

AstaZine[®] Natural
Astaxanthin
THE SUPPLEMENT YOU CAN FEEL

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WHITEPAPER



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“The Healthy Ten”

Ten Clinically-Validated Benefits of Natural Astaxanthin

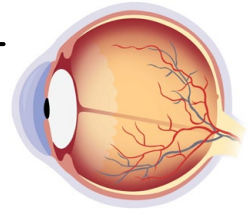


Brain Health

- 10 Human Clinical Studies
- 137 Supporting Pre-Clinical Trials

Eye Health

- 17 Human Clinical Studies
- 26 Supporting Pre-Clinical Trials

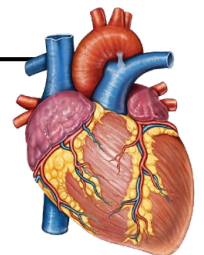


Skin Health & UV Protection

- 13 Human Clinical Studies
- 44 Supporting Pre-Clinical Trials

Cardiovascular Support

- 20 Human Clinical Studies
- 83 Supporting Pre-Clinical Trials

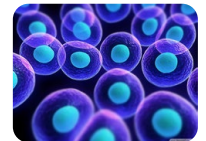


Immune System Modulation

- 5 Human Clinical Studies
- 36 Supporting Pre-Clinical Trials

Anti-Aging & Cellular Health

- 79 Human Clinical Studies
- 343 Supporting Pre-Clinical Trials



Reproductive and Hormonal Health

- 6 Human Clinical Studies
- 33 Supporting Pre-Clinical Trials

Athletic Performance & Energy Levels

- 23 Human Clinical Studies
- 43 Supporting Pre-Clinical Trials

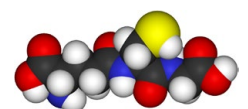


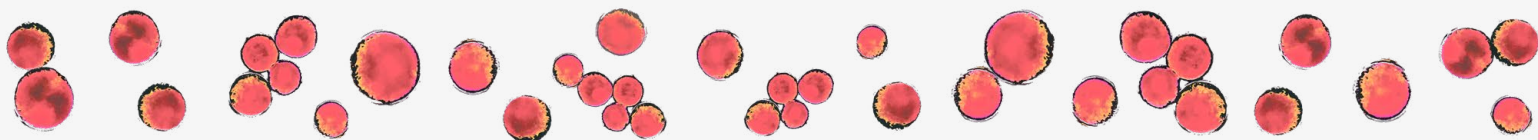
Joint, Tendon and Muscle Support

- 13 Human Clinical Studies
- 95 Supporting Pre-Clinical Trials

World's Strongest & Highest Quality Natural Antioxidant

- 18 Human Clinical Studies
- 125 Supporting Pre-Clinical Trials





Natural Astaxanthin has to be about the best supplement available for word-of-mouth advertising. And the reason for this is that most consumers can feel it working in their bodies. There aren't a lot of supplements that you can feel working. In fact, probably about 95% of supplements are taken on faith—people think that they're doing something beneficial for their health, so they take them. And getting people to continue taking these "can't feel 'em" supplements for the long range is an ongoing battle for supplement brands.

Give your customers a supplement that they'll see and feel working in their bodies and that they'll tell their friends and family about!

Not so with Natural Astaxanthin. When people take this product, after about a month or so they start seeing and feeling the benefits for themselves. Benefits that people claim to feel from Natural Astaxanthin (all of which have been validated in human clinical research) include:

- Less joint, tendon and muscle discomfort and better flexibility
- Better workouts, increased strength and quicker recovery from exercise
- Improved immunity and less colds & flu

- Improved skin quality and appearance
- More energy
- Even improved vision and better functioning brains

How many other supplements can bring even one of these "feelable" benefits to consumers? AstaZine® Natural Astaxanthin is the champ when it comes to "feelability." And this is great for sales. Once people start taking AstaZine®, most of them will become unpaid spokespeople and start doing some free advertising for your brand. They'll tell their moms and dads about how their aches and pains have diminished. They'll tell their brothers and sisters about how much more energetic they feel and how their workouts are much better. They'll tell their friends about how they're able to read better without their glasses. And they'll tell their coworkers about how they're not getting colds like they used to.



#1 Priority When Sourcing Astaxanthin: Shop for Quality!

We've seen some horror stories coming from disreputable suppliers who are calling products "Natural Astaxanthin" that are actually synthetically produced from petrochemicals. Synthetic Astaxanthin is a completely different molecule than its distant natural relative, and it's never been shown to have any of the clinically-validated health benefits that Natural Astaxanthin has. Plus, there are huge differences in bottom-line efficacy between the



two—for example, in head-to-head tests of antioxidant strength, Natural Astaxanthin has consistently performed a minimum of 20 times better than Synthetic! (Capelli, et al, 2013)

And the quality situation isn't only about Synthetic Astaxanthin—we've seen some "Astaxanthin" products on the market that don't even have any Astaxanthin in them! It's become a bit of a "Wild West" in the Astaxanthin business lately. There have even been companies showing up with booths at major trade shows trying to sell Synthetic Astaxanthin as "natural" to unsuspecting customers.

Finally, when talking about Astaxanthin quality it's important to note that there are key differences within the honest suppliers of Natural Astaxanthin that discerning supplement brands should be aware of. For example, make sure you know the water source for the Astaxanthin algae cultures that your producer uses. BGG uses crystal clear water that

originates in the Himalayan Mountains high in the Tibetan plateau. And our proprietary closed culture system uses natural sunlight to yield the optimum conditions—the algae grow in tubes that are protected from the many contaminants that constantly show up in other growing systems—but at the same time the intense natural sunlight raises our Astaxanthin content to the very highest among all producers. In fact, we can yield about double the Astaxanthin content compared to some of the other reputable producers. Plus, it's important to remember that Natural Astaxanthin from algae is a carotenoid cocktail. And we have the highest ratio of pure Astaxanthin to other carotenoids ever attained. The champion ingredient in this natural carotenoid cocktail that's providing the health benefits is Astaxanthin, and we have it in abundance. BGG's AstaZine® is quite simply the purest, most concentrated Natural Astaxanthin product available in the world today.



What Do Consumers Feel When They Take Astaxanthin?

As we mentioned above, more than any other supplement we've encountered, consumers can actually feel a variety of benefits when taking Natural Astaxanthin. One of the most important things to remember though: Astaxanthin doesn't work overnight. This is a natural product and its effects, all of which are clinically-validated, take time to manifest. Some consumers will feel it start to work in 2 weeks or less, but the majority of people won't feel any benefits until 4 to 6 weeks. And there are outliers who may take over 2 months before it really starts to kick in. We'll examine the research later, but for now, just to whet the reader's appetite, here are some of the various, clinically-proven effects of Natural Astaxanthin that consumers say they can see and feel:

- Most consumers say that Natural Astaxanthin decreases joint, tendon and muscle pain and improves flexibility.
- Increased energy levels and improvements in athletic performance are also common results.
- Improvements in eye and brain performance are another frequent testimonial, particularly in areas like preventing eye fatigue and eye strain from overuse of computers.
- Lots of people say that Astaxanthin boosts their immunity and they start getting less colds & flu after they've been using it for a while.
- And of course, many people happily claim that Astaxanthin has improved their skin quality.

Besides these noticeable effects that people can feel, Natural Astaxanthin's extreme antioxidant potential and its modulation of inflammation also yield several other clinically-validated benefits that people don't necessarily "feel." For example, there are benefits for cardiovascular health as well as potential that has been demonstrated in pre-clinical animal trials that Natural Astaxanthin may have anti-cancer effects and benefits for diabetics. It's easy to see why Natural Astaxanthin is a must-take supplement for anyone approaching middle age. Yet, at the same time, people in their 20's and 30's will get immediate results as well as long-term benefits from daily Astaxanthin use—energy, recovery from exercise, strength—all sorts of things that young adults crave. Basically, there's no other supplement on the market that can help so many different people in so many diverse ways (Capelli and Cysewski, 2014).



What is Astaxanthin?

Astaxanthin is a carotenoid. In many different comparative antioxidant studies, it has consistently proven to be the strongest natural antioxidant. In Figure 1, for example, you can see that it was many times more powerful in singlet oxygen quenching than any other antioxidant it was tested against. In



fact, it was 800 times more active than CoQ10 and a whopping 6000 times more active against singlet oxygen than Vitamin C.

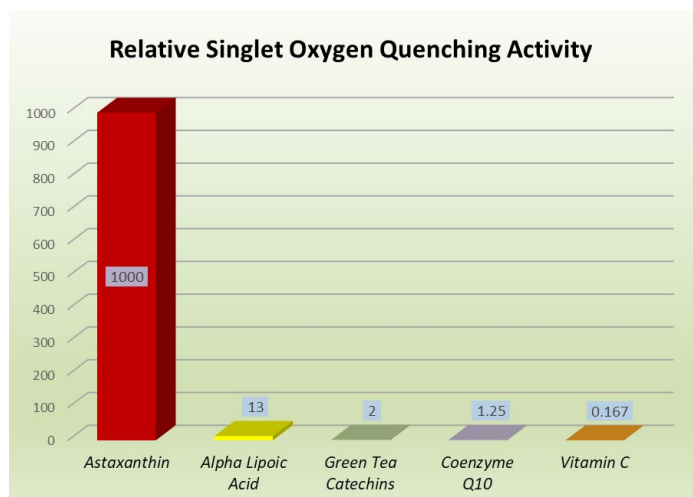


Figure 1. Relative singlet oxygen quenching activity among different nutrients

Astaxanthin is a member of the xanthophyll family within the broader carotenoid classification, and as such it contains not only carbon and hydrogen but also oxygen atoms (Figure 2). In nature, Astaxanthin is found in algae and is what gives salmon, lobster, crab and shrimp as well as flamingoes their red



color. Within the animal world, Astaxanthin is found in the highest concentration in the muscles of salmon—in fact, it's what gives salmon the strength and

endurance to swim up rivers and waterfalls for days on end, the greatest display of stamina in nature.

Chemistry of Astaxanthin

Astaxanthin consists of two terminal rings joined by a polyene chain. This molecule has two asymmetric carbons located at the 3, 3' positions of the β -ionone ring with hydroxyl group (-OH) on either end of the molecule. In one case, the hydroxyl group reacts with a fatty acid and then forms a mono-ester, whereas when both hydroxyl groups react with fatty acids the result is termed a di-ester. Astaxanthin exists in stereoisomers, geometric isomers, free and esterified forms. All of these forms are found in natural sources. The stereoisomers (3S, 3'S) and (3R, 3'R) are the most abundant in nature. *Haematococcus* biosynthesizes the (3S, 3'S)-isomer whereas the yeast *Xanthophyllomyces dendrorhous* (also known as *Phaffia rhodozyma*) produces (3R, 3'R)-isomer. Synthetic Astaxanthin comprises isomers of (3S, 3'S) (3R, 3'S) and (3R, 3'R).

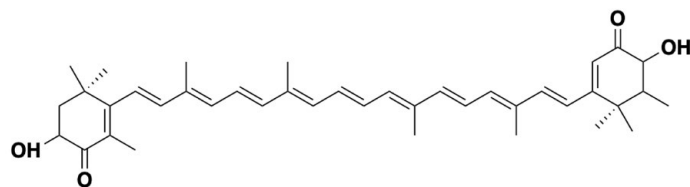


Figure 2. Astaxanthin's Chemical Structure

Astaxanthin has the molecular formula $C_{40}H_{52}O_4$. Its molar mass is 596.84 g/mol (Ambati, et al, 2014).

