>
</tbody>
</table>
This table clearly demonstrates that Astaxanthin has several positive effects on vision and eye function, especially those related to accommodation of the lens and related symptoms such as “eye fatigue”, difficulties to focus on nearby objects with increasing age (presbyopia) and asthenopia symptoms (shoulder stiffness, ocular pain and headache). Although the underling mechanism for these effects is still unknown, an interesting hypothesis was proposed by Miyawaki, et al (2008) who showed improved blood transit times (rheology) following Astaxanthin ingestion. The authors suggested that Astaxanthin, via its anti-oxidative activity, may improve erythrocyte membrane flexibility thus enabling better blood fluidity which in turn may improve eye function.
(15) Astaxanthin and Male Fertility

Infertility results from the coincidence of genetic disorders, environmental factors and pathogens of the reproductive organs. Evidence has accumulated supporting the role of ROS in the pathogenesis of sperm dysfunction among infertile men. Spermatozoa are highly sensitive to ROS and possess little defense against oxygen damage, which induces changes in the sperm membranal composition and harms their DNA. Due to the high concentration of polyunsaturated fatty acids (PUFA), spermatozoa membranes are highly vulnerable to oxidation, thus reducing their fluidity and fusogenic capacity. The combination of Astaxanthin’s unique membranal protection and strong antioxidative ability makes it a good candidate for intervention in male infertility treatments.

El Garem, et al (2002) evaluated the effect of Astaxanthin supplementation on semen quality of infertile male. Twenty sub-fertile men received either 16 mg/day Astaxanthin or placebo during three months, in addition to the conventional treatment. Decreased ROS activity and improved sperm motility and morphology were seen in the supplemented group, while no change was observed in the placebo group. At the end of the treatment period, five couples out of ten successfully conceived in the Astaxanthin-supplemented group, compared to one out of ten couples in the placebo group. The researchers proposed that the Astaxanthin-improved quality of spermatozoa, may explain the increased frequency of conception.

In another study, Comhaire, et al (2005) treated 30 infertile men with Astaxanthin (16 mg/day), or placebo for three months in addition to conventional treatment. The effects of Astaxanthin on sperm parameters, ROS level, serum hormones and pregnancy rate were evaluated. Astaxanthin reduced ROS and Inhibin B and increased sperm linear velocity. Pregnancy rate among the placebo cases (10.5 %) was significantly lower compared with the Astaxanthin group (54.5 %) [Table 11].
Table 11: Effect of Astaxanthin on human male infertility: Semen characteristics and pregnancy rate at baseline and after 3 months of treatment. [Adapted from Comhaire (2005)]

<table>
<thead>
<tr>
<th>Criteria measured</th>
<th>Placebo Pre-intervention</th>
<th>Placebo Post-intervention</th>
<th>Astaxanthin Pre-intervention</th>
<th>Astaxanthin Post-intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm concentration</td>
<td>28.3</td>
<td>28.2</td>
<td>38.2</td>
<td>48.6</td>
</tr>
<tr>
<td>Linear velocity</td>
<td>25.3</td>
<td>22.9</td>
<td>22.1</td>
<td>29.6</td>
</tr>
<tr>
<td>ROS</td>
<td>376</td>
<td>490</td>
<td>394</td>
<td>99</td>
</tr>
<tr>
<td>Pregnancy rate</td>
<td>0</td>
<td>10.5</td>
<td>0</td>
<td>54.5</td>
</tr>
</tbody>
</table>

Although these studies show promising findings, the results need to be confirmed in a larger trial before recommending Astaxanthin for the complementary treatment of infertile men.
(16) Astaxanthin and the metabolic syndrome

Metabolic syndrome (MS) identifies clinical symptoms and biochemical markers, including obesity, insulin resistance, hyperglycemia, hyperlipidemia, and hypertension, all leading to an increased risk of cardiovascular disease (CVD). These conditions cause metabolic dysregulation, elevated fatty acids (FFA), and increased secretion of pro-inflammatory "adipokines". Left untreated, MS causes lipotoxicity (especially to the liver), chronic inflammation, hypertension, atherosclerosis, type 2 diabetes (T2D) and CVD (Roberts 2009). Increasing evidence shows that MS is characterized by increased oxidative stress (Hopps 2010). Moreover, one of the primary cellular sites for the formation and subsequent damage of oxidative stress is the mitochondria, which are implicated in several aspects of MS, such as insulin resistance, obesity, inflammation, and liver abnormalities (Kim 2008).

The exact causes for MS are not fully understood, however, it is clear that genetic predisposition in combination with high-fat diet are major risk factors (Roche 2005). In line with the evidence regarding the pivotal role of oxidative stress in MS, Astaxanthin, as well as other antioxidants, were tested for their effects in animal models of T2D and obesity and in hyperlipidemic subjects. The effects of Astaxanthin on hyperlipidemia were discussed in chapter 10, dealing with its connection to cardiovascular diseases. The following section will describe the effects of Astaxanthin on hyperlipidemia as part of MS and T2D.

(16.1) Astaxanthin and onset of Type 2 Diabetes

Oxidative stress induced by hyperglycemia is probably involved in tissues resistance to insulin which may eventually deteriorate to dysfunction of pancreatic β cells, two factors that define Type 2 Diabetes (T2D). Research is therefore conducted at both improving tissue sensitivity to insulin and preventing the exhaustion of pancreatic cells that leads to their final destruction. Uchiyama, et al (2002) used diabetic db/db mice, a well-known obese model of T2D, and showed that the higher level of blood glucose observed in db/db mice was decreased after treatment with Astaxanthin. The ability of
pancreatic cells to secrete insulin was preserved in the Astaxanthin-treated group. Similar results were achieved by Hussein, et al (2007), using the hypertensive rat (SHR) model. These rats mimic an environmentally-induced MS however, if pre-treated with Astaxanthin, SHR rats have significantly lower levels of MS biochemical markers including lower fasting blood glucose, higher sensitivity to insulin, improved adiponectin level, a significant increase in HDL-cholesterol, and a significant decrease in triglycerides, and non-esterified fatty acids (Figure 40a,b,c). Mean blood pressure was also reduced in hypertensive rats following treatment with Astaxanthin (Hussein 2005) [Figure 41].

Figure 40a: Triglycerides (TG) level in Astaxanthin (ASX) or olive-oil (OL)-treated normal Wistar or SHRcp rats, ex vivo. [Adapted from Hussein (2007)]
Figure 40b: High-density lipoprotein (HDL)-cholesterol level in Astaxanthin (ASX) or olive-oil (OL)-treated normal Wistar or SHRcp rats, ex vivo. [Adapted from Hussein (2007)]

Figure 40c: Non esterified fatty acids (NEFA) level in Astaxanthin (ASX) or olive-oil (OL)-treated normal Wistar or SHRcp rats, ex vivo. [Adapted from Hussein (2007)]
Additionally, Astaxanthin showed significant effects on the white adipose tissue by decreasing the size of the fat cells. These results suggest that Astaxanthin ameliorates insulin resistance by mechanisms involving the increase of glucose uptake, and by modulating the level of circulating lipid metabolites and adiponectin. Complementry to these results are the findings obtained in the obese rat model (Ikeuchi 2007). Obese rats treated with Astaxanthin presented an inhibition of the increase in body weight and weight of adipose tissue resulting from high-fat diet feeding. In addition, Astaxanthin reduced liver weight, liver TG, plasma TG and total cholesterol. Similar results were obtained by Kim, et al (2009) in mice fed high-fat diet. The authors suggested that Astaxanthin may be of value in reducing obesity and MS in humans. A support to this notion comes from a recent study in humans (Yoshida 2010), showing that administration of Astaxanthin to mildly hypertensive subjects resulted in increase in HDL-cholesterol and adiponectin.

Mitochondria are a major source for ROS production and one of the first to be damaged by them. Oxidative stress leading to mitochondrial dysfunction is a critical factor in MS and the efficacy of Astaxanthin on mitochondrial function was hence
investigated in Hela cell line using Astaxanthin’s concentration close to what can be achieved using supplements and/or diet (Wolf 2010). It was found that Astaxanthin decreased physiologically occurring oxidative stress and protected cultured cells against strong oxidative stress induced with a respiratory inhibitor. Moreover, Astaxanthin improved the maintenance of a high mitochondrial membrane potential and stimulated respiration. Astaxanthin at nanomolar concentrations was effective in maintaining mitochondria in a reduced state. Additionally, Astaxanthin improved the ability of mitochondria to remain in a reduced state under oxidative challenge. Supportive evidence to the protective effect of Astaxanthin on mitochondria was obtained in two additional studies in cell cultures: Liu, et al (2009) showed that in dopaminergic SH-SY5Y cells Astaxanthin accumulated in mitochondria (Figure 42) and inhibited cellular oxidative toxicity via mitochondria-targeted protective mechanism (Figure 43).

![Figure 42: Astaxanthin accumulation in SH-SY5Y cells. Astaxanthin content in different fractions was determined by HPLC analysis and expressed as % of the added total concentration. ND – Not detected. [Adapted from Liu (2009)]](image-url)
Figure 43: Protective effect of Astaxanthin on DHA-OOH or 6-OHDA-induced damage to mitochondria of SH-SY5Y cells. Mitochondrial damage was followed by determining the amount of mitochondrial cytochrom C release to the cytosol. [Adapted from Liu (2009)]

In a more diabetes-related system, Manabe et. al. (2008) showed that Astaxanthin protects kidney mesangial cells from hyperglycemia-induced oxidative signaling through accumulation in the mitochondria and reduction of the production of ROS-modified proteins in them (Figure 44). Taken together, these results suggest that Astaxanthin may improve MS via its beneficial effects on mitochondria function.

Figure 44: Astaxanthin (ASX) inhibits glucose-induced production of oxidative Stress-modified proteins in mitochondria of normal human mesangial cells (NHMCs). [Adapted from Manabe (2008)]
(16.2) Astaxanthin and T2D-induced nephropathy

As mentioned above, ROS may be involved not only in the onset of T2D, but also in many of its complications. One of these common complications is kidney dysfunction called diabetic nephropathy, caused by chronic damage to the capillaries in the kidney. Astaxanthin was investigated for its effect on characteristic histological, biochemical and genetic features of nephropathy. In a recent study (Kim YJ 2009), the protective action of Astaxanthin against high-glucose-induced oxidative stress was examined in proximal tubular epithelial cells (PTECs). The efficacy of Astaxanthin was assessed by measuring several key markers and activities, including lipid peroxidation, total reactive species (RS), superoxide (\(\cdot \text{O}(2)\)), nitric oxide (NO*), and peroxynitrite (ONOO(-)). Results showed that Astaxanthin effectively suppressed all the above mentioned oxidative damage markers (Kim 2009). Similar protective effect of Astaxanthin against progression into diabetic nephropathy was found by Nakano et. al (2008) in ODS rats. The effect of Astaxanthin could be further enhanced in combination with vitamine E.

Finally, it was shown by Naito, et al (2004) that Astaxanthin prevented the progression of diabetic nephropathy induced by oxidative stress in diabetic db/db mice. Astaxanthin-treated mice showed a lower level of blood glucose and a significantly smaller mesangial area compared to non-treated db/db group. The increases in urinary albumin (Figure 45) and DNA oxidation marker at 12 weeks of treatment were significantly inhibited by supplementation with Astaxanthin. The results suggested that the antioxidative activity of Astaxanthin reduced oxidative stress on the kidneys and prevented renal cell damage.
In an effort to reveal the mechanism underlying these effects, the profile of gene expression patterns in glomerular cells of Astaxanthin-treated diabetic mice were followed (Naito 2006). The analysis of the most affected genes (3.1%) showed that the mitochondrial oxidative phosphorylation pathway was the most significantly affected pathway. The affected genes were associated with proteins located in the mitochondrial inner membrane, and the expression levels of these genes were decreased in mice treated with Astaxanthin as compared to the levels in the controls. In addition, the expression of many genes associated with oxidative stress, collagen synthesis, and transforming growth factor-β signaling was enhanced in the diabetic mice, and this enhancement was slightly inhibited in the Astaxanthin-treated mice. This genetic approach stresses the likelihood that Astaxanthin affects T2D outcomes not only by counteracting ROS but also by modulating the expression of related genes.
(17) Summary

As seen in this review, Astaxanthin supplementation is a practical strategy in protecting the body from oxidative damage, which has impact in a number of health conditions. Natural Astaxanthin ingested in the presence of fat or oil has greater bioavailability and is distributed systemically. Thanks to its exceptionally high antioxidative potency, Astaxanthin can exert its effects in relatively low concentrations, thus making it suitable to serve as dietary supplement and in functional food/beverages. Astaxanthin is completely safe, as evident from numerous clinical, pre-clinical and acute toxicity studies. Promising findings suggest that Astaxanthin may improve the function of many physiologic systems both as a preventive and as a therapeutic treatment. These beneficial effects are believed to result from a concerted action of Astaxanthin via a number of pathways rather than acting merely as an antioxidant. Thus, accumulating evidence from various systems suggests that Astaxanthin acts via scavenging reactive oxide species (ROS), inhibiting lipid peroxidation in membranes and stabilizing the structure and fluidity of the latters and modulating the expression of genes such as structural (connexins) genes, mitochondrial and inflammatory ones. Current and future research in these fields will undoubtedly shed more light on Astaxanthin potential in human health.
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