CAROT


Astaxanthin
Prime
AstaRex
NATURAL ASTAXANTHIN
Astaxanthin

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ASTAXANTHIN

Astaxanthin (3, 3'-dihydroxy-beta, beta-carotene-4, 4'-di-one) is a powerful naturally occurring pigment that belongs to the family of xanthophylls, the oxygenated derivatives of carotenoids and mainly found in marine environments. Chlorophyte algae *Haematococcus pluvialis* accumulate the highest amount of astaxanthin in the form of 3S, 3'S. One of the most powerful antioxidants found in nature, Astaxanthin, also popularly known as "the king of carotenoids". Astaxanthin is a carotenoid nutrient with molecular properties that precisely position it within cell membranes and circulating lipoproteins, thereby imbuing them with potent antioxidant actions and offers protection against free radical damage to preserve healthy lipids, proteins and DNA, and help the human body maintain a healthy state. Astaxanthin protects the entire cells as it works in both water soluble and fat-soluble part of the cell.The antioxidant activity of astaxanthin is stronger than β-carotene and vitamin E by 40x and 500x respectively. Besides, astaxanthin differs from other antioxidants in its ability to penetrate the blood brain and retina barriers. Therefore, it is believed to protect the brain and nervous system from neurodegenerative diseases (e.g. cerebral thrombosis and stroke) and aging. Meanwhile, astaxanthin has been documented to prevent age-related macular degeneration (AMD) and enhance immune functions. Furthermore, recent studies revealed the wrinkling and moisturizing effect of astaxanthin which suggest its potential cosmeceutical applications in protection against skin aging.

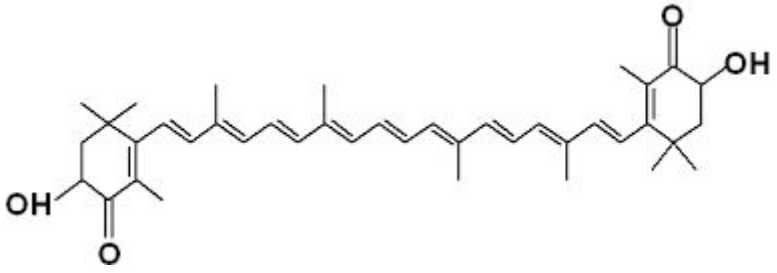

Fig1: Structure of Astaxanthin

SUPERIOR POSITION IN CELL MEMBRANE

Astaxanthin contains conjugated double bonds, hydroxyl and keto groups. It has both lipophilic and hydrophilic properties. Conjugated double bond acts as a strong antioxidant by donating the electrons and reacting with free radicals to convert them to be more stable product and terminate free radical chain reaction. Astaxanthin has better biological activity than other antioxidants, because of its unique ability to span through the double layer cell membrane. β-carotene and vitamin C only reside inside and outside the lipid bilayer membrane respectively. The astaxanthin molecule is exposed both in- and outside of the cell giving better overall protection.

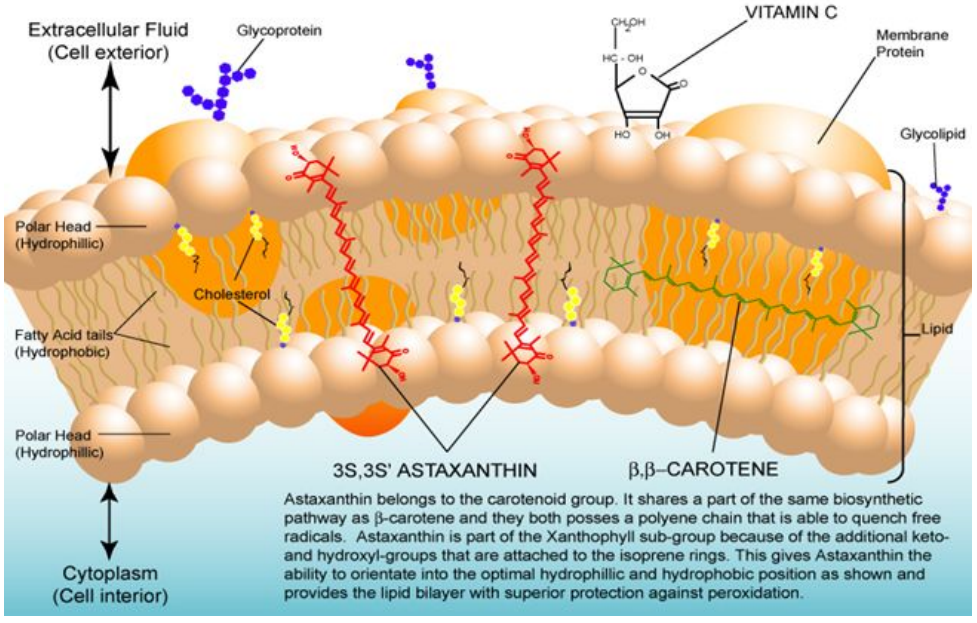

Fig2: Superior position in cell membrane

SAFE ANTIOXIDANT

Carotenoids are divided into three classes: i) without significant antioxidative properties, ii) anti- and pro-oxidants and iii) pure antioxidants. Non-polar carotenoids such as lycopene and β-carotene can become pro-oxidants. High intake pro – oxidants disordered the membrane bilayer enriched with polyunsaturated fatty acids and increases lipid peroxidase level. Astaxanthin classified as "pure anti-oxidants" not possessing any pro-oxidative properties like β-carotene and lycopene. Astaxanthin decreases lipid peroxidase level. Astaxanthin is enormously more powerful in quenching singlet oxygen than other antioxidants

- 6000 times stronger than vitamin C
- 3000 times stronger than resveratrol
- 800 times stronger than coenzyme Q10
- 560 times stronger than green tea catechins
- 500 times stronger than vitamin E
- 200 times stronger than lutein
- 75 times stronger than alpha lipoic acid
- 40 times stronger than beta carotene

NATURAL ASTAXANTHIN AND SYNTHETIC ASTAXANTHIN

An enchanting point to consider is why synthetic astaxanthin is significantly inferior to natural astaxanthin as an antioxidant and may not be safe for use as human nutraceutical supplement. Natural astaxanthin accumulates in *Haematococcus pluvialis* as a survival mechanism against environmental stress. Synthetic astaxanthin produced from petrochemicals, has been used as a feed ingredient, primarily to pigment the flesh of salmonids.

The main differences between natural astaxanthin and synthetic astaxanthin are

- Natural astaxanthin is comprised of 95% esterified molecules, both monoesterified and diesterified (Natural astaxanthin have either one or two fatty acid molecules attached to the ends of the astaxanthin molecule). Synthetic astaxanthin is free

Astaxanthin (Synthetic astaxanthin is not esterified and has no fatty acids attached to the ends of the molecule).

- Natural and synthetic astaxanthin share the same molecular formula but shaped differently. Natural astaxanthin contains 100% 3S,3'S enantiomer. Synthetic astaxanthin contains a combination of three different enantiomers: It has 25% 3S,3′S (the same shaped molecules as natural astaxanthin), but it contains primarily molecules shaped differently from natural astaxanthin: 50% is meso-astaxanthin comprised of the 3R,3′S enantiomer and 25% is pure "R" enantiomer 3R,3′R.
- Synthetic astaxanthin contains no supporting carotenoids, while natural astaxanthin is naturally complexed in *Haematococcus* microalgae with other carotenoids. When lipids are extracted from the algae, the resulting extract contains primarily natural astaxanthin, but it also contains three other naturally occurring carotenoids. The resulting "natural carotenoid complex" contains approximately 70% monoesterified astaxanthin, 10% diesterified astaxanthin, 5% free astaxanthin, 6% beta-carotene, 5% canthaxanthin and 4% lutein.

In vitro studies conducted at Creighton University and Brunswick Laboratories showed natural astaxanthin to be over 50 times stronger than synthetic Astaxanthin in singlet oxygen quenching, approximately 20 times stronger in eliminating superoxide ion and 3.5 times stronger against peroxyl radicals . For these reasons, synthetic astaxanthin would have to be used at a rate 14–55 times more than natural astaxanthin to obtain the same antioxidant protection. Current dosage recommendations for humans range from 2 to 16mg/day based on extensive human clinical trials showing a wide range of health benefits. Based on this dosage range of natural astaxanthin, the synthetic astaxanthin recommended dosage range would be a minimum of 28 mg/day to a maximum of 880 mg/day when considering the differences in antioxidant activity. With an average difference of antioxidant measurements in the range of 20×–30×, and an average human dosage of 8 mg/day, the average dose for synthetic astaxanthin would be in the proximity of160–240 mg/day. Before release to human consumers,long-range safety trials should be conducted at this dosage level to ensure that, unlike synthetic beta-carotene and synthetic canthaxanthin, there are no concerns with

synthetic astaxanthin in areas such as carcinogenesis or retinal crystal formation (Bob Capelli et al, 2013).

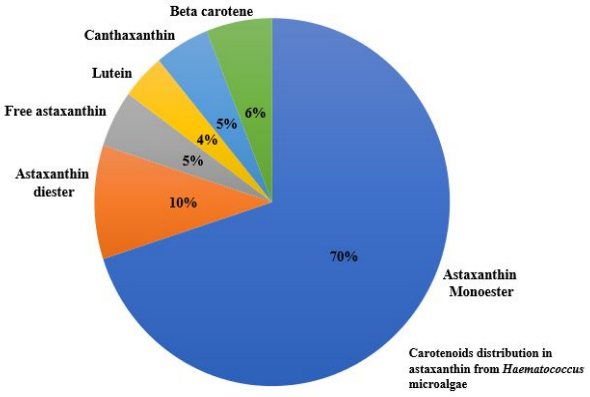

Fig3: Carotenoids distribution in *Haematococcus*

CLINICAL STUDIES: ASTAXANTHIN FROM *Haematococcus pluvialis*

Dietary needs and physical activities of every organism are genetically determined. Unhealthy eating habits and lack of physical activity are the major factors contributing to lifestyle diseases. Lifestyle diseases are defined as diseases linked with the way people live their lives. Diseases that impact on our lifestyle are alzheimer's disease, arthritis, atherosclerosis, asthma, cancer, chronic liver disease or cirrhosis, chronic obstructive pulmonary disease, type 2 diabetes, heart disease, metabolic syndrome, chronic renal failure, osteoporosis, stroke, depression, obesity and vascular dementia. Stress, irregular lifestyle, unbalanced diet & smoking are contributing factors to the generation of free radicals, active oxygen which accelerates the process of ageing.

Astaxanthin, a xanthophyll carotenoid, has a unique structure featured by the presence of polar moieties on both end of its polyene chain. This structural property of astaxanthin confers a great antioxidant activity and allows it to align in the cell membrane for various biological activities.Astaxanthin has more hydroxyl groups than other xanthophylls which may account for its superior antioxidant activity and profound health benefits in human.

ASTAXANTHIN: A POTENTIAL THERAPEUTIC AGENT IN CARDIOVASCULAR DISEASE

Cardiovascular diseases (CVD) are the major cause of mortality globally, as well as in India.

CVD are caused by disorders of the heart and blood vessels, and includes coronary heart disease (heart attacks), cerebrovascular disease (stroke), raised blood pressure (hypertension), peripheral artery disease, rheumatic heart disease, congenital heart disease and heart failure. An increasing trend in proportionate CVD mortality has been reported by Registrar General of India (RGI) with 20.6% deaths in 1990, 21.4% in 1995, 24.3% in 2000, 27.5% in 2005, and 29.0% in 2013. According to 2010-2013 RGI data, proportionate mortality from CVD increased to 23% of total and 32% of adult deaths in years 2010-2013.

Oxidative stress and inflammation are the wide contributors of cardiovascular diseases. Treatment of oxidative stress related health problems leads to the use of antioxidants as a therapeutic agent.Astaxanthin is a potent antioxidant with anti-inflammatory activity and its effect examined both in experimental animals and humans. Oxidative stress and inflammation are the inducers of atherosclerotic cardiovascular disease. A series of clinical and experimental research has shown that astaxanthin is a potential therapeutic agent against atherosclerotic cardiovascular disease. Experimental evidence suggests that oral supplementation with astaxanthin in studies in healthy human volunteers and patients with reflux oesophagitis demonstrated a significant reduction in oxidative stress and biomarkers of inflammation, improves lipid profiles, promotes better blood flow in capillaries and lowers blood pressure in hypersensitive individuals.

ASTAXANTHIN HELPS TO KEEP BLOOD LIPID LEVELS AT A NORMAL RANGE

The first human clinical trial on astaxanthin's ability to control blood lipids was done in Japan. This study used 61 volunteers of ages 25 – 60 who were not obese and did not have related issues such as high blood pressure or diabetes. The subjects were given a range of astaxanthin doses over a period of 12 weeks. Optimum results were found at 12 mg per day. It was found that astaxanthin improves blood lipid profiles by decreasing low density lipoprotein (LDL, the bad cholesterol) and triglycerides, and by increasing high density lipoprotein (HDL, the good cholesterol) (Yoshida et al, 2010).

In an open labelled study involving 24 healthy volunteers who ingested astaxanthin in doses from 1.8 to 21.6 mg/day for two weeks, LDL lag time, as a measure of susceptibility of LDL

to oxidation, was significantly greater in astaxanthin treated participants indicating inhibition of the oxidation of LDL (Iwamoto et al, 2000)

ASTAXANTHIN SUPPORTS HEALTHY BLOOD FLOW

In a single blind placebo–controlled study, 20 human volunteers were supplemented with 6 mg of natural astaxanthin for ten days. The experimental group was 57.5 ± 9.8 years of age and the placebo group was 50.8 ± 13.1 years of age.Study concluded that continuous ingestion of astaxanthin 6 mg per day for a ten-day period improves blood rheology (Miyawaki et al, 2008).

ASTAXANTHIN KEEPS CRP LEVELS AT A NORMAL RANGE

C-reactive protein (CRP) is one of the most common blood markers used to detect stress in the body. CRP is produced in the liver and released into the bloodstream when the body is fighting the aches and pains associated excess oxidation.

In a randomized, double blind placebo-controlled study, Park et al (2010) examined 14 healthy female subjects for pro-inflammatory marker CRP. Natural astaxanthin given to people at 2 mg per day experienced a reduction in CRP levels in just eight weeks and a reduction in plasma 8-hydroxy-2′-deoxyguanosine observed after four week in those taking astaxanthin.

A 2006 human clinical study conducted by The Health Research and Studies Center in Los Altos, California, studied 25 people for eight weeks. Sixteen people were given natural astaxanthin and nine received a placebo. The group given astaxanthin experienced a 20 percent reduction in CRP levels in just eight weeks, whereas the placebo group had an increase in CRP levels (Spiller et al., 2006).

In a randomized, double blind placebo-controlled study, Karppi, et al, (2007) examined 40 healthy non-smoking Finnish males for 12 weeks. Natural Astaxanthin given to people at 8 mg per day was found to reduce blood plasma levels of two different hydroxy fatty acids.

Blood levels of the hormone adiponectin were also higher in the people who took astaxanthin. Adiponectin is a natural hormone that helps promote healthy blood sugar and

blood fat levels.

An open-label uncontrolled study was conducted to evaluate the mental and physical effects, as well as safety of 8-week oral treatment with astaxanthin at a daily dose of 12 mg. Of 35 healthy female volunteers screened, 20 women with increased oxidative stress burden, selected were included in the study. Results show that astaxanthin may enhance antioxidant capacity, reduce lower limb vascular resistance (increase ankle brachial pressure), decrease blood pressure, and improve physical symptoms in women with high oxidative stress (Masaaki Iwabayashi et al 2009.)

In a study, Kim et al (2004) examined 20 healthy postmenopausal women (55 ± 4.8 years old) with high levels of oxidative stress. Subjects were supplemented with 12 mg of natural astaxanthin daily. After 8 weeks, lower limb vascular resistance was significantly decreased.

ASTAXANTHIN AND SKIN HEALTH

The skin is the largest organ of the body with a total area of about 20 square feet. The skin protects us from microbes and the elements, helps to regulate the body temperature, and permits the sensations of touch, heat and cold. Epidermis, dermis and hypodermis are the three layers of the skin. Each layer having unique and important functions. Internal oxidative stress from bad diet, metabolic byproducts formation can lead to skin damage. Exposure to sunlight results in sunburn and can also lead to photo induced inflammation, immunosuppression, aging and even in carcinogenesis of the skin. Clinical research and experiments have shown that dietary antioxidants could reduce oxidative stress. A large body of research has found that astaxanthin possess various health benefits and it can contribute large health benefits in the field of dermatology.

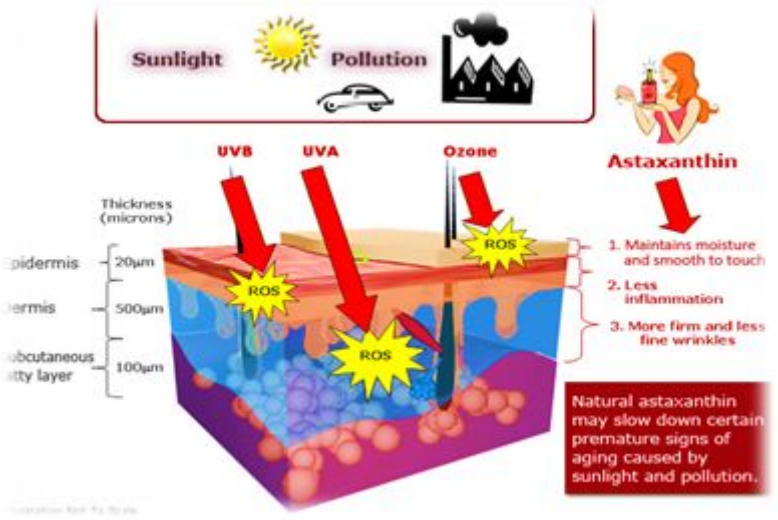

Fig4: Astaxanthin in Skin Health

In a study of Chalyk, 2017, 31 middle aged volunteers were supplemented with 4mg of astaxanthin. It was found that continuous consumption of astaxanthin for four weeks decreases the plasma levels of malondialdehyde (by 11.2% on day 15 and by 21.7% on day 29).The analysis of RSSC samples revealed decreased levels of corneocyte desquamation and microbial presence at the end of the study.

Tominaga et al 2017, conducted an in vitro study and in parallel, a randomized, double-blind, parallel-group, placebo-controlled study with 65 healthy female subjects for 16 weeks to verify the effects of oral astaxanthin supplementation (6 or 12 mg) on skin integrity. The authors demonstrated that pre- and post-treatment with astaxanthin dose-dependently decreased the secretion of inflammatory cytokines and MMP-1 from UVB-irradiated keratinocytes. Furthermore, the clinical study demonstrated that skin moisture content and deep wrinkles were not significantly changed in the astaxanthin supplemented groups, whereas these parameters significantly worsened in the placebo group during the study period. Interestingly, IL-1α levels in the stratum corneum were maintained only in the high-dose group. In addition, skin elasticity improvements were observed in the high-dose group compared with that of the placebo group in participants with high skin moisture content.

In a study, 36 healthy female mice were divided into a control group and a group receiving 78.9 µM topical astaxanthin treatment twice daily for 15 days to identify the effect of astaxanthin on cutaneous wound healing. Astaxanthin-treated wounds showed noticeable contraction by day 3 of treatment and complete wound closure by day 9, whereas the wounds of control mice revealed only partial epithelialization and still carried scabs. Wound healing biological markers including Col1A1 and bFGF were significantly increased in the astaxanthin-treated group since day 1. The results indicate that astaxanthin is an effective compound for accelerating wound healing (Meephansan,et al 2017).

In a randomized, double-blind placebo-controlled study with 44 healthy subjects showed that a combination of astaxanthin (2 mg/day) and collagen hydrolysate (2 mg/day) for 12 weeks improves elasticity and barrier integrity in human skin. These improvements were associated with molecular changes such as the induction of pro collagen type I and decreased levels in the expression of the collagen-degrading enzyme MMP-1and the elastin-degrading enzyme MMP-12 (Yoon et al 2014).

In a study using hairless mice, Arakane (2002) demonstrated the astaxanthin's ability to suppress the formation of UVB photo-induced wrinkles. UVB doses of 65-95 mJ/cm2 were applied five times per week for 18 weeks on the back skin of the mice. After each UVB treatment, topical application of astaxanthin (350 µM) was coated on the exposed areas. After 5 weeks, the appearance of new wrinkles was significantly reduced up until the end of the study period ($P<0.01$) at 18 weeks). Concurrently stained skin secretion revealed that astaxanthin preserved the integrity of derma layer by protecting the collagen network.

In 2001, Seki et al. conducted a small pilot study with astaxanthin from *H. pluvialis* to investigate the same wrinkle reduction effect on the skin of 45 healthy subjects. The authors observed an anti-wrinkle effect in female human subjects (n = 3), using a topical cream containing astaxanthin combined with other active ingredients. A dermatological assessment revealed significant reduction of wrinkles and puffiness on the lower eye and cheeks after two weeks of use. In a separate test using female subjects (n=11), instrument analysis recorded significant moisture improvement ($p < 0.05$) after 3 weeks of use.

A second preliminary human study performed by Yamahita et al in 1995 showed in healthy male subjects (n = 7) that topical natural astaxanthin from krill significantly reduces erythema by 60% at 98 h after UV-B exposure. In a second study, the same author administered 2 mg of astaxanthin or placebo to 49 healthy female subjects (mean age of 47 years). After six weeks of treatment, significant improvements were observed in skin moisture and elasticity.

In another study by Tominaga et al. (2009) the effect of astaxanthin on wrinkle reduction and skin elasticity was investigated in 28 female subjects (20–55 years). The combined use of a dietary supplement and a topical product containing astaxanthin for eight weeks showed a reduction in the overall average wrinkle depth.

A double-blind placebo controlled study (Yamashita 2002), showed that astaxanthin in combination with tocotrienol, (a superior form of vitamin E), improved several aspects of overall skin condition. Eight female subjects with dry skin conditions (mean age 40 yrs) received daily doses containing 2 mg astaxanthin and 40 mg natural tocotrienols. Several types of data were collected at 2 and 4 weeks and compared to the initial baseline readings. Measurable differences were observed starting just 2 weeks after supplementation. By the 4th week, the treated subjects with dry skin characteristics exhibited the following: increased moisture levels, reduction in pimples and reduction of wrinkles.

In an open-label uncontrolled study, 30 healthy female subjects received for eight weeks 6 mg per day of oral supplementation combined with 2 mL (78.9-µM solution) per day of a topical application of astaxanthin. Significant improvements were observed in skin wrinkle, age spot size, elasticity, and skin texture (Tominaga et al, 2012).

Tominaga et al, 2012. also conducted a randomized double-blind placebo-controlled study involving 36 healthy male subjects supplemented with 6 mg of astaxanthin for six weeks. At the end of the study period, astaxanthin improved wrinkles, elasticity, transepidermal water loss (TEWL), moisture content, and sebum oil level. These results demonstrate that astaxanthin may improve skin condition in both men and women.

To evaluate the anti-inflammatory effect of astaxanthin on skin deterioration, Kumi Tominaga et al 2017, conducted a 16-week clinical study with 65 healthy female participants. Participants were orally administered either a 6 mg or 12 mg dose of astaxanthin or a placebo. Wrinkle parameters and skin moisture content significantly worsened in the placebo group after 16 weeks. However, significant changes did not occur in the astaxanthin groups. Interleukin-1α levels in the stratum corneum significantly increased in the placebo and low-dose groups but not in the high-dose group between weeks 0 and 16. This study was performed in Japan from August to December, when changing environmental factors, such as UV and dryness, exacerbate skin deterioration. Study suggests that long-term prophylactic astaxanthin supplementation may inhibit age-related skin deterioration and maintain skin conditions associated with environmentally induced damage via its anti-inflammatory effect.

To study the astaxanthin effect on sun exposure, scientists tested a group of people to see how much UV light was needed to redden their skin or cause mild sunburn. The group was given 4 milligrams a day of Astaxanthin for two weeks, followed by a repeat of the skin-reddening test. The results were that 4 milligrams of astaxanthin per day increased the amount of time it took for UV radiation to redden the skin, demonstrating astaxanthin's ability to support the structure of the skin during sun exposure. This study proved that in just two weeks astaxanthin was working as an internal sunscreen. Astaxanthin was found to decrease melanin production by 40%. Astaxanthin included in topical formulas as whitening agents (Arakane, K, 2001).

In 1995, a study was conducted on special hairless mice to test the protective effects of astaxanthin, beta carotene or retinol against ultraviolet light. From birth, the mice were fed different diets containing combinations of the three substances, the substances alone or a control diet with none of the three substances. After four months, half of each group was exposed to UV light, at which three markers for skin damage were tested. After irradiation, Astaxanthin alone or in combination with retinol was remarkably effective in preventing photo aging of the skin as measured by these markers (Savoure, et al, 1995).

Enriched astaxanthin extract from *Haematococcus pluvialis* enhances collagen production in human dermal fibroblasts through inhibited MMP1 and MMP3 mRNA expression and induced TIMP1, the antagonists of MMPs protein, gene expression. Astaxanthin is an alternative treatment for collagen production (Chou et al 2016).

EYE HEALTH

Eyes are organs of the visual system. They provide organisms with vision, the ability to receive and process visual detail, as well as enabling several photo response functions that are independent of vision. The eye is subjected to higher levels of oxidation due to the depletion of the ozone layer, which directly affects the eyes. Excessive exposure to sunlight and to the highly oxygenated environment causes free radicals to be generated in the eye.

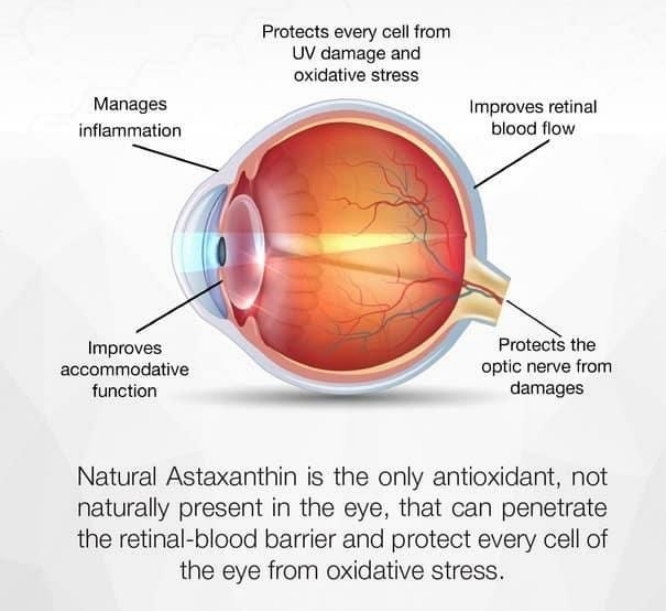

Fig5: Astaxanthin in Eye Health

Two of the leading causes of visual impairment and blindness are age related macular degeneration (AMD) and age-related cataracts. Carotenoids such as lutein and zeaxanthin reduced the risk of AMD and other degenerative conditions. Various clinical studies have proven that astaxanthin is superior to all other nutraceuticals. Astaxanthin is capable of crossing the blood–retinal barrier and will deposit in the retina and offer antioxidant efforts that reduce retinal destruction by preventing light-induced oxidation (Domínguez et al 2007).

In a double blind study conducted by Nagaki et al in 2010 examined the effect of astaxanthin in the efficacy of natural astaxanthin on accommodation ability. 42 subjects were supplemented with 9mg /day natural astaxanthin Vs placebo for 4 weeks. It was found that astaxanthin group had significantly higher accommodation ability compared to the control group.

In a double-blind study researcher evaluated the effects of astaxanthin on accommodation, critical flicker fusion (CFF), and pattern visual evoked potential (PVEP) in visual display terminal (VDT) workers. Authors reported that intake of 5mg/day astaxanthin for four weeks reduces eye strain, blurring and diplopia. They also found that accommodation amplitude improved after astaxanthin supplementation in VDT workers. (Nagaki, et al, 2006).

Different dosage levels were tested for eye fatigue by a group led by Dr. Nakamura in 2004, evaluated the effect of astaxanthin on visual function in 49 eyes of 49 healthy volunteers. They were over 40 years of age. They were divided into 4 groups matched for age and gender. Each group was given per oral astaxanthin once a day. The dosage was 0 mg, 2 mg, 4 mg, or 12 mg for each group. After ingestion of astaxanthin for consecutive 28 days, the uncorrected far visual acuity significantly improved in groups receiving 4 mg or 12 mg. The accommodation time significantly shortened in groups receiving 4 mg or 12 mg. There was no change in refraction, flicker fusion frequency, or pupillary reflex. (Nakamura, et al, 2004).

In a randomized, double-blind, placebo-controlled study, Saito et al 2012, examined 20 healthy volunteers who ingested 12 mg astaxanthin or placebo capsules over a 4-week period. It was found that administration of astaxanthin over a 4-week period can elevate the choroidal blood flow velocity without any adverse effects.

In a randomized, double-blind, placebo-controlled study, NagakiYasunori et al 2005., randomized thirty-six volunteers into two groups. Astaxanthin group that consisted of 18 subjects who received oral astaxanthin, 6mg/day, for 4 weeks and a placebo group that

consisted of 18 subjects who received an identical looking oral placebo for 4 weeks. It was found that intake of 6mg/day astaxanthin for 4 weeks increase retinal capillary blood flow, while retinal capillary blood flow in the placebo group after placebo treatment was unchanged.

In a double blind study, Shiratori et al., 2005 investigated the efficacy of natural astaxanthin on accommodation speed. 40 healthy subjects suffering asthenopia took a 6mg/day natural astaxanthin supplement vs placebo for weeks. It was found that astaxanthin group showed significant improvement in accomodation speed.

Hshimoto et al 2013 conducted a study with 35 patients who underwent bilateral cataract surgery on one side before and the other side after intake of astaxanthin (6 mg/day for 2 weeks). After astaxanthin intake, the superoxide scavenging activity was significantly elevated, while the level of total hydroperoxides was significantly lowered. Astaxanthin intake clearly enhanced the superoxide scavenging activity and suppressed the total hydroperoxides production in human aqueous humor, indicating the possibility that astaxanthin has suppressive effects on various oxidative stress-related diseases.

Hshimoto et al 2016, conducted a study with 35 patients who underwent bilateral cataract surgery. Patients were supplemented with astaxanthin (6 mg/day) immediately after receiving surgery in one eye, then underwent surgery in the other eye after 2 weeks. Aqueous humor was taken from each of the eyes during surgery for analysis of $O_2^{\bullet-}$ scavenging activity and levels of H_2O_2, and vascular endothelial growth factor (VEGF). It was found that astaxanthin intake may have affected vascular endothelial growth factor level through its antioxidant effects by increasing $O_2^{\bullet-}$ scavenging activity and suppressing peroxide production.

Nitta et al, 2005 conducted a double-blind study to evaluate astaxanthin's effect on eye fatigue and visual accommodation. Forty subjects were divided into placebo and treatment

groups, with the treatment group receiving 6 mg of astaxanthin for four weeks. This research established an optimum daily dose for eye fatigue at 6 mg per day.

Keiko Kono et al 2014, conducted a study involving 48 subjects aged 45 to 64 years (mean age: 52.8 years, 25 men and 23 women) supplemented with 4mg of natural astaxanthin for four weeks. At the end of the study it was found that astaxanthin improves accommodative ability.

Sawaki, et al, in 2002 examined healthy men for analysing the effect of natural astaxanthin on the eyes.18 healthy adult male volunteers that were equally divided into two groups (treatment and control). The treatment group was given 6 mg of natural astaxanthin per day for four weeks. It was found that the deep vision and the critical flicker fusion of the treated groups were significantly improved compared to the control group. No effects of treated group were observed on static and kinetic visual acuity. It was suggested that supplementation of astaxanthin is effective for the improvement of visual acuity. The greatest enhancement was seen in depth perception which improved by 46% in the group supplementing with natural astaxanthin.

In a randomised controlled trial open labelled placebo study with 27 volunteers over 12 months found improvement in central retinal dysfunction in age related macular degeneration when 4 mg of natural astaxanthin administered with other antioxidants.

Cataracts are a very common and growing problem in our aging human population. In one pre-clinical animal trial, astaxanthin was found to have potent antioxidant effects in the prevention of cataracts in rats' eyes (Wu, et al, 2002). A similar study with both in-vitro and in-vivo components revealed that astaxanthin helped prevent the formation of cataracts (Liao, et al, 2009). Another study took the lens from the eyes of pigs and tested the ability of Astaxanthin to protect them from induced oxidative damage. This experiment found that, astaxanthin was capable of protecting the lens proteins from oxidation. In fact, Astaxanthin performed better than the antioxidant glutathione which is produced by the pig's own body (Wu, et al, 2006).

Ishikawa et al., 2015, revealed that administration of 100µl of 100 mg/ml of astaxanthin dissolved in DMSO protects glucocorticoid-induced cataract in chick embryo.

Astaxanthin's extreme antioxidant activity on eye health was borne out from another mammal study done in Japan. In this study, the researchers concluded that Astaxanthin had neuroprotective effects against retinal damage in-vitro and in-vivo, and that its protective effects may have been partly mediated via its antioxidant effects" (Nakajima, et al, 2008).

MALE FERTILITY

Enhanced production of reactive oxygen species in the sperm has been documented among the men with infertility. Several studies suggested that astaxanthin supplements are used for sperm quality improvement treatment.

In one double-blind randomized controlled trial to evaluate astaxanthin's use in protecting sperm function and male fertility, thirty men from infertile couples (where the female partner had no fertility issues) received either astaxanthin (16 mg/day) or a placebo for three months. The men provided semen for intra uterine insemination (IUI) during the three months and the occurrence of pregnancy was recorded. By the end of three months, sperm motility was significantly increased and semen free radical production was decreased in the astaxanthin group versus the placebo group. Most noted in this study was the pregnancy rate, which was 54.5 percent for the astaxanthin group compared to 10.5 percent for the placebo group (Comhaire et al 2005).

EFFECTS OF ASTAXANTHIN ON CIRCULATION

As people age, their red blood cells (RBCs) can be more susceptible to oxidative attack, resulting in peroxidative damage to the RBC membrane phospholipids, impairing its oxygen -carrying capacity (Marotta et al 2006). In a 2011 double-blind RCT healthy subjects, ages

50-69 years (n=30), were randomly allocated to receive astaxanthin at 6 mg/day or 12 mg/day or a placebo for 12 weeks. Both astaxanthin intakes significantly lowered RBC hydroperoxide levels ($p<0.05$ for both doses versus placebo); the 12 mg/day dose did not work significantly better than the 6 mg/day dose (Nakagaw et al 2011).

ASTAXANTHIN'S ROLE IN BRAIN HEALTH

The human brain is the central organ of the human nervous system, and with the spinal cord makes up the central nervous system. The nervous system is rich in both unsaturated fats (which are prone to oxidation) and iron (which has strong prooxidative properties). These, together with the intense metabolic aerobic activity and rich irrigation with blood vessels found in tissues of the nervous system, make tissues particularly susceptible to oxidative damage. There is substantial evidence that oxidative stress is a causative or at least ancillary factor in the pathogenesis of major neurodegenerative diseases (Alzheimer's, Huntington's, Parkinson's and amyotrophic lateral sclerosis, ALS) and those diets high in antioxidants offer the potential to lower the associated risks (Grant, et al. 1997).

A randomised, double-blind, placebo-controlled human trial was conducted to assess the efficacy of 12-week astaxanthin supplementation (6 or 12 mg/d) on both astaxanthin and Phospholipid hydroperoxides (PLOOH) levels in the erythrocytes of thirty middle-aged and senior subjects. After 12 weeks of treatment, erythrocyte astaxanthin concentrations were higher in both the 6 and 12 mg astaxanthin groups than in the placebo group. In contrast, erythrocyte PLOOH concentrations were lower in the astaxanthin groups than in the placebo group. In the plasma, somewhat lower PLOOH levels were found after astaxanthin treatment. These results suggest that astaxanthin supplementation results in improved erythrocyte antioxidant status and decreased PLOOH levels, which may contribute to the prevention of dementia (kiyotaka Nakagawa et al 2011)

In a randomized double blind, placebo - controlled study, 12mg/day supplementation of natural astaxanthin for 3 months improved memory, mental quickness and multitasking in senior subjects complaining of age-related forgetfulness (Satoh, et al, 2009).

One trial tested male subject ages 50–69 had symptoms of mild cognitive impairment and treated with astaxanthin doses of 20 mg/day for 12 weeks. The subject displayed improvements in performances on the CogHealth and P300 cognitive tests when compared to the baseline data (Satoh et al. 2009b).

In a controlled and double-blinded clinical trial that used low and high doses (6 and 12 mg/day) for 12 weeks, with male and female subjects ages 45–64, the subjects were tested for CogHealth as well as the Groton Maze Learning Test (GMLT). By the end of the study, astaxanthin treated subjects had improved significantly, making fewer errors in the maze test in both low and high doses. The high-dose group also demonstrated faster reaction times in the CogHealth tests. The researchers concluded that, the 12 milligrams a day of astaxanthin significantly improves cognitive function and psychomotor functions (Katagiri et al. 2012)

Zanotta et al., 2014 conducted a clinical study in patients diagnosed with mild cognitive impairment (MCI). Patients were supplemented with 4 mg of astaxanthin daily in combination with Bacopa monnieri and other antioxidants for 60 days. Study suggest that astaxanthin supplementation improves disease assessment scale cognitive subscale. Other clinical studies have found that astaxanthin treatment significantly raises blood levels of carotene, and improves the health of circulating red blood cells – reducing free radical related damage that can eventually produce nerve and brain cell damage. Even low dosages of one and three milligrams a day have shown a strengthening effect among red blood cells.

A 2009 study from Taiwan's Hungkuang University using brain cells, found that astaxanthin suppressed about 75% of the reactive oxygen species production along with other parts of the oxidative process involved in the beta-amyloid production process found in Alzheimer's disease (Chang, et al, 2010).

National Institute on Drug Abuse found that "Astaxanthin can reduce ischemia-related injury in brain tissue through the inhibition of oxidative stress, reduction of glutamate release, and antiapoptosis [prevention of cell death]. Astaxanthin may be clinically useful for patients vulnerable or prone to ischemic events." (Ischemia is the condition where there is a deficient supply of blood to the brain as a result of an obstruction of the arteries, which results in stroke, brain cell death and impaired brain function). (Shen, et al, 2009 and Curek, et al, 2010).

A study on a human neuroblastoma cell line showed that astaxanthin can protect from cell death. Astaxanthin suppresses MPP+-induced oxidative stress in PC12 cells via the HO-1/NOX2 axis. The Fujian Medical University researchers concluded that astaxanthin should be strongly considered as a potential neuroprotectant and adjuvant therapy for patients with Parkinson's disease. (Ikeda, et al, 2008).

Brazilian scientists noted that many neuronal dysfunctions and psychotic behaviours such as anxiety and depression are related to different brain regions being subjected to oxidative stress. They found that Astaxanthin could restore normal oxidative conditions in plasma and positively affect the forebrains of animals (Mattei, et al, 2011)

In an animal study, mouse were supplemented with 2 mg/kg of astaxanthin for 4 weeks. Decreased oxidative stress makers and increased antioxidant enzyme activities in different regions of the brain including the frontal cortex, hypothalamus, striatum, pariental cortex, hippocampus, and cerebellum in young and old animals (Al-Amin et al. 2015)

Lu et al. 2015 found that supplementation of rat with 75mg/kg of astaxanthin reduced neuronal damage, decreased oxidative stress, and apoptosis in hippocampus.

ANTI-DIABETIC ACTIVITY

Diabetes is recognized as an important cause of premature death and disability. Diabetes and obesity are closely intertwined disorders that cause long term negative consequences. Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease. Global prevalence of diabetes has

grown from 4.7 % in 1980 to 8.5 % in 2014, during which time prevalence has increased or at best remained unchanged in every country. Over the past decades, diabetes prevention has risen faster in low and middle income countries. Diabetes caused 1.5 million death in 2012. It was the eighth leading cause of death among both sexes and fifth leading causes of death in women in 2012. Globally, an estimated 422 million adults were living with diabetes in 2014, compared to 108 million in 1980. The global prevalence (age-standardized) of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5% in the adult population (WHO, 2016). Diabetes is a major component of metabolic syndrome, can be managed effectively with the help of astaxanthin. Prevalence of diabetes is predicted to double globally from 171 million in 2000 to 366 million in 2030 with a maximum increase in India. Diabetes mellitus is strongly associated with oxidative stress, which can be a consequence of increased free radical production, reduced antioxidant defenses or both. Clinical studies have proven that astaxanthin supplementation could improve diabetic conditions.

In 2002 Uchiyama, K et al conducted a study with mice, a well-known mice model of type 2 diabetes to determine the effect of astaxanthin. It was found that astaxanthin supplementation at a daily dosage of 1 mg for 12 weeks could reduce the oxidative stress caused by hyperglycemia in pancreatic β-cells and also improve glucose tolerance and serum insulin levels and decrease blood glucose levels. Astaxanthin can protect pancreatic β-cells against glucose toxicity. It was also shown to be a good immunological agent in the recovery of lymphocyte dysfunctions associated with diabetic rats (Uchiyama, K et al 2002).

Nakano et al.conducted a study against oxidative damage in streptozotocin induced diabetic rats to identify the effect of astaxanthin in combination with other antioxidants. Supplementation of astaxanthin (0.1 g/kg) together with α-tocopherol (0.1 g/kg) for 20 weeks ameliorated oxidative injury through the suppression of oxidative stress induced by diabetes. (Nakano et al, 2008).

In another study by Nakano et al, 2008 found that streptozotocin-induced diabetic rats showed decreased levels of lipid peroxides in plasma, liver and kidney, and of plasma triglyceride after 12 weeks of a diet containing astaxanthin and flavangenol, a pine bark extract, at doses of 0.1g/kg and 2.0 g/kg, respectively (Nakano et al, 2008).

In mice with diabetes induced by alloxan that promotes oxidative stress, postprandial hyperglycemia was suppressed by feeding astaxanthin at doses of 5 mg/ kg and 10 mg/kg daily for 7 days (Wang, et al 2012).

Astaxanthin supplementation of 20 mg/kg for 30 days reversed the elevated lipid peroxidation and protein carbonyl groups, which are the indicators of oxidative damage to biomolecules in alloxan induced diabetic rats (Marin et al 2011).

Consumption of a high fat/high fructose diet supplemented with astaxanthin at a dose of 6 mg/kg body weight for 60 days ameliorated high fat/high fructose diet-induced hyperinsulinemia and insulin resistance in Swiss albino mice (Bhuvaneswari et al 2010).

Mice fed with high fat/high fructose diet containing astaxanthin at a dose of 2mg/kg body weight for 45 days improved insulin sensitivity by decreasing serine phosphorylation of insulin receptor substrates (IRS), increasing the association of IRS and phosphatidylinositol 3-kinase (PI3K), and increasing Akt phosphorylation in the liver (Bhuvaneswari et al 2012).

In a study Swiss albino mouse were fed a high fat/high fructose diet supplemented with ASTX at 6 mg/kg for 60 days, ASTX improved hyperglycemia and hyperinsulinemia, and decreased plasma levels of TNFα and IL-6 (ArunKumar etal 2012).

Some of the studies demonstrated that astaxanthin prevents diabetic nephropathy by reduction of the oxidative stress and renal cell damage (Manabe, E et al 2008). Consumption of 0.02% astaxanthin for 12 weeks prevented the progression of diabetic nephropathy, evidenced by reduced glomerular mesangial area, improved hyperglyceridemia and oxidative stress in db/db mice (Naito et al 2004) Also, astaxanthin supplementation decreased the expression of genes involved in the mitochondrial oxidative phosphorylation pathway, such as complexes I, III, and IV, in primary glomerular cells from the kidney of db/db mouse fed 0.02% astaxanthin for 6 weeks (Naito et al 2006).

Nishigaki et al. found that astaxanthin could inhibit the non-enzymatic glycation and glycated protein/iron chelate-induced cytotoxicity in human umbilical-vein endothelial cells by preventing lipid and protein oxidation (Nishigaki, et al 2010).

Hussein et al. investigated the effects of astaxanthin in a metabolic syndrome animal model

of spontaneously hypertensive corpulent rat, and found that astaxanthin significantly lowered the levels of blood glucose, nonesterified fatty acids and triglycerides, and significantly increased the levels of high-density lipoprotein cholesterol and adiponectin, indicating that astaxanthin ameliorates insulin resistance and improve insulin sensitivity by mechanisms involving the increase of glucose uptake, and by modulating the levels of circulating adiponectin and blood lipids (Hussein et al. 2007)

ASTAXANTHIN SUPPORTS A HEALTHY IMMUNE SYSTEM

Immune system cells are very sensitive to free radical damage. The cell membrane contains poly unsaturated fatty acids (PUFA). Drs. Jean Soon Park and Boon P. Chew at Washington State University discovered that astaxanthin boosts immunity in animals. In an important study, they were able to show that it also increases the health of the immune system in humans. Astaxanthin showed higher immuno-modulating effects in mouse model when compared to β-carotene (Jyonouchi, H et al 1991).

In a double-blind, placebo controlled study researchers examined the effect of astaxanthin on healthy females of an average age of 21. Subjects were supplemented with 2 to 8 mg /day of astaxanthin for eight weeks. An increased level of NK cells and decreased damage to cellular DNA was found after eight weeks of astaxanthin intake. An overall improvement in the markers of immune system health was found. At the 2-mg/day dose, total T- and B-cell numbers were significantly increased over placebo. At the 8-mg/day dose, natural killer cell cytotoxic activity increased. Skin delayed-type hypersensitivity (DTH), cell and humoral mediated immunity, was significantly improved by the 2-mg/ day dose ($p<0.05$). The researchers concluded astaxanthin promotes overall immune competence (park et al., 2010).

In another randomized, double blind placebo controlled study 42 subjects were examined for the pro inflammatory marker CRP. Plasma C-reactive protein concentration was lower on week 8 in subjects given 2 mg astaxanthin. A higher percentage of leukocytes expressed the LFA-1 marker in subjects given 2 mg astaxanthin on week 8. Subjects fed 2 mg astaxanthin had a higher tuberculin response than unsupplemented subjects. There was no difference in TNF and IL-2 concentrations, but plasma IFN-gamma and IL-6 increased on week 8 in subjects given 8 mg astaxanthin. Therefore, dietary astaxanthin decreases a DNA damage

biomarker and acute phase protein, and enhances immune response in young healthy females. (Park et al 2010).

Astaxanthin was also tested for skin immunity in another double-blind RCT. Patients (ages 19-51 years; n=27) with mild-to-moderate atopic dermatitis received either 12 mg/day astaxanthin or a placebo for four weeks. Although astaxanthin did not significantly improve the dermatitis severity, a significant shift in T-helper1/T-helper2 (Th1/Th2) balance was observed – with a shift toward Th1 dominance. The researchers judged this an important finding since atopic dermatitis is considered a Th2-dominant disease. Those in the astaxanthin group also experienced significant improvement in anxiety and other quality of life symptoms compared to placebo (Satoh et al.2010)

Yamada et al 2010, performed a clinical study of astaxanthin in six Sjögren's syndrome (SS) patients and six normal individuals, quantifying the volume of saliva secretion and the level of oxidative stress markers in the saliva. The level of oxidative stress marker, hexanoyl-lysine, in the saliva was reduced after astaxanthin intake. Study suggested that intake of 12mg/day astaxanthin for 2 weeks might act as an ROS scavenger, providing benefits to SS patients with impaired salivary secretion.

In a study forty trained male soccer players were randomly assigned to astaxanthin and placebo groups to investigate the effect of astaxanthin supplementation on salivary IgA (sIgA) and oxidative stress status in plasma. Astaxanthin group was supplemented with 4 mg of astaxanthin. Saliva and blood samples were collected at the baseline and after 90 days of supplementation in pre-exercise conditions. This study indicates that astaxanthin supplementation improves sIgA response and attenuates muscle damage, thus preventing inflammation induced by rigorous physical training (Baralic et al 2015).

MUSCLE PERFORMANCE

Thirty-two male elite soccer players were randomly assigned in a double-blind fashion to astaxanthin and placebo (P) group. Astaxanthin group was supplemented with 4 mg of astaxanthin for 90 days. After the 90 days of supplementation, the athletes performed a 2

hours acute exercise bout. Study suggested that soccer training and soccer exercise are associated with excessive production of free radicals and oxidative stress, which might diminish antioxidant system efficiency. Supplementation with 4 mg astaxanthin could prevent exercise induced free radical production and depletion of non-enzymatic antioxidant defense in young soccer players (Djordjevic et al 2012).

In a study forty soccer players were randomly assigned in a double-blind fashion to astaxanthin and placebo (P) group. Study suggested that astaxanthin supplementation for 90 days increases total sulphydryl group content and improve paraoxonase activity through protection of free thiol groups against oxidative modification (Baralic et al., 2013)

Earnest et al 2011., examined the effect of astaxanthin on substrate metabolism and cycling time trial (TT) performance. It was found that intake of 4mg/day of astaxanthin for 28 days improves TT performance.

In the randomized, double-blind, placebo-controlled study Park et al.2010 found that intake of 2 or 8mg of astaxanthin for an 8-week period may lower muscle pain, stiffness and fatigue by reducing inflammation and oxidation in muscles.

Malmsten et al 2008, conducted an experiment with young healthy male students not subjected to any medication. The students (n=40) were randomly divided into two groups of equal size. Before starting the dietary supplementation, each individual carried out the standardized test exercises to obtain starting point values. A double-blind study on male students found that intake of 4 mg of astaxanthin for 24 weeks improves muscle endurance.

Sawaki et al 2004 conducted an experiment with 16 adult male volunteers to establish the effect of astaxanthin supplementation on the build-up of lactic acid before and after running 1,200 meters, the treated groups ingested an astaxanthin capsule per day for 4 weeks (6mg astaxanthin per day) and the control groups received a placebo capsule. Serum lactic acid concentration at 2 minutes after activity (1,200 m running) of the treated group was

significantly lower than that of the control one, no other effects related to supplementation of astaxanthin on serum biological and hematological examinations were observed. It was suggested that supplementation of astaxanthin is effective for the improvement of muscular fatigue that may lead to sports performance benefits.

RHEUMATOID ARTHRITIS

Rheumatoid arthritis is an auto-immune disorder in which the sufferer's own immune system attacks itself.It is a chronic destructive disorder that can cripple sufferers. Unfortunately, many traditional therapies are not very effective, and prescription drugs may be unsafe as well as ineffective. Alternative therapies and nutraceuticals before natural astaxanthin have yielded inconsistent results.

Randomized, double-blind, placebo-controlled human clinical trials conducted to identify the efficacy of astaxanthin in the pain relief and rheumatoid arthritis. Clinical trials were initiated with twenty one subjects, fourteen receiving 12mg /day astaxanthin and seven receiving a placebo. Fourteen receiving astaxanthin and seven receiving a placebo. The results showed that pain scores for the treatment group decreased by approximately 10% after four weeks, and by more than 35% after eight weeks. The pain scores for the placebo group remained relatively constant. The subjects taking natural astaxanthin self-rated satisfaction scores improved by approximately 15% after four weeks and by over 40% after eight weeks. These results were extremely significant, and the researchers concluded that "Astaxanthin-based supplements appear to be an effective addition in the treatment of rheumatoid arthritis (Nir and Spiller, 2002b).

CARPAL TUNNEL SYNDROME (REPETITIVE STRESS INJURY)

Carpal tunnel syndrome (CTS) is a debilitating disease of the wrist that manifests itself in numbness, pain and in extreme cases even paralysis. There is no cure for this condition; current medical procedures are to put a splint on the wrist to immobilize it or, at the very least, restrict movement to a minimum. If the condition doesn't improve after immobilization, most often wrist surgery is recommended. Unfortunately, not all patients respond to surgery.

The clinical trial done on CTS sufferers with twenty subjects. Thirteen people received 4 milligrams of natural astaxanthin three times a day, and seven people received a placebo. The result showed that the group taking natural astaxanthin reported a 27% reduction in daytime pain after four weeks and a 41% reduction after eight weeks. (Nir and Spiller, 2002a). This study as well as extensive anecdotal evidence from carpal tunnel sufferers shows that natural astaxanthin may be a viable alternative to surgery.

ASTAXANTHIN AND TENNIS ELBOW (TENDONITIS)

A study was conducted by Dr. Spiller from the Health Research and Studies Centre on patients suffering from tennis elbow. Tennis elbow is a form of tendonitis. One of the debilitating results of this condition is the decrease in grip strength and pain that is generated when gripping something in the hand. It appears that Astaxanthin can have a significant effect on inflammation of the tendons.

A single-centre, double-blind, placebo-controlled study was conducted over an 8 week duration to analyse the effect of astaxanthin supplementation on the grip strength of tennis elbow sufferers. This study was comprised of thirty three subjects who completed the eight week course of supplementation or placebo. Researchers found that astaxanthin group had less arm soreness and improved grip strength. After eight weeks of taking astaxanthin, the treatment group showed a remarkable average improvement in grip strength of 93%, while at the same time self-assessment of their pain level decreased (Spiller, et al 2006).

ASTAXANTHIN AND CANCER

Natural Astaxanthin dose and time-dependently inhibited cell growth in colon cancer cells, leading the researchers to conclude that it may protect from colon cancer (Palozza, et al, 2009)

Rats with colon cancer fed Astaxanthin and showed a decrease in proteins involved in carcinogenesis and an increase in colon cancer cell death. The conclusion drawn was that Astaxanthin exhibited anti-inflammatory and anti-cancer effects through cancer cell death and through modulation of multiple blood markers (Nagendraprabhu, et al, 2011).

Astaxanthin showed an anti-cancer effect in rats by inducing cell death in liver carcinoma cells (Song, et al, 2011).

A mouse study involving Drs. Chew and Park showed that Astaxanthin delayed tumor growth and modulated the immune response, but only when it was given before tumor initiation. In a study researcher found that bone cancer in dogs was positively affected by Astaxanthin (Wakshlag, et al, 2010).

In an in-vitro study, mouse tumor cells were put into a solution supplemented with astaxanthin and into the same solution without Astaxanthin. After one and two days, it was found that the tumor cells in the astaxanthin solution had lower cell numbers as well as a lower DNA synthesis rate (Sun, et al, 1998).

In another study of mouse breast tumor cells, it was found that Astaxanthin reduced the proliferation of the tumor cells by 40% in a dose-dependent fashion (Kim, et al, 2001). A very interesting study pitted Astaxanthin against eight other carotenoids to see which was most effective at inhibiting liver tumor cells in culture. It was found that Astaxanthin surpassed every other carotenoid in this test (Kozuki, 2000).

The proliferation of human cancer cell lines has also been inhibited by Astaxanthin in vitro. Human colon cancer cell lines were placed in a culture containing Astaxanthin versus no Astaxanthin. After four days, the cell lines in the culture containing astaxanthin were significantly less viable (Onogi, et al, 1998). Also, in work with human prostate cancer cells, Astaxanthin and lycopene both showed significant inhibitory effects (Levy, et al, 2002).

Researchers transplanted tumor cells into mice and found that astaxanthin inhibited the growth of the cancerous tumors, again in a dose-dependent fashion (Sun, et al, 1998).

Study was done to see at what stage the Astaxanthin would have its positive effects. It was found that when Astaxanthin supplementation was started both at one week and also at three weeks prior to the tumor inoculation, growth was inhibited. However, when the supplementation with Astaxanthin began at the same time as the tumor inoculation, the benefit was not found. The conclusion of this study was that Astaxanthin may work better in the early stages of tumor development, but not in the later stages. This study also pointed out

that, unlike chemotherapy drugs, Astaxanthin's abilities to reduce tumors cannot be due to toxicity. Even dietary concentrations as high as 2% did not induce toxicity in rats, mice or ferrets. The theory espoused by these researchers from the University of Minnesota's School of Medicine and led by the "Grandmother of Astaxanthin Research" Dr.Jyonouchi, is that Astaxanthin's anti-tumor activity is related to its enhancement of the immune response (Jyonouchi, et al, 2000).

Other mice studies have also shown very promising results. An early study led by Dr. Chew found that astaxanthin reduced the growth of transplanted breast tumors. This study was very interesting in that it tested Astaxanthin against two other carotenoids—beta carotene and canthaxanthin. The researchers found that "Mammary tumor growth inhibition by astaxanthin was dose-dependent and was higher than that of canthaxanthin and beta carotene. Lipid peroxidation activity in tumors was lower ($P < 0.05$) in mice fed 0.4% Astaxanthin, but not in that fed beta-carotene and canthaxanthin" (Chew, et al, 1999)

Another study demonstrated that Astaxanthin suppressed spontaneous liver carcinogenesis (Nishino, et al, 1999). Further studies have shown that introduction of carcinogens such as benzopyrene to mice was positively affected when they were fed astaxanthin; two specific types of cancer that appeared in the control group were inhibited in the astaxanthin group (Lee, et al, 1997 and Lee, et al, 1998).

Astaxanthin consumed in the diet reduced the incidence of tumor-promoting substances in the skin of hairless mice that were exposed to UVA and UVB radiation (Savoure, et al, 1995). Related research done at the Veterans Affairs Medical Center in Texas showed that Astaxanthin and beta carotene (but not lycopene) prevented UV-mediated carcinogenesis in mice (Black, H, 1998).

Astaxanthin, along with some other carotenoids, was found to be an effective anti-tumor agent in a series of studies on mice and rats at the Gifu University School of Medicine in Japan (Mori, et al, 1997). One of these studies found that Astaxanthin significantly reduced both the incidence and the proliferation of chemically-induced bladder cancer in mice. In this study, Astaxanthin was tested against canthaxanthin. It was found that the results with

canthaxanthin were not statistically significant, while those with Astaxanthin were (Tanaka, et al, 1994).

Two other studies showed the same effects in the oral cavity and the colon of rats; Astaxanthin reduced the incidence and the proliferation of cancers when carcinogenic chemicals were introduced (Tanaka, et al, 1995a). Lastly, a few different studies have shown Astaxanthin's positive effects on cancer of the liver in rats (Gradelet, et al, 1997, Gradelet, et al, 1998, and Kurihara, et al, 2002).

Mechanism that prevent or shrink cancer (Rousseau, et al, 1992):

1. Its potent biological antioxidant action

2. Its abilities as an immune system enhancer

3. Its action as a regulator of gene expression

In regards to regulating gene expression, basically the cell to cell communication through the gap junctions is deficient in many human tumors. Improvement in this cell to cell communication tends to decrease tumor cell proliferation (Bertram, J, 1999). Astaxanthin is known to improve this intercellular communication.

OTHER VITAL INFORMATION

BIOAVAILABILITY AND PHARMACOKINETICS OF ASTAXANTHIN

Absorption of astaxanthin is dependent on the type of oil that is consumed with astaxanthin. Astaxanthin with combination of fish oil promoted hypolipidemic/hypocholesterolemic effects in plasma and it increased phagocytic activity of activated neutrophils when compared with astaxanthin and fish oil alone (Barros, et al 2012). Astaxanthin was superior to fish oil in particular by improving immune response and lowering the risk of vascular and infectious diseases. The proliferation activity of T- and B-lymphocytes was diminished followed by lower levels of O_2, H_2O_2 and NO production, increased antioxidant enzymes - superoxide dismutase, catalase and glutathione peroxidase (GPx), and calcium release in cytosol after administration of astaxanthin with fish oil. Astaxanthin absorption was higher when it was

emulsified with olive oil than with corn oil in rat duodenum. Astaxanthin bioavailability in human plasma was confirmed with single dosage of 100 mg (Osterlie et al 2000).

Astaxanthin bioavailability in humans was enhanced by lipid based formulations; high amounts of carotenes solubilized into the oil phase of the food matrix can lead to greater bioavailability. Carotenoids are absorbed into the body like lipids and transported via the lymphatic system into the liver. A high cholesterol diet may increase carotenoid absorption while a low fat diet reduces its absorption. Astaxanthin mixes with bile acid after ingestion and make micelles in the intestinum tenue. The micelles with astaxanthin are partially absorbed by intestinal mucosal cells. Intestinal mucosal cells incorporate astaxanthin into chylomicra. Chylomicra with astaxanthin are digested by lipoprotein lipase after releasing into the lymph within the systemic circulation, and chylomicron remnants are rapidly removed by the liver and other tissues. Astaxanthin is assimilated with lipoproteins and transported into the tissues. Of several naturally occurring carotenoids, astaxanthin is considered one of the best carotenoids being able to protect cells, lipids and membrane lipoproteins against oxidative damage (Ranga Rao Ambati et al 2014).

Scavenger receptor class B, type I (SR-BI) may mediate the intestinal absorption of astaxanthin since its shares several structural similarities with β-carotene and xanthophylls, including β-cryptoxanthin, lutein and zeaxanthin. Evidence has suggested that astaxanthin isomers may be absorbed at a different degree. In humans, after oral administration of a mixture of all-*cis*-astaxanthin and all-*trans*- astaxanthin at a ratio of 1:14, the isomers appeared in the plasma at ~1:2 ratio. The observation suggests that all-*cis*-astaxanthin may be preferentially absorbed or selectively accumulated in the circulation **(**Yue Yange et al 2013).

DOSAGE AND SAFETY OF ASTAXANTHIN

Multiple animal studies and human trials revealed that Astaxanthin is safe, with no side effects when it is consumed with food. In a randomized, double-blind and placebo-controlled trial that gave daily supplementation of 6 mg of astaxanthin from *H. pluvialis* to healthy adults for 8 weeks, there were no significant changes in blood pressure, plasma metabolic panels and blood cell blood count whereas ASTX supplementation slightly increased serum levels of calcium, total proteins and eosinophils within healthy ranges (spiller et al 2003).

Moreover, administration of a single dose of 100 mg ASTX in middle-aged male, daily dose of 40 mg for 4 weeks in patients with functional dyspepsia (. Kupcinskas et al 2008), or daily dose of 4 mg for 12 months in subjects with macular degeneration (Parisi et al 2008) did not induce any adverse side-effects. To date, no adverse side-effects of astaxanthin supplementation have been reported in humans. In 2010, the U.S. Food and Drug Administration acknowledged the "generally recognized as safe (GRAS)" status of astaxanthin extracted from *H. pluvialis*. Astaxanthin dosage effects on human health benefits were presented in table 1.

Table 1: Astaxanthin dosage and health benefits.

Astaxanthin use	Dosage
Antioxidant	2- 4 mg/day
Arthritis	8- 12 mg/day
Tendonitis or Carpal tunnel Syndrome	8- 12 mg/day
Silent Inflammation	4- 12 mg/day
Internal sunscreen	4- 8 mg/day
Internal beauty and Skin improvement	4- 8 mg/day
Immune system enhancer	2-4 mg/day
Cardio vascular health	4- 8 mg/day
Strength and endurance	4- 12 mg/day
Competitive Athletes	8-12 mg/day
Brain and central nervous system health	4- 8 mg/day
Eye health	6- 8 mg/day
Topical use	20- 100 parts per million

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