Astaxanthin contains conjugated double bonds, hydroxyl and keto groups. It has both lipophilic and hydrophilic properties. The red color is due to the conjugated double bonds at the center of the compound. This type of conjugated double bond acts as a strong antioxidant by donating the electrons



and reacting with free radicals to convert them to be a more stable product. It can terminate the free radical chain reaction in a wide variety of living organisms. Astaxanthin shows better biological activity than other antioxidants, because it can link with the cell membrane from inside to outside (Figure 3). It can span the cell membrane and, unlike most other antioxidants, it can have one end in the water-soluble part of the cell and the other end of the molecule in the fat-soluble part of the cell. This unique feature of Astaxanthin allows it to effectively protect the entire cell, making it far superior to other antioxidants.

Health Benefits of Astaxanthin

The benefits of Natural Astaxanthin are so diverse that it seems almost unbelievable. How can one supplement help the body in so many different ways? But if you examine the different benefits more closely, it becomes clear that they all are linked to the two foundational properties Astaxanthin has that separate it from other supplements (including other members of its own carotenoid family):

- Astaxanthin is nature's strongest and highest quality antioxidant
- Astaxanthin safely and naturally modulates the inflammatory response through multiple pathways

The World's Strongest and Highest Quality Natural Antioxidant

We referenced above one antioxidant study that compared Astaxanthin to several other well-known and widely used antioxidants (Figure 1). In that study, Astaxanthin was shown to be:

- 800X stronger than CoQ10
- 550X stronger than Green Tea Catechins
- 75X stronger than Alpha Lipoic Acid
- 6000X stronger than Vitamin C (Nishida, et al, 2007)

This is but one of many studies that have all consistently shown the same end result—Astaxanthin's antioxidant activity is exceptional. Whether comparing with carotenoids from the same family or with other heavily touted antioxidants like Pycnogenol®, Astaxanthin regularly comes up 20 or more times stronger. And it's not only Astaxanthin's strength

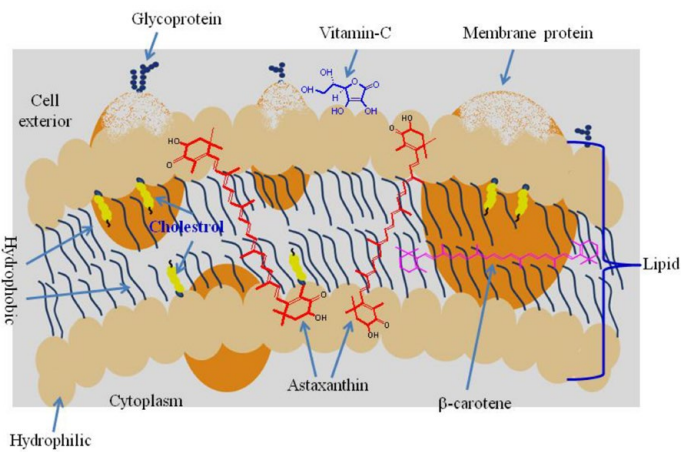


Figure 3. Structure of different chemicals on the cell membrane

Interesting Fact: Another important difference between Astaxanthin and many other antioxidants is that it can never become a pro-oxidant. Many antioxidants, under certain conditions, can change into pro-oxidants and actually start causing additional oxidation in our bodies. This can lead to acceleration of disease formation (heart disease, cancer, and many of the other life-threatening maladies). But Astaxanthin has been tested and found to never turn into a pro-oxidant in two different studies (Beutner, et al, 2001; Martin, et al, 1999).



that makes it such a remarkable antioxidant; it also has unique properties not commonly found in other antioxidants. One that we mentioned above is key: It can span the cell membrane and have one end of the molecule in the water-soluble part of the cell, while the other end of the molecule is in the fat-soluble part of the cell. Other antioxidants like beta carotene and vitamin E can only protect the fat-soluble part of our cells; while water-soluble antioxidants like vitamin C can protect only the water-soluble part of our cells. But Astaxanthin protects the entire cell. And many good antioxidants cannot cross the blood-brain and blood-retinal barriers and bring their antioxidant protection to the brain and eyes. But Astaxanthin can. And as we noted above, Astaxanthin can never become a pro-oxidant. It's very important to remember that Astaxanthin is not a special and unique antioxidant simply because it's so powerful; it also acts very differently than other antioxidants. Remember this: It's the best natural antioxidant both quantitatively and qualitatively (Capelli and Cysewski 2014).

Many human clinical trials have demonstrated Astaxanthin's powerful antioxidant activity. We won't cite all of them here (a comprehensive Abstract List is available from BGG with over 1,000 studies listed), a few of the most recent, interesting studies include:

- Astaxanthin decreased levels of oxidative stress marker malondialdehyde and inflammatory marker interleukin-6 in patients with Type-2 diabetes (Mashhadi, et al, 2021).
- Astaxanthin added to dark chocolate decreased oxidative stress in aging subjects (Petyaev, et al, 2018).
- Astaxanthin improved exercise tolerance, reduced oxidative stress and improved cardiac contractility in heart failure patients (Kato, et al, 2020).

Modulation of the Inflammatory Response

Astaxanthin's ability to modulate the inflammatory response is very different than its profound antioxidant activity. In fact, its modulation of the inflammatory response is much gentler and works on multiple inflammatory pathways (Lee, 2003 and Ohgami, 2003). It takes longer to work than most commonly-used anti-inflammatories you find in a pharmacy.



All of the over-the-counter anti-inflammatories such as aspirin and NSAID's like Tylenol® tend to work much faster than Natural Astaxanthin. And of course, prescription drugs like Celebrex® and Vioxx® also work faster. These drugs work in a very intense manner on only one inflammatory pathway, the Cox-2 enzyme. So consumers get quick relief from pain. But alas, that comes at a huge cost: Safety! All of these quicker anti-inflammatories have side effects: Aspirin is the least serious of the group, but it can cause stomach bleeding and ulcers. NSAID's are one step above aspirin on the safety concern list as prolonged use can result in liver damage. And worst of all are the prescription pain pills like Celebrex® and Vioxx®: They can cause cardiovascular disease. (Vioxx® caused many heart attacks and



was taken off the market, while Celebrex® remains on the market, albeit with a slew of disclaimers.)



On the other hand, Natural Astaxanthin has been shown in many different medical research studies as well as consumer surveys to work just about as well for pain relief as the drugs with side effects we mentioned above, but in over 25 years of consumer use and hundreds of studies, it has never been shown to have any side effect or contraindication (Capelli and Cysewski, 2014). Astaxanthin has been through various safety tests in humans and animals, both chronic toxicity studies and acute toxicity studies, and it has consistently proven to be completely safe. But once again, it won't work as fast as the drugs—it's a natural product which will take about 2 to 6 weeks to start working as it accumulates in our bodies. It can sometimes take even longer to work in some people. (And we find that about 20% of people who use Astaxanthin don't feel any results. This is probably because their bodies are very poor at absorbing carotenoids. The absorption of carotenoids in humans ranges from as little as 5% absorption to over 90% absorption, so a portion of the population will not feel Astaxanthin's results even if they take an upper-limit 12mg - 24mg daily dose.) But ultimately, the big question that consumers have to ask themselves: Do they want to risk same-day pain relief with the possibility of life-threatening side effects, or would they prefer to wait a few weeks for something that works just as well but is safe and natural?

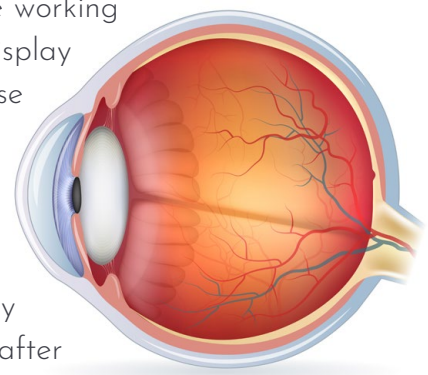
Again, the human clinical trials on Astaxanthin's anti-inflammatory effects are too numerous to men-

tion here, but a few of the most interesting ones are:

- Astaxanthin reduced pain and improved satisfaction with life of patients suffering from incurable rheumatoid arthritis (Nir and Spiller, 2002).
- Astaxanthin decreased muscle inflammation and improved recuperation in elite soccer players (Baralic, et al, 2015).
- In a University of Memphis study, Astaxanthin prevented joint soreness after intense knee exercise (Fry, 2001).
- Astaxanthin reduced inflammation and injury to the vocal fold after vocal loading (Kishimoto, et al, 2017).

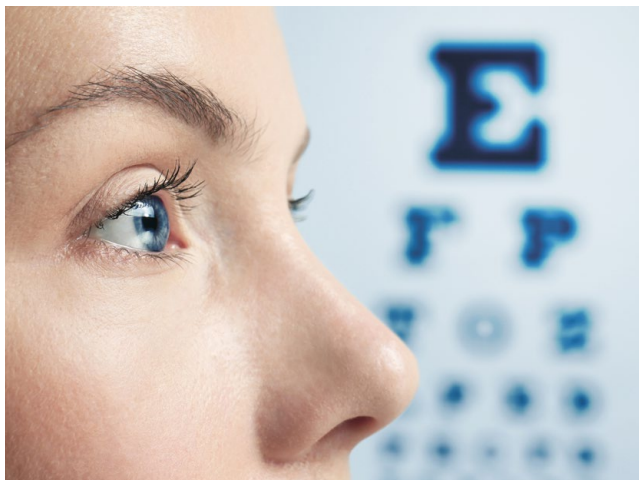
Eye & Brain Health Benefits

Way back in the 1950's, it was demonstrated that Astaxanthin is capable of crossing the blood-brain and blood-retinal barriers to bring its super-antioxidant protection and broad-spectrum anti-inflammatory activity to these vital organs in rodents (Massonet 1957 and Grangaud 1951). This is particularly important since evidence indicates that most diseases associated with the eyes and brain are the result of oxidation and/or inflammation. In today's world, our eyes are subjected to much higher levels of oxidation and overuse than our ancestors. For example, eye fatigue and eye strain are serious problems among people working long hours at visual display terminals. Many of these people complain they are suffering from eye soreness, blurring and diplopia (double vision). In a double-blind study performed in Japan, after





four weeks of supplementation with 5 mg of Astaxanthin per day (extracted from *Haematococcus* algae meal) the authors reported a 46% reduction in the number of eye strain subjects (Nagaki et al., 2002). Free radicals and singlet oxygen oxidize the polyunsaturated fatty acids in the retina which leads to functional impairment of the retinal cell membranes, which in turn, can cause permanent damage to the retinal cells. Once damage occurs in the retinal cells, they cannot be repaired. However, research has found that Astaxanthin can attenuate the apoptosis of retinal ganglion cells in diabetic mice by inhibition of oxidative stress (Dong, et al, 2013). In addition, another group of scientists has investigated the protective effects of Astaxanthin against light-induced retinal damage. The results suggest that Astaxanthin can effectively alleviate the damage via the mechanism of its antioxidative effect (Otsuka et al., 2013). In fact, a pharmaceutical manufacturer is considering adding Astaxanthin into eye drops to protect eyes from UV damage (Lennikov et al., 2011).



Other human research on Natural Astaxanthin's positive effects in eye health has shown that it can improve visual acuity (the ability to see fine

detail) and depth perception. A study on healthy 20-year old men was done in Japan over the course of four weeks. Each subject in the treatment group took 6mg per day of Astaxanthin. At the end of four weeks, there was a significant improvement in both depth perception and acuity. In fact, depth perception improved drastically by over 40% in just four weeks (Sawaki, et al, 2002). And another key factor for good eye health was tested in a separate human clinical trial, also at a dose of 6mg per day. This study showed that Natural Astaxanthin improved retinal capillary blood flow, thus better feeding the eyes with health-giving blood (Yasunori, et al, 2005). Lastly, in an innovative area not previously studied, Astaxanthin was shown to perform as an antioxidant in the eyes during cataract surgery (Hashimoto, et al, 2019).

There were two studies published using BGG's AstaZine® Natural Astaxanthin this year in the area of eye health with excellent results. The first was on AstaZine® as a standalone supplement, and the second was done on AstaZine® in combination with BGG's AbsoLutein™ and Zeaxanthin. Both of these studies focused on eye health in people over-using visual display terminals (VDTs). In today's world, many people spend hours a day on computers and smart phones or in front of televisions. This can cause greatly increased oxidation in the eyes, which leads to myriad issues such as eye fatigue and eye strain, blurry vision, eye soreness, the loss of visual acuity (the ability to see fine details), and in some cases, even double vision or eye dryness. A supplement that can effectively reduce the oxidation in the eye that causes all these conditions in over-users of modern technological devices would be a great boon to hundreds of millions or even billions of people around the world.



AstaZine®

THE WORLD'S PUREST NATURAL ASTAXANTHIN

The study on AstaZine® as a standalone was done on 60 healthy adults. The method was a state-of-the-art clinical trial: placebo-controlled, double-blind, randomized and parallel, and subjects in the treatment group took 9 mg per day of AstaZine® over a six-week period, while the control group took a similar-looking placebo. After six weeks, the AstaZine® group aged over 40 years of age had corrected visual acuity in the dominant eye, which

the researchers proffered as proof of the protective effect of AstaZine® against the increased oxidation caused by overuse of VDTs. "These results suggest Astaxanthin reduces oxidative stress caused by visual display terminal work. Age-related reduction in ciliary muscle strength is likely the main detractor of visual acuity. Correspondingly, Astaxanthin reduced visual display terminal work-induced visual stress in the middle-aged and elderly" (Sekikawa, et al, 2023). Results were published in the Journal of Clinical Biochemistry and Nutrition.

For many years, Lutein and Zeaxanthin have been the carotenoids most associated with eye health. In fact, these two excellent carotenoids, and Lutein in particular, are probably the best known and most



consumed nutrients for people's eyes in the world today. And while there is no doubt that they are outstanding nutrients for the eyes, we feel that Astaxanthin will ultimately be proven to be the best carotenoid of all for eye health due to its super antioxidant profile, both from a quantitative and qualitative perspective. There is currently just more published research on Lutein and Zeaxanthin, but as we see from the preceding pages, Astaxanthin is quickly catching up.

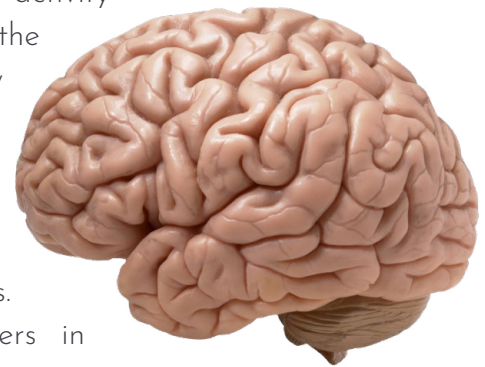
In any event, combining these three together makes for a blockbuster supplement for the eyes. And that is exactly what was done in this final eye health clinical study we'll examine. The object of this study was to test this eye health formula for two symptoms caused by overuse of video display terminals. Many people logging long hours on VDTs experience compromised eye-to-hand coordination. In addition, an aspect of vision that is also sometimes adversely affected by VDT overuse is smooth-pursuit eye movement (a type of eye movement in which the eyes remain fixated on a moving object). The formula in this study given to the treatment group contained 6 mg of AstaZine®, 10 mg of BGG's AbsoLutein®, and 2 mg of Zeaxanthin over a duration of eight weeks. Again, this study was cutting edge: Placebo-controlled, randomized, double-blind with a parallel group.

The results for eye-to-hand movement were excellent—the treatment group had significantly improved eye-to-hand coordination compared to the placebo group. In addition, macular pigment optical density showed a significant increase in the group taking the eye formula. There was not a statistically significant result on smooth-pursuit eye movement. The researchers concluded, "Consumption of a supplement containing Astaxanthin, Lutein

and Zeaxanthin mitigates the decline of eye-hand coordination after VDT operation" (Yoshida, et al, 2023). It's very exciting to see new findings emerge for Astaxanthin and Astaxanthin-based formulas in the area of eye health after so many years of research.

Brain health is an important concern in the aging process. Considerable experimental evidence suggests that brain aging has been implicated in the development of neurodegenerative diseases such as Alzheimer's Disease (AD) and Parkinson's Disease (PD). Astaxanthin has received great attention for its potential ability to help the brain due to its extreme antioxidant activity and its support of the body's inflammatory response. A number of studies have been published showing potential brain health benefits.

University researchers in China conducted an experiment using mice with early brain injury after experimental subarachnoid hemorrhage, and the results showed that Astaxanthin could significantly promote normal brain function (Zhang et al., 2014). Wu et al. also investigated the protective effect and mechanism through administration of Astaxanthin. They found Astaxanthin treatment significantly improved the activities of glutathione peroxidase and superoxide dismutase (SOD), plus it increased glutathione content and total antioxidant capacity, but decreased malondialdehyde, protein carbonylation and 8-hydroxy-2-deoxyguanosine levels in the brains of aging rats. Additionally, Astaxanthin ameliorated histopathological changes in the hippocampus and restored brain-derived neurotrophic





factor levels in both the brains and hippocampus of aging rats. These results suggest that Astaxanthin can promote healthy brain aging, which may be due to attenuating oxidative stress, ameliorating hippocampus damage, and upregulating BDNF expression (Wu et al., 2014).



In humans, Astaxanthin proved to be effective in improving cognitive function in healthy older people. A randomized, double-blind, placebo-controlled study was conducted on 96 subjects. They ingested a capsule containing Astaxanthin-rich *Haematococcus pluvialis* extract or a placebo capsule for 12 weeks. Somatometry, haematology, urine screens, and Cognitive Health and the Groton Maze Learning Test were performed before and after every 4 weeks of administration. Changes in cognitive performance were evaluated. Cognitive health scores improved in people taking 12 mg of Astaxanthin per day after 12 weeks (Katagiri, et al, 2012). Other human research has shown that Natural Astaxanthin can help older people with age-related forgetfulness (Satoh, et al, 2009). And an excellent double-blind, placebo-controlled study in 2011 showed that Astaxanthin supplementation “may contribute to the prevention of dementia in humans as we age” (Nakagawa, et al, 2011).

Lastly, a Japanese study showed that Astaxanthin supplementation helped improve processing and psychomotor speed in patients with mild cognitive impairment (Ito, et al, 2018).

In addition, Astaxanthin has demonstrated other ways it can help with brain health above and beyond cognitive improvement. In a BGG-sponsored study, Astaxanthin demonstrated it can improve mood state in endurance athletes. In eight weeks of supplementation, the Astaxanthin group in this study suffered 57% less feelings of depression while mental fatigue decreased by 36%. Overall, subjects taking Astaxanthin felt their general mental health improved by 11%, all of which were statistically significant results (Talbot, et al, 2017). The mental fatigue element of this study corroborated a study published the previous year. In that study, Astaxanthin was shown to decrease both mental and physical fatigue (Hongo, et al, 2016).

Cardiovascular Health

Heart disease and stroke are the leading causes of death in the world. Every 33 seconds someone in the United States dies from cardiovascular disease; this is roughly the equivalent of a September 11th-like tragedy repeating itself every 24 hours, 365 days a year. Currently, an estimated 80 million Americans have one or more types of heart disease (“Heart Disease: Scope and Impact,” 2014). The situation is more serious in developed countries. As the wealth level increases, people have become less active and more overweight. More and more people are dying from heart disease in the 21st century. A supplement that can help maintain heart health could be beneficial for billions of people around the world. Fortunately, plenty of studies have found that Natural Astaxanthin is effective to minimize the risk of



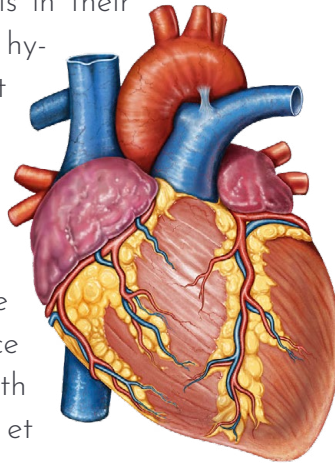
heart issues and promote a healthy cardiovascular system. Astaxanthin's antioxidant power and its ability to modulate the inflammatory response are two major cardiovascular benefits illustrated by many researchers. One group found that ingestion of Natural Astaxanthin each day significantly improved blood flow time and reduced blood pressure in humans (Miyawaki, et al, 2008).

There are different kinds of lipoproteins which exist in our blood vessels: low density lipoprotein (LDL, bad cholesterol) and triglycerides, high density lipoproteins (HDL, good cholesterol) and others. The buildup of bad cholesterol in the blood vessels will lead to a blockage of blood flow and increase blood pressure, which is a potential threat for many kinds of cardiovascular disease. Research

has found Natural Astaxanthin can help support healthy blood lipid levels. Experiments were conducted first on rats, and then a human clinical trial was conducted in Japan. A very promising effect on LDL reduction was found. The researchers found that Astaxanthin inhibits LDL oxidation and can possibly contribute to the prevention of atherosclerosis (Iwamoto et al., 2000). Later, Dr. Hussein confirmed the assumption done by the previous study. He found a significant positive effect between the ingestion of Natural Astaxanthin and reduction of blood pressure (Hussein et al., 2007). In 2010, scientists at Washington State University concluded that Natural Astaxanthin may have benefits for cardiac protection after their rodent study showed that Astaxanthin positively impacted the heart's mitochondrial membrane (Nakao et al.,



2010). Recently, Xu et. al found a combination of flaxseed oil and Astaxanthin can improve oxidative stress, lipid abnormalities and inflammation, providing evidence that this combination could be a promising functional food in cardiovascular health promotion (Xu et al., 2014). Additionally, a human clinical study done in Europe found that at an 8mg per day dosage, subjects supplementing with Astaxanthin had lower levels in their plasma of two different hydroxyl fatty acids (Karppi, et al, 2007). A study related to cardiovascular health also showed Astaxanthin's potential to reduce blood pressure, improve glucose metabolism, and reduce visceral fat in patients with Type-2 diabetes (Mashhadi, et al, 2018). And, in a BGG-sponsored study, in amateur athletes preparing for a half marathon, Astaxanthin decreased heart rate by 10% during long-distance running, allowing the heart to function well without having to work as hard (Talbot, et al, 2017).



Immune System Benefits

Immunity has become a hot topic in the supplement industry and among consumers in the advent of COVID-19 recently. Immune system cells are extremely sensitive to free-radical damage, in part because their cell membranes contain a high percentage of polyunsaturated fatty acids (a common target of free radicals). Antioxidants in general and Astaxanthin in particular, offer important protection against free-radical onslaught to preserve immune system defenses. In the first human study examining Natural Astaxanthin's effect on the immune



response, researchers from Washington State University divided 42 women into one of three groups: placebo, 2mg Astaxanthin, or 8 mg Astaxanthin daily. After eight weeks of supplementation, blood levels of Astaxanthin in both Astaxanthin groups were found to be significantly higher and, not surprisingly, were highest in the subset of women taking the highest amount of Astaxanthin. Importantly, both levels of Astaxanthin supplementation improved the function of natural killer cells (which target and destroy cells that have become infected with viruses). Taking Astaxanthin also raised levels of T and B cells, key functional cells in the immune system. This study also found that Plasma-8OHdG (a biomarker of DNA damage) was much lower in the Astaxanthin groups compared to the placebo group. In addition, C-reactive protein (a marker of inflammation) was also significantly lower in those supplementing with Astaxanthin (Park, et al, 2010). The doctors from Washington State University concluded that Natural Astaxanthin helps to improve the human immune system. They claim that Natural Astaxanthin:

- Stimulates lymphocyte proliferation
- Increases the total number of antibody-producing B-cells
- Produces increased number of T-cells



- Amplifies natural killer cell cytotoxic activity
- Significantly increases delayed-type hypersensitivity response
- Dramatically decreases DNA damage (Park, et al, 2010)

To summarize, these results show that Astaxanthin works through many different pathways to support healthy immune function in humans. Many pre-clinical trials and four other human clinical studies support these findings in humans.

Skin Health: The Internal Beauty Pill

Photo-aging is caused by the super-positioning of chronic ultraviolet (UV) induced damage on the intrinsic aging process and accounts for the majority of age-associated changes in skin appearance. Aged skin, especially photo-aged skin, manifests as a decrease of skin thickness and elasticity, skin dryness, epidermal barrier dysfunction, and changes in

pigmentation. By preventing UV damage, skin can be protected from these conditions. Recent research found that Natural Astaxanthin not only prevents UV damage from occurring, but may actually help to reverse these external signs of aging from the inside out (Yoon et al., 2014). The buzz about Natural Astaxanthin for Internal Beauty has started to catch on: a Hollywood star (Gwyneth Paltrow) and a Supermodel (Heidi Klum) have revealed their beauty secret—both have been using Natural Astaxanthin to enhance their beauty and help their skin (Rawi, Nov. 2011). Due to its strong antioxidant activity, Natural Astaxanthin has the ability to scavenge skin-damaging free radicals. Astaxanthin is widely distributed through most organs in the body, including the skin where it accumulates and makes its way into all skin layers (topical sunscreens can reach only the outermost layers). This can provide potent protection against ultraviolet radiation, the most powerful environmental risk factor for skin cancer (Hawkins, April, 2013).





A landmark clinical trial done in the USA in 2006 reported that Natural Astaxanthin has several different skin benefits. Forty-six middle aged women were divided into two groups, placebo and those taking 4 mg of Natural Astaxanthin per day. Results were examined in a variety of ways such as measurement by dermatological devices; visual assessment by a dermatologist; before and after pictures; and self-assessment. All results showed noticeable benefits. This study claims that, as an internal beauty pill, Natural Astaxanthin:

- Reduces wrinkles
- Improves skin elasticity
- Increases skin moisture levels
- Reduces visible signs of UV-aging within four to six weeks of use
- Maintains a youthful appearance (Yamashita, 2006)

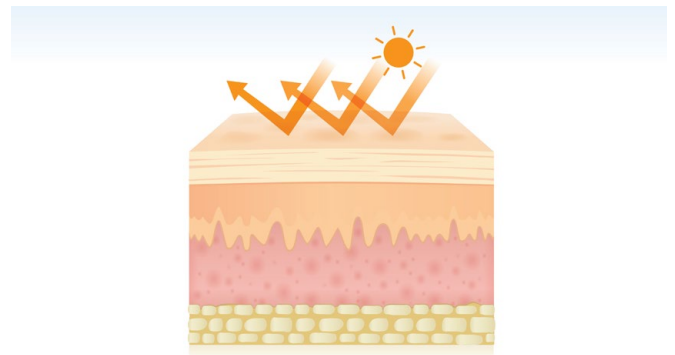
Other human clinical studies have been performed. One was an open-label study involving 30 healthy female subjects for 8 weeks. Significant improvements were observed by combining 6 mg per day oral supplementation and 2 ml (78.9 μ M solution) per day topical application of Natural Astaxanthin. Natural Astaxanthin dual use resulted in improvements in wrinkles (crow's feet), age spot size (cheek), elasticity (crow's feet), skin texture (cheek), moisture content of corneocyte layer (cheek) and corneocyte condition (cheek). These results suggest that Astaxanthin may improve skin condition in all layers such as corneocyte layer, epidermis, basal layer and dermis by combining oral supplementation and topical treatment (Tominaga, et al, 2012).

Some excellent recent human trials corroborate the above. In summary:

- Astaxanthin prevents skin deterioration

due to environmental factors such as UV and dehydration (Tominga, et al, 2017).

- Astaxanthin is effective against UV-induced skin deterioration (Ito, et al, 2018).
- Astaxanthin rejuvenates facial skin and reduces oxidative stress in skin (Chalyk, et al, 2017).



Astaxanthin protects from UV damage in multiple skin layers

Astaxanthin for Athletes and Active People

Besides all the outstanding benefits already listed above for athletes and active people, Natural Astaxanthin can help in other ways. Exerting energy causes a vast increase in free radicals in our bodies, particularly when doing grueling work for long hours or participating in intense sports activities. So taking a strong antioxidant is a tremendous bonus for people participating in difficult work or sports activities to prevent cell damage and to recover better after physical exertion. This is why many athletes are using Natural Astaxanthin—just like salmon that have high concentrations of Astaxanthin in their muscles and get the incredible strength and energy to swim upstream for weeks, athletes and hard-working people can increase their strength and energy levels by supplementing with Astaxanthin each day.



As far back as the 1990's, research was being done in this area. A human clinical trial in Sweden in 1998 took healthy, active young men and gave them either 4mg per day of Natural Astaxanthin or a placebo for six months. At the end of the trial, the strength of the men taking Astaxanthin had increased three times faster than the men taking placebo. This was measured by the amount of deep knee bends they could do at the start and again at the end of the study—after six months the men taking Astaxanthin could do 62% more deep knee bends (Malmsten, 1998).

Even more exciting, a human clinical study on Natural Astaxanthin was sponsored by Gatorade with excellent results. Gatorade is one of the leading companies for consumable sports products in the world, so they're very interested in finding effective

nutrients that can help athletes. They did this study on competitive bicyclists. The study lasted only four weeks, and each athlete took either 4mg of Astaxanthin or a placebo. Astaxanthin made these athletes stronger and gave them more energy in just four weeks, and at a relatively low dose of only 4mg per day. Statistically significant improvements were found in the treatment group's power output and in their time in a 20km time trial. In fact, the cyclists taking Natural Astaxanthin shaved an average of 2 minutes off their time trials which was about a 5% improvement. In addition, their power output increased by a whopping 15% (Earnest, et al, 2011). This is an incredible advantage for a competitive athlete—to be that much stronger that they can improve in a race by 5% in just a month. And of course, this can help anyone who has to work hard or compete in sports on any level.

A related study showed that Astaxanthin helped improve performance, enhanced whole-body fat oxidation rates, and reduced respiratory exchange ratio in recreational cyclists (Brown, et al, 2021).

And it's not just for athletes: Astaxanthin can help everyone:

- Astaxanthin improved exercise tolerance, reduced oxidative stress and improved cardiac contractility in heart failure patients (Kato, et al, 2020).
- Astaxanthin increased physical activity and improved self-assessment of mental and physical quality of life in patients with heart failure (Ishiwata, et al, 2020).
- Astaxanthin improved metabolic adaptation and improved muscle endurance in elderly subjects during aerobic training (Liu, et al, 2021).

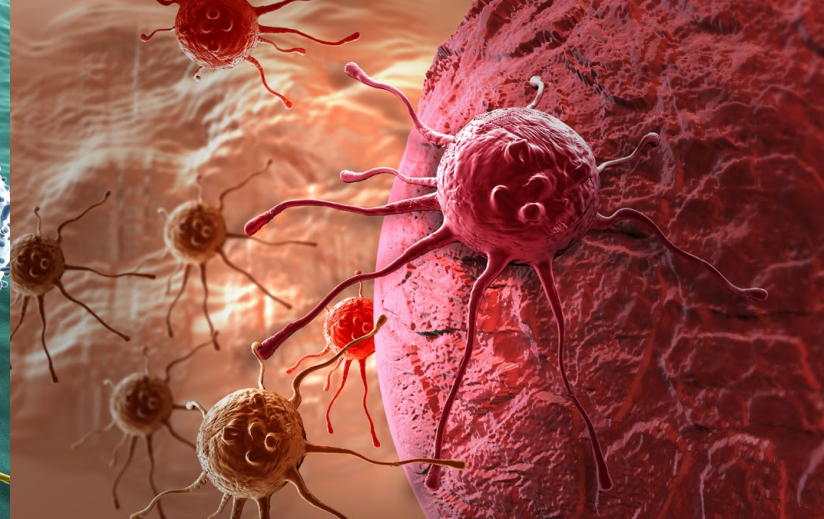
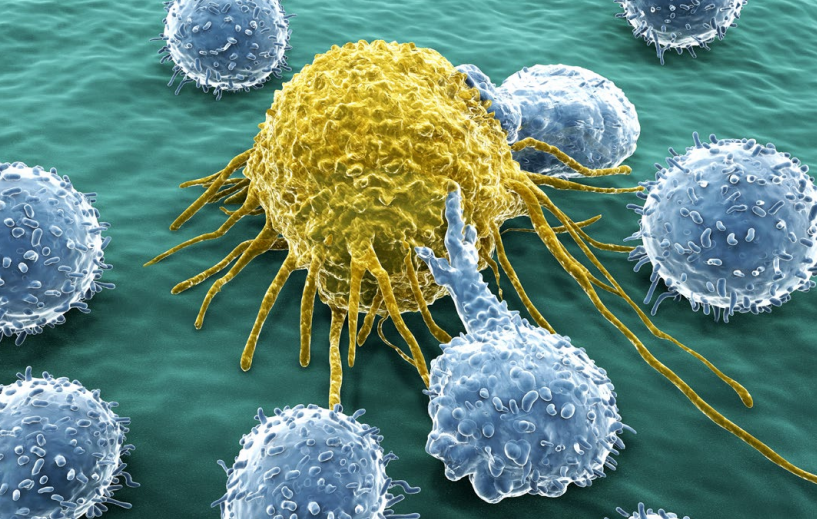
Recent Emerging Research for Diabetics

Research has been emerging recently that shows great promise for diabetics. In a 2021 study, Astaxanthin decreased levels of the oxidative stress marker malondialdehyde and the inflammatory marker interleukin-6 in patients with Type-2 diabetes (Mashhadi, et al, 2021). And a previous study by the same research group showed that Astaxanthin reduced blood pressure, improved glucose metabolism, and reduced visceral fat, again in Type-2 diabetics (Mashhadi, et al, 2018). This research is corroborated by over 30 pre-clinical trials related to diabetes.

Anti-Cancer Activity

We want to make it extremely clear that there has not been any research on cancer prevention or tumor reduction in humans; all of the research has been done on animals, mostly rodents, or through in-vitro experiments. Nevertheless, we





feel it's important to let our readers know about the interesting research in this area.

Early in 1994, Tanaka and co-workers at the Gifu University School of Medicine in Japan, published in the journal *Carcinogenesis* about the possible chemopreventative activity of Astaxanthin against bladder carcinogenesis (Tanaka et al., 1994). The scientists showed that Astaxanthin reduced the incidence of chemically-induced carcinoma by suppression of cell proliferation.

Unlike many pharmaceuticals, Astaxanthin shows beneficial effects for cellular health at each stage of cancer development according to a very promising study (Hawkins, 2013):

- It may prevent cancer initiation by protecting DNA from ultraviolet and oxidative damage.
- It can improve early detection and destruction of cells that have undergone malignant transformation by boosting immune surveillance.
- It has shown an ability to prevent cancerous growth in cells that evade immune detection by reducing inflammatory changes such as those that appear in aging.
- It blocks the rapid cell replication of tumors in their growth phase by stopping the cancer cell reproductive cycle and restoring cancer cells' ability to die off by apoptosis.

- It prevents cell replication from spreading by reducing tumor production of tissue-melting proteins.

Over 60 studies also validate the protective effect of Natural Astaxanthin in cells and demonstrate potential for cancer prevention and tumor reduction. Kavitha et al found that Astaxanthin exerted chemopreventative effects by concurrently inhibiting phosphorylation of transcription factors and signaling kinases and inducing intrinsic apoptosis (Kavitha, et al, 2013). Another group of researchers investigated the effect of Astaxanthin in the treatment of cancer in a rodent model (mice). They found Astaxanthin mono- and di-esters have a better ability to prevent malignant cell growth than Synthetic Astaxanthin or Total Carotenoids. These results suggest that Natural Astaxanthin, which is esterified, has a better bioavailability effect than Synthetic Astaxanthin, and this manifested as a better effect against cancer in rodents (Rao et al., 2013).

Most Recent Trends in Astaxanthin's Medical Research

Several recent clinical trials show new benefits or corroborate earlier findings. Among the most promising studies:

- Self-reported physical activity and quality of life improved in patients supplementing with Astaxanthin for three months (Ishiwa-



ta, et al, 2021).

- Supplementing with Astaxanthin improved metabolism during aerobic training in elderly subjects (Liu, et al, 2021).
- Pre-diabetic, yet healthy subjects showed significant improvements in blood glucose levels and reduction of malondialdehyde-modified LDL cholesterol (Urakaze, et al, 2021).
- Nursing home residents could walk farther and blood lactate levels decreased after 16 weeks of Astaxanthin supplementation (Nakanishi, et al, 2022).
- Astaxanthin has previously shown beneficial effects on male fertility. In a recent study, when treated with Astaxanthin, motility of sperm improved and the number of decondensed sperm decreased in the semen of 30 subjects with normal sperm levels (Dede and Saylan, 2022).
- Obese men who supplemented with Astaxanthin showed decreases in cardiovascular risk factors and adipokine levels (Saeidi, et al, 2023).
- Smokers who supplemented with Astaxanthin had reduced potential disease risks due to Astaxanthin's antioxidative and anti-inflammatory pathways (Pratomo, et al, 2023).

Dosage

The recommended daily dosage of Astaxanthin is 4 - 8 mg per day for normal health maintenance, with a higher dosage of 12 - 24 mg per day when used in therapy for conditions such as infertility or severe joint discomfort. Also, athletes (particularly

those doing high endurance activity) generally report better results at 12 - 24 mg per day. However, carotenoids are absorbed at very different rates by different people. As mentioned above, the absorption rate varies from as low as 5% to over 90%. So some people will have to take a full 12 mg dose or even more if their bodies are only absorbing at a 5% level in order to get the same health benefits as someone whose body absorbs at a 90% level who takes a 4 mg dose per day.



Natural Astaxanthin versus Synthetic Astaxanthin

Natural Astaxanthin accumulates in living microalgae (*H. Pluvialis*) as compared to Synthetic Astaxanthin which is manufactured in a laboratory from petrochemicals or petrochemical derivatives. The #1 concern with Synthetic Astaxanthin is that it has never been directly safety-tested in humans. This compares with Natural Algae Astaxanthin which has a long history of direct use in humans



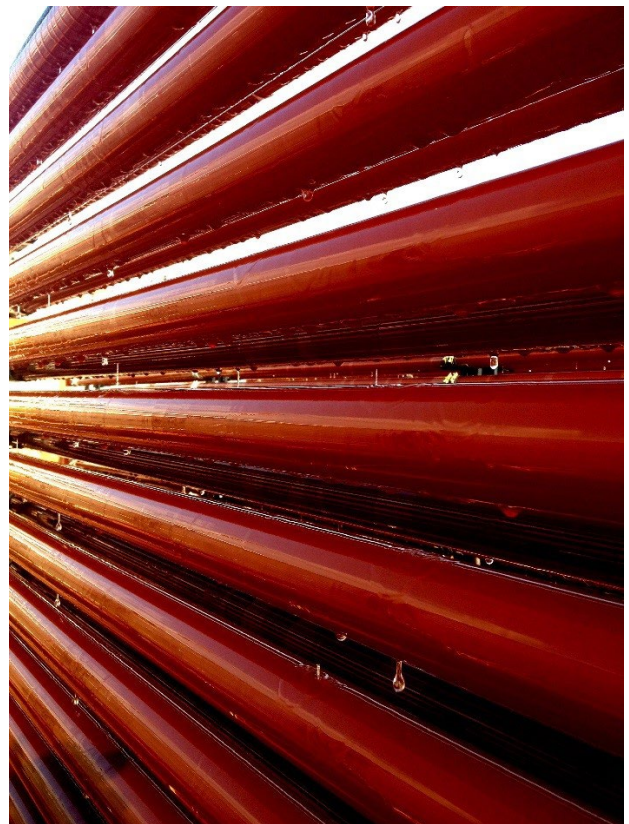
and extensive safety testing including chronic and acute human toxicity studies.

If you've gotten past this first hurdle, and somehow you're still thinking of buying Synthetic Astaxanthin because it's a little cheaper than Natural, please first consider this: Not only has Synthetic Astaxanthin never been safety tested in humans, but it has also never been clinically tested to show ANY health benefit in humans. All of that positive human clinical research we mentioned above on cardiovascular health, eye & brain health, immunity, joint health, and skin health was done exclusively on Natural Astaxanthin.



There may be some readers still thinking "Astaxanthin is Astaxanthin. If they can call Synthetic Astaxanthin 'Astaxanthin,' it must be the same as Natural Astaxanthin." Nothing could be further from the truth. First of all, the Synthetic Astaxanthin molecule is shaped completely differently from the Natural Astaxanthin molecule. Secondly, Natural Astaxanthin is approximately 95% esterified, meaning that it has fatty acids attached to one or both ends of the molecule, while Synthetic Astaxanthin is not esterified. Next, Natural Astaxanthin comes complexed with other, naturally occurring carot-

enoids in the algae as a sort of "carotenoid cocktail." Synthetic Astaxanthin does not share this natural, synergistic formula. And here's the real kicker: When tested side-by-side in different antioxidant surveys, Natural Astaxanthin has consistently proven to be 20 to 90 times stronger than Synthetic Astaxanthin! (Capelli, et al, 2013; Capelli, et al, 2019). So if you wanted the same antioxidant effect, you'd have to take at least 20X higher dose of Synthetic Astaxanthin than Natural. In other words, instead of taking 4 to 8 mg per day, you'd have to take 80 to 160 mg each day. And after all that, you still wouldn't be guaranteed any of the clinically-proven benefits for eye, brain, cardiovascular, immune, joint or skin health that Natural Astaxanthin has displayed in human clinical trials. How they can even call Synthetic Astaxanthin "Astaxanthin" in the marketplace is unbelievable.





Synthetic Astaxanthin Synopsis: Synthetic Astaxanthin is not adequate for human consumption. It is synthesized from petrochemicals and sold into animal-feed markets. Farm-raised salmon, for instance, are fed less-expensive Synthetic Astaxanthin to give their flesh a more attractive reddish hue – otherwise the fish would be a pale gray color, and not attractive to consumers. Synthetic Astaxanthin is not GRAS (Generally Recognized as Safe by the US FDA), and is not accepted in the human food or supplement ingredient categories in any country.

Yeast-Based Astaxanthin Synopsis: Astaxanthin can also be derived from mutated strains of *Phaffia rhodozyma* yeast, however the chemical structure of this form of Astaxanthin is totally different from algae found in nature. *Phaffia rhodozyma* is subjected to UV light, gamma radiation and mutagenic chemicals to produce this mutation. This type of Astaxanthin is sometimes used in trout and salmon feeds, but is not allowed for human consumption in various countries. *Phaffia rhodozyma*, like Synthetic Astaxanthin is a non-esterified form. The United States FDA has serious restrictions on the use of *Phaffia rhodozyma*, limiting the maximum dose per day to 2 mg for adults, and this form is not permitted for use by children. Also, the FDA does not recommend that Astaxanthin from *Phaffia* yeast is used on a daily basis. Meanwhile, the FDA has no restrictions on Natural Astaxanthin from Algae—it's OK for children and fully permitted for daily use.

Chemical Detail: Differences Between Natural Astaxanthin and Other Sources

Astaxanthin has two chiral centers and can exist as three isomers in nature as (3*S*, 3*S'*), (3*R*, and 3'*R*) and (3*R*, 3'*S*). The first, 3*S*, 3*S'*, is the most common in nature. Additionally, the three isomers can exist in

four configurations. An All-E configuration (straight chained) and three Z-isomers (bent chain). The All-E isomer is most prominent in nature. In rainbow trout, the retention of all-E-Astaxanthin was much higher than that of the Astaxanthin Z-isomers.

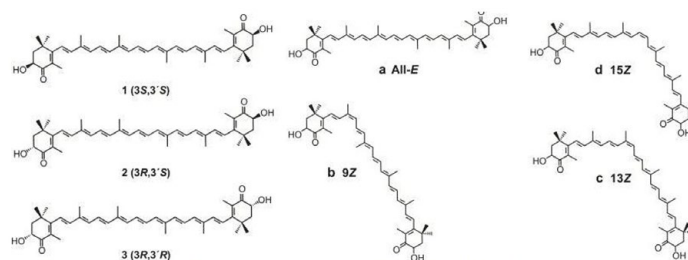


Figure 4. Structure of different Astaxanthin isomers

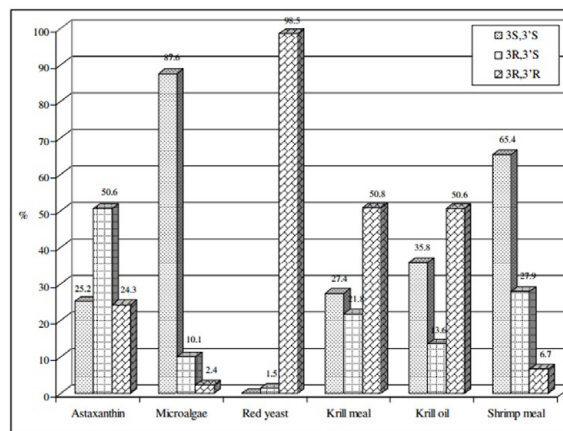


Figure 5. distribution of Astaxanthin isomers from different sources

The above figure clearly shows the distribution of the Astaxanthin isomers in different organisms. Synthetic Astaxanthin is usually present as a mixture with a typical ratio of 1:2:1 (3*S*, 3'*S*: 3*R*, 3'*S*: 3*R*, 3'*R*). *Haematococcus pluvialis* algae meal contains 87.6% of the 3*S*, 3'*S* enantiomer, 10.1% of the meso form and 2.3% of the 3*R*, 3'*R* enantiomer, while the red yeast *Phaffia rhodozyma* contains 1.5% of the meso form and 98.5% of the 3*R*, 3'*R* enantiomer. Of the three, the 3*S*, 3'*S* isomers are the most stable



and this is why academia believes that Natural Astaxanthin from algae is able to be stored in tissues without excessive oxidation. This is the key as to why Natural Astaxanthin has a much higher antioxidant effect than Synthetic Astaxanthin. And this is also probably why Natural Astaxanthin is so effective with so many different health concerns in humans.

The following table shows the Astaxanthin content in each natural source.

SOURCE	AMOUNT OF ASTAXANTHIN (MG PER KG)
<i>Haematococcus pluvialis</i>	10,000-80,000
Krill	46-130
Krill oil	727
<i>Phaffia rhodozyma</i>	30-800
Rainbow trout	1-13
Red seabream	2-14
Salmon (sockeye)	26-37
Salmon (Atlantic)	3-11

Table 1. Astaxanthin content in different natural sources

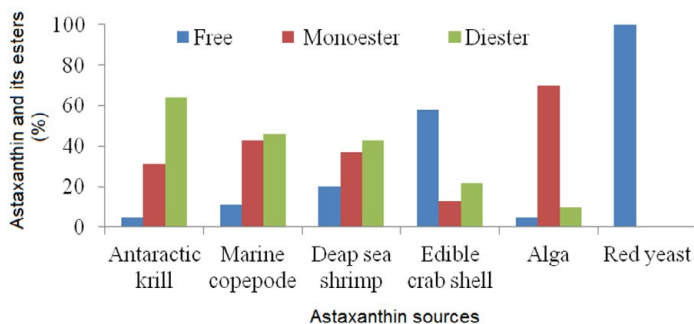


Figure 6. Astaxanthin and its esters from various sources

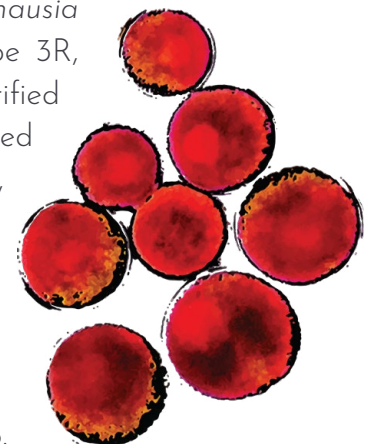
Compared with other sources, *Haematococcus pluvialis* microalgae have been shown to produce the

highest and most sustainable source of Astaxanthin. Due to its high yield, it is possible to collect large amounts of Astaxanthin from the algae biomass. In addition, when microalgae are used as a Natural Astaxanthin production source it becomes more cost-effective than other sources due to a shorter cultivation time and simplified extraction processes. In addition, Astaxanthin from *Haematococcus pluvialis*, resembles the most bioavailable form of Astaxanthin found in nature. A study on the primary stereoisomer of Astaxanthin found

in Antarctic krill (*Euphausia superba*) was found to be 3R, 3'R which is primarily esterified form, whereas in farmed Atlantic salmon it is 3S, 3'S which occurs as the free form. The relative percentage of Astaxanthin and its esters in krill, copepod, shrimp and crab shell is shown in Figure 6.

Natural Astaxanthin from microalgae is usually esterified and predominantly of (3S, 3'S) configuration, occurs as the 3S, 3'S stereoisomer and primarily as monoesters (>90%), with diesters comprising ~8% and the free molecule ~1%.

Logic would assume that these products will perform differently in antioxidant testing because they're inherently different molecules. And while all the human clinical research showing a wide variety of health benefits has been performed exclusively on Natural Astaxanthin from algae, it is unclear how Synthetic Astaxanthin would function in our bodies with extended use.





Production of AstaZine® Natural Astaxanthin

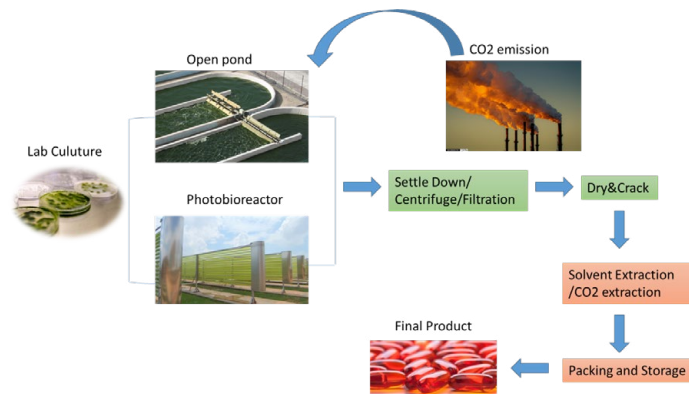
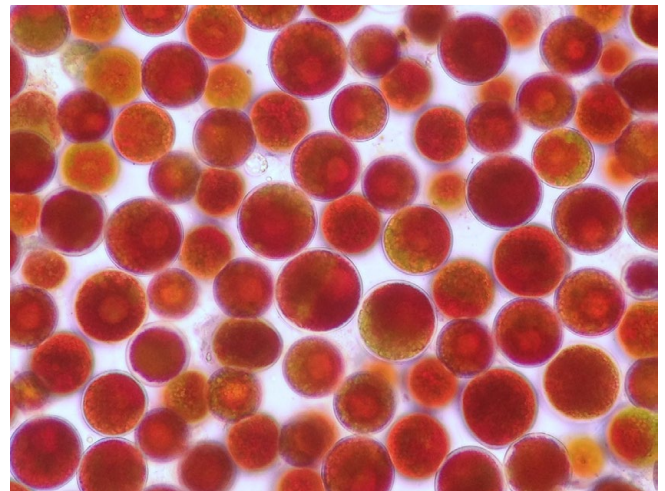


Figure 7. Astaxanthin production from microalgae

Figure 7 describes the production process of Astaxanthin. Generally, a two-stage culture system is adopted as a successful strategy for the commercial production of *H. pluvialis*: green stage for cell growth and induction stage for Astaxanthin accumulation. In the green stage, cell growth is fast, but Astaxanthin content is low. However, in the induction stage, cells grow slowly but can accumulate Astaxanthin quickly. Hence, *H. pluvialis* is cultured to obtain enough cell density in the green stage, and then is induced to increase Astaxanthin content in the induction stage by changing culture conditions (Wan et al., 2014). The initial production of *Haematococcus* usually takes place in closed culture systems, some as large as 40,000 liters, after a series of inoculation and cultivation. Then the microalgae culture is transferred to either open ponds or Photobioreactors for the cultivation and induction.

Some producers grow Astaxanthin with giant open culture ponds, up to 500,000 liters, during the final reddening cycle - *Haematococcus pluvialis* microal-

gae cells gradually turn from green to red as they accumulate Astaxanthin. When the algae are sufficiently infused with Astaxanthin, these companies harvest, wash and then dry the algae. Finally, they extract the lipids from the dried biomass.



Haematococcus pluvialis under a microscope

Other suppliers of Astaxanthin grow *Haematococcus* entirely in closed systems, using biodomes, giant tanks or tubes. Advocates of this process argue that this method results in higher concentrations of Astaxanthin in the biomass, and that there is no exposure to heavy metal contamination or microbiological contamination from birds. But proponents of the open pond system contend that closed-culture systems are risky due to their high surface area and the many nooks and crannies in such systems that make them susceptible to contamination. Unfortunately, open pond systems are plagued by varying temperatures and heavy rainfall, and generally produce a low yield product which has been exposed to a barrage of contaminants such as heavy metals and other pollution as well as bird excrement, and of course, the habitual invaders of open pond systems such as invasive microbes and competing algal species.



BGG's Astaxanthin (AstaZine®) is cultivated in a 100% closed system that is located in a GEO Park (Figure 8) in a sparsely settled area. This region is well known for its mild climate, clean air and abundant, pure water resources. In fact, the water used in our algae cultures is Himalayan Mountain water that originates on the Tibetan plateau. This area has consistent sunlight over 200 days per year, with moderate rainfall and humidity, which remains consistent throughout the year. Daytime temperatures in this area range from 23-28 C (73-84 F) (so it's an ideal location for photo induction), while nighttime temperature remain below 28°C (Wan et al., 2014).



Figure 8. BGG's 100% Glass Tube Photobioreactor Production System

The enclosed system permits a very strict quality control process which ensures the best Astaxanthin yield and lowest contamination. BGG's AstaZine® is the purest, most concentrated Natural Astaxanthin product in the world. In addition, the requirement for temperature control is crucial to the production of *Haematococcus* biomass. This species of algae does not tolerate high temperatures. If left out of control, higher temperatures would kill off the microalgae.

For this reason, a drip system of flowing water is constantly maintained to cool the glass tubes where the algae are grown. BGG's farm for *Haematococcus* cultivation (as shown in the Figure 9) initiated in 2006, and after several expansions, is now by far the largest Natural Astaxanthin production facility in the world.

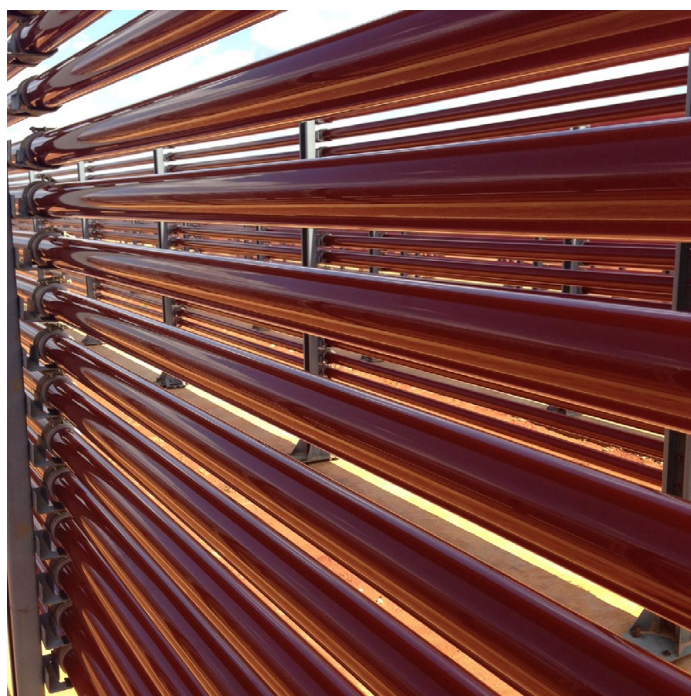


Figure 9. Glass Tubes of Photobioreactor used for BGG Astaxanthin production

BGG employs an advanced Photobioreactor (PBR) system (which facilitates better control of the culture environment), nutrients such as carbon dioxide, a consistent supply of cool, underground water, optimal temperature, efficient exposure to light, culture density, pH levels, gas supply rate, mixing regime, etc. All of the parameters are strictly monitored 24 hours per day during cultivation to ensure that the highest quality, purest Astaxanthin is produced. The photobioreactor has a built-in CIP cleaning system that internally cleans the tubes to prevent contam-



ination. The whole system is highly automated and remotely controlled; with little human labor required in the production and maintenance. This advanced management allows for high consistency and stability for every batch of production. Advantages to BGG's Astaxanthin production are summarized as follows:

- Better protection from outside contamination.
- Cultivation of algae is in controlled conditions, hence greater potential for much higher productivity.
- Large surface-to-volume ratio. PBRs offer maximum efficiency in using light and therefore greatly improve productivity. Typically, the culture density of algae produced is 10 to 20 times greater than bag culture (in which algae culture is done in bags) and can be even greater.
- Better control of gas transfer.
- Reduction in evaporation of growth medium.
- More uniform temperature.
- Space saving - Can be mounted vertically, horizontally or at an angle.
- Reduced Fouling - Recently employed tube self-cleaning mechanisms can dramatically reduce fouling.

All of these benefits and close management by the production Team ensures that the absolute best quality Astaxanthin is produced.

Although *H. pluvialis* is one of the richest sources of Astaxanthin, its massive culture for commercial purposes has been little exploited because of its slow growth rate and complex life cycle (Fujii et al. 2006). Considerable scope therefore exists for



Figure 10. BGG microalgae strain research center

developing more productive strains, which can be done by using simple strain selection or by using more advanced methods (which are proprietary). BGG has developed the top R&D team for microalgae strain development. Great efforts have been employed in selecting the best strains for cultivation in last few years. Currently the best strain can reach over 8% Astaxanthin content in the dried microalgae biomass as compared to some other leading producers which are in the 2 - 3% range. Ours is by far the highest level of Astaxanthin content achieved to date by producers anywhere, which is a key measure of our ultra-advanced technology.



Figure 11. Astaxanthin production plant

Extraction of Astaxanthin can be obtained by solvents. However, since it is impossible to remove all traces of toxic solvents, the EEC directive (88/344) and the Food and Drug Administration have imposed great restrictions in the use of organic solvents, namely hexane, methyl acetate and methylene chloride. Supercritical CO₂ fluid extraction (SFE) is presently an important alternative to the traditional separation methods (Palavra et al, 2011). This SFE extraction technique is especially indicated when thermo-labile compounds are present, in addition to avoiding the use of toxic solvents, since CO₂ is a GRAS solvent type. The major advantage of supercritical fluid extraction over conventional extraction is that this technique does not require subsequent processing steps to separate the solvent, since CO₂ is a gas at normal temperature and pressure. The conventional extraction processes produce extracts that are very dilute, and containing materials that are strongly susceptible to oxidation. So the subsequent steps of separation of these solvents can promote the degradation of the compounds of interest (Sánchez-Camargo, et al, 2011).



References

- Ambati**, R. R., Phang, S. M., Ravi, S., & Aswathanarayana, R. G. (2014). Astaxanthin: Sources, Extraction, Stability, Biological Activities and Its Commercial Applications-A Review. *Marine Drugs*, 12(1), 128-152. doi: 10.3390/md12010128
- Beutner**, S., Frixel, S., Blanco, I., Hoffman, T., Martin, H., Mayer, B., Noack, P., Ruck, C., Schmidt, M., Schulke, I., Sell, S., Ernst, H., Haremza, S., Seybold, G., Sies, H., Stahl, W., Walsh, R. (2001). "Quantitative assessment of antioxidant properties of natural colorants and phytochemicals: carotenoids, flavonoids, phenols and indigoids. The role of beta carotene in antioxidant functions." *Journal of the Science of Food and Agriculture* 81:559-568 (online 2001).
- Brown**, D. R., Warner, A. R., Deb, S. K., Gough, L. A., Sparks, S. A., & McNaughton, L. R. (2021). The effect of astaxanthin supplementation on performance and fat oxidation during a 40 km cycling time trial. *Journal of science and medicine in sport*, 24(1), 92-97. <https://doi.org/10.1016/j.jsams.2020.06.017>
- Capelli**, B., Cysewski, G. (2014). The worlds' best kept health secret: Natural Astaxanthin.
- Capelli**, B., Bagchi, D., Cysewski, G. (Dec 2013). Synthetic Astaxanthin is significantly inferior to algal-based Astaxanthin as an antioxidant and may not be suitable as a human nutritional supplement. *NutraFoods* December 2013. doi: 10.1007/s13749-013-0051-5
- Capelli**, B., Talbott, S., Ding, L. (2019). "Astaxanthin sources: Suitability for human health and nutrition." *Functional Foods in Health and Disease* 2019;9(6):430-445.
- Chalyk**, N. E., Klochkov, V. A., Bandaletova, T. Y., Kyle, N. H., & Petyaev, I. M. (2017). Continuous astaxanthin intake reduces oxidative stress and reverses age-related morphological changes of residual skin surface components in middle-aged volunteers. *Nutrition research (New York, N.Y.)*, 48, 40-48. <https://doi.org/10.1016/j.nutres.2017.10.006>
- Dong**, L. Y., Jin, J., Lu, G., & Kang, X. L. (2013). Astaxanthin Attenuates the Apoptosis of Retinal Ganglion Cells in db/db Mice by Inhibition of Oxidative Stress. *Marine Drugs*, 11(3), 960-974. doi: 10.3390/md11030960
- Earnest**, C., Lupo, M., White, K, Church, T. (2011). "Effect of Astaxanthin on cycling time trial performance." *International Journal of Sports Medicine* 2011;32:882-88.
- Grangaud**, R. (1951). "Research on Astaxanthin, a New Vitamin A Factor." Doctoral Thesis at University of Lyon, France.
- Hashimoto**, H., Arai, K., Takahashi, J., & Chikuda, M. (2019). Effects of astaxanthin on VEGF level and antioxidant in human aqueous humor: difference by sex. *Journal of clinical biochemistry and nutrition*, 65(1), 47-51. <https://doi.org/10.3164/jcfn.18-110>
- Hawkins**, L. (April, 2013). Astaxanthin Provides Broad Spectrum Protection. *Life Extension Magazine*. Heart Disease: Scope and Impact. (2014).



- Hongo, N.** et al. "Randomized controlled trial of the anti-fatigue effects of astaxanthin on mental and physical loads simulating daily life." *Journal of Clinical Therapeutics & Medicines* 32.7 (2016): 277-91.
- Hussein, G., Nakagawa, T., Goto, H., Shimada, Y., Matsumoto, K., Sankawa, U., & Watanabe, H.** (2007). Astaxanthin ameliorates features of metabolic syndrome in SHR/NDmcr-cp. *Life Sciences*, 80(6), 522-529. doi: <http://dx.doi.org/10.1016/j.lfs.2006.09.041>
- Ishiwata, S., Kato, T., Kasai, T., Sato, A., Yatsu, S., Matsumoto, H., Shitara, J., Murata, A., Shimizu, M., Suda, S., Matsue, Y., Naito, R., Hiki, M., & Daida, H.** (2021). Changes in self-reported physical activity and health-related quality of life following 3-month astaxanthin supplementation in patients with heart failure: results from a pilot study. *Annals of palliative medicine*, 10(2), 1396-1403. <https://doi.org/10.21037/apm-20-1378>
- Ito, N., Saito, H., Seki, S., Ueda, F., & Asada, T.** (2018). Effects of Composite Supplement Containing Astaxanthin and Sesamin on Cognitive Functions in People with Mild Cognitive Impairment: A Randomized, Double-Blind, Placebo-Controlled Trial. *Journal of Alzheimer's disease : JAD*, 62(4), 1767-1775. <https://doi.org/10.3233/JAD-170969>
- Ito, N., Seki, S., & Ueda, F.** (2018). The Protective Role of Astaxanthin for UV-Induced Skin Deterioration in Healthy People-A Randomized, Double-Blind, Placebo-Controlled Trial. *Nutrients*, 10(7), 817. <https://doi.org/10.3390/nu10070817>
- Iwamoto, T., Hosoda, K., Hirano, R., Kurata, H., Matsumoto, A., Miki, W., . . . Kondo, K.** (2000). Inhibition of low-density lipoprotein oxidation by Astaxanthin. *J Atheroscler Thromb*, 7(4), 216-222.
- Karppi, J., Rissanen, T., Nyysönen, K., Kaikkonen, J., Olsson, A., Voutilainen, S., Salonen, J.** (2007). "Effects of astaxanthin supplementation on lipid peroxidation." *International Journal for Vitamin Nutrition Research* 77(1):3-11.
- Katagiri, M., Satoh, A., Tsuji, S., & Shirasawa, T.** (2012). Effects of Astaxanthin-rich *Haematococcus pluvialis* extract on cognitive function: a randomised, double-blind, placebo-controlled study. *Journal of Clinical Biochemistry and Nutrition*, 51(2), 102-107. doi: 10.3164/jcbn.D-11-00017
- Kato, T., Kasai, T., Sato, A., Ishiwata, S., Yatsu, S., Matsumoto, H., Shitara, J., Murata, A., Shimizu, M., Suda, S., Hiki, M., Naito, R., & Daida, H.** (2020). Effects of 3-Month Astaxanthin Supplementation on Cardiac Function in Heart Failure Patients with Left Ventricular Systolic Dysfunction-A Pilot Study. *Nutrients*, 12(6), 1896. <https://doi.org/10.3390/nu12061896>
- Kavitha, K., Kowshik, J., Kishore, T. K. K., Baba, A. B., & Nagini, S.** (2013). Astaxanthin inhibits NF-kappa B and Wnt/beta-catenin signaling pathways via inactivation of Erk/MAPK and PI3K/Akt to induce intrinsic apoptosis in a hamster model of oral cancer. *Biochimica Et Biophysica Acta-General Subjects*, 1830(10), 4433-4444. doi: 10.1016/j.bbagen.2013.05.032
- Kishimoto, Y., Kaneko, M., Suzuki, R., Kawai, Y., Tateya, I., & Hirano, S.** (2017). Protective Effect of Astaxanthin on Vocal Fold Injury and Inflammation Due to Vocal Loading: A Clinical Trial. *Journal of voice : official journal of the Voice Foundation*, 31(3), 352-358. <https://doi.org/10.1016/j.jvoice.2016.06.017>
- Lee, S., Bai, S., Lee, K., Namkoong, S., Na, H., Ha, K., Han, J., Yim, S., Chang, K., Kwon, Y., Lee, S., Kim, Y.**



- (2003). "Astaxanthin Inhibits Nitric Oxide Production and Inflammatory Gene Expression by Suppressing I κ B Kinase-dependent NFR-kB Activation." *Molecules and Cells*. 16(1):97- 105.
- Lennikov, A.,** Kitaichi, N., Fukase, R., Murata, M., Noda, K., Ohno, S., . . . Ishida, S. (2011). Amelioration Of Ultraviolet-induced Photokeratitis Treated With Astaxanthin Eye Drops In Mice. *ARVO Annual Meeting Abstract Search and Program Planner, 2011, 2004*.
- Liu, S. Z.,** Valencia, A. P., VanDoren, M. P., Shankland, E. G., Roshanravan, B., Conley, K. E., & Marcinek, D. J. (2021). Astaxanthin supplementation enhances metabolic adaptation with aerobic training in the elderly. *Physiological reports*, 9(11), e14887. <https://doi.org/10.14814/phy2.14887>
- Malmstem, C.** (1998). "Dietary supplementation with Astaxanthin-rich algal meal improves muscle endurance—a double blind study on male students." Karolinska Institute, Gustavsberg, Sweden.
- Martin, H.,** Jager, C., Ruck, C., Schmidt, M. (1999). "Anti- and Pro-Oxidant Properties of Carotenoids." *Journal fur praktische chemie* 1999, 341, No. 3.
- Mashhadi, N. S.,** Zakerkish, M., Mohammadiasl, J., Zarei, M., Mohammadshahi, M., & Haghhighizadeh, M. H. (2018). Astaxanthin improves glucose metabolism and reduces blood pressure in patients with type 2 diabetes mellitus. *Asia Pacific journal of clinical nutrition*, 27(2), 341-346. <https://doi.org/10.6133/apjcn.052017.11>
- Massonet, R.** (1958). "Research on Astaxanthin's Biochemistry." Doctoral Thesis at University of Lyon, France.
- Miyawaki, H.,** Takahashi, J., Tsukahara, H., & Takehara, I. (2008). Effects of Astaxanthin on Human Blood Rheology. *Journal of Clinical Biochemistry and Nutrition*, 43(2), 69-74. doi: 10.3164/jcbrn.2008048
- Nagaki, Y.,** Hayasaka, S., Yamada, T., Hayasaka, Y., Sanada, M., & Uonomi, T. (2002). Effects of Astaxanthin on accommodation, critical flicker fusion, and pattern visual evoked potential in visual display terminal workers. *Journal of traditional medicines*, 19(5), 170-173.
- Nakagawa, K.,** Kiko, T., Miyazawa, T., Carpennero Burdeos, G., Kimura, F., Satoh, A. (2011). "Antioxidant effect of astaxanthin on phospholipid peroxidation in human erythrocytes." *The British Journal of Nutrition*. 105(11):1563-71.
- Nakao, R.,** Nelson, O. L., Park, J. S., Mathison, B. D., Thompson, P. A., & Chew, B. P. (2010). Effect of Astaxanthin supplementation on inflammation and cardiac function in BALB/c mice. *Anticancer Res*, 30(7), 2721-2725.
- Nishida, Y.,** Yamashita, E, and Miki, W. (September 2007). Comparison of Astaxanthin's Singlet Oxygen Quenching Activity with Common Fat and Water-soluble Antioxidants *e21st annual Meeting on Carotenoid Research*.
- Ohgami, K.,** Shiratori, K., Kotake, S., Nishida, T., Mizuki, N., Yazawa, K., Ohno, S. (2003). "Effects of Astaxanthin on lipopolysaccharide-induced inflammation in vitro and in vivo." *Investigative Ophthalmology and Visual Science*. 44(6):2694-701.
- Otsuka, T.,** Shimazawa, M., Nakanishi, T., Ohno, Y., Inoue, Y., Tsuruma, K., . . . Hara, H. (2013). The Protective Effects of a Dietary Carotenoid, Astaxanthin, Against Light-Induced Retinal Damage. *Journal of Pharmaceutical Sciences*, 123(3), 209-218. doi: 10.1254/jphs.13066FP



- Palavra, A. M. F., Coelho, J. P., Barroso, J. G., Rauter, A. P., Fareleira, J. M. N. A., Mainar, A., . . . Novais, J. M.** (2011). Supercritical carbon dioxide extraction of bioactive compounds from microalgae and volatile oils from aromatic plants. *The Journal of Supercritical Fluids*, 60(0), 21-27. doi: <http://dx.doi.org/10.1016/j.supflu.2011.04.017>
- Park, J. S., Chyun, J. H., Kim, Y. K., Line, L. L., & Chew, B. P.** (2010). Astaxanthin decreased oxidative stress and inflammation and enhanced immune response in humans. *Nutr Metab (Lond)*, 7, 18. doi: 10.1186/1743-7075-7-18
- Petyaev, I. M., Klochkov, V. A., Chalyk, N. E., Pristensky, D. V., Chernyshova, M. P., Kyle, N. H., & Bashmakov, Y. K.** (2018). Markers of Hypoxia and Oxidative Stress in Aging Volunteers Ingesting Lycosomal Formulation of Dark Chocolate Containing Astaxanthin. *The journal of nutrition, health & aging*, 22(9), 1092-1098. <https://doi.org/10.1007/s12603-018-1063-z>
- Rao, A. R., Sindhuja, H. N., Dharmesh, S. M., Sankar, K. U., Sarada, R., & Ravishankar, G. A.** (2013). Effective Inhibition of Skin Cancer, Tyrosinase, and Antioxidative Properties by Astaxanthin and Astaxanthin Esters from the Green Alga *Haematococcus pluvialis*. *Journal of Agricultural and Food Chemistry*, 61(16), 3842-3851. doi: 10.1021/jf304609j
- Rawi, M.** (Nov. 2011). Extended life pill: 'Miracle supplement' promises to fight the signs of ageing. *Daily Mail (UK)*.
- Ryu, S. K., King, T. J., Fujioka, K., Pattison, J., Pashkow, F. J., & Tsimikas, S.** (2012). Effect of an oral Astaxanthin prodrug (CDX-085) on lipoprotein levels and progression of atherosclerosis in LDLR^{-/-} and ApoE^(-/-) mice. *Atherosclerosis*, 222(1), 99-105. doi: 10.1016/j.atherosclerosis.2012.02.002
- Sánchez-Camargo, A. P., Martínez-Correa, H. A., Paviani, L. C., & Cabral, F. A.** (2011). Supercritical CO₂ extraction of lipids and Astaxanthin from Brazilian redspotted shrimp waste (*Farfantepenaeus paulensis*). *The Journal of Supercritical Fluids*, 56(2), 164-173. doi: <http://dx.doi.org/10.1016/j.supflu.2010.12.009>
- Satoh, A., Tsuji, S., Okada, Y., Murakami, N., Urami, M., Nakagawa, K., Ishikura, M., Katagiri, M., Koga, Y., Shirasawa, T.** (2009). "Preliminary clinical evaluation of toxicity and efficacy of a new astaxanthin-rich *Haematococcus pluvialis* extract." *Journal of Clinical Biochemistry and Nutrition* 44(3):280-4.
- Sawaki, K., Yoshigi, H., Aoki, K., Koikawa, N., Azumane, A., Kaneko, K., Yamaguchi, M.** (2002). "Sports Performance benefits from taking Natural Astaxanthin characterized by visual acuity and muscle fatigue improvements in humans." *Journal of Clinical Therapeutics & Medicines* 18:(9)73-88.
- Sekikawa, T., Kizawa, Y., Li, Y., Miura, N.** (2023). Effects of diet containing Astaxanthin on visual function in healthy individuals: a randomized, double-blind, placebo-controlled, parallel study." *Journal of Clinical Biochemistry and Nutrition* 2023 Jan;72(1):74-81.
- Shokri-Mashhadi, N., Tahmasebi, M., Mohammadi-Asl, J., Zakerkish, M., & Mohammadshahi, M.** (2021). The antioxidant and anti-inflammatory effects of astaxanthin supplementation on the expression of miR-146a and miR-126 in patients with type 2 diabetes mellitus: A randomised, double-blind, placebo-controlled clinical trial. *International journal of clinical practice*, 75(5), e14022. <https://doi.org/10.1111/ijcp.14022>



- Talbott, S., Hantla, D., Capelli, B., Ding, L., Li, Y., Artaria, C. (2017).** "Effect of Astaxanthin Supplementation on Cardiorespiratory Function in Runners." *EC Nutrition* 11.6 (2017): 253- 259.
- Talbott, S., Hantla, D., Capelli, B., Ding, L., Li, Y., Artaria, C. "Astaxanthin Supplementation Reduces Depression and Fatigue in Healthy Subjects." EC Nutrition 14.3 (2019): 239-246. Sekikawa, T., Kizawa, Y., Li, Y., Takara, T. "Cognitive Function Improvement with Astaxanthin Intake: A Randomized, Double-Blind, Placebo-Controlled Study." *Pharmacometrics* 97 (1/2) 1-13 (2019).**
- Tanaka, T., Morishita, Y., Suzui, M., Kojima, T., Okumura, A., & Mori, H. (1994).** Chemoprevention of mouse urinary bladder carcinogenesis by the naturally occurring carotenoid Astaxanthin. *Carcinogenesis*, 15(1), 15-19.
- Tominaga, K., Hongo, N., Karato, M., & Yamashita, E. (2012).** Cosmetic benefits of Astaxanthin on humans subjects. *Acta Biochim Pol*, 59(1), 43-47.
- Tominaga, K., Hongo, N., Fujishita, M., Takahashi, Y., & Adachi, Y. (2017).** Protective effects of astaxanthin on skin deterioration. *Journal of clinical biochemistry and nutrition*, 61(1), 33-39. <https://doi.org/10.3164/jcfn.17-35>
- Wan, M., Zhang, J., Hou, D., Fan, J., Li, Y., Huang, J., & Wang, J. (2014).** The effect of temperature on cell growth and Astaxanthin accumulation of *Haematococcus pluvialis* during a light-dark cyclic cultivation. *Bioresource Technology*, 167(0), 276-283. doi: <http://dx.doi.org/10.1016/j.biortech.2014.06.030>
- Wu, W. Q., Wang, X., Xiang, Q. S., Meng, X., Peng, Y., Du, N., . . . Liu, X. B. (2014).** Astaxanthin alleviates brain aging in rats by attenuating oxidative stress and increasing BDNF levels. *Food & Function*, 5(1), 158-166. doi: 10.1039/c3fo60400d
- Xu, J. Q., Gao, H., Zhang, L., Chen, C., Yang, W., Deng, Q. C., . . . Huang, F. H. (2014).** A combination of flaxseed oil and Astaxanthin alleviates atherosclerosis risk factors in high fat diet fed rats. *Lipids in Health and Disease*, 13. doi: 10.1186/1476-511x-13-63
- Yamashita, E. (2006).** "The Effects of a Dietary Supplement Containing Astaxanthin on Skin Condition." *Carotenoid Science*, 2006.
- Yasunori, et al. (2005).** "The effect of astaxanthin on retinal capillary blood flow in normal volunteers." *Journal of Clinical and Therapeutic Medicine* 21(5):537-542.
- Yoon, H. S., Cho, H. H., Cho, S., Lee, S. R., Shin, M. H., & Chung, J. H. (2014).** Supplementating with Dietary Astaxanthin Combined with Collagen Hydrolysate Improves Facial Elasticity and Decreases Matrix Metalloproteinase-1 and -12 Expression: A Comparative Study with Placebo. *Journal of Medicinal Food*, 17(7), 810-816. doi: 10.1089/jmf.2013.3060
- Yoshida, K., Sakai, O., Honda, T., Kikuya, T., Takeda, R., Sawabe, A., Inaba, M., Koike, C. (2023).** Effects of Astaxanthin, Lutein and Zeaxanthin on Eye-Hand Coordination and Smooth-Pursuit Eye Movement after Visual Display Terminal Operation in Healthy Subjects: A Randomized, Double-Blind, Placebo-Controlled Intergroup Trial. *Nutrients*. 2023 Mar 17;15(6):1459
- Zhang, X. S., Zhang, X., Zhou, M. L., Zhou, X. M., Li, N., Li, W., . . . Shi, J. X. (2014).** Amelioration of oxidative stress and protection against early brain injury by Astaxanthin after experimental subarachnoid hemorrhage. *Journal of Neurosurgery*, 121(1), 42-54. doi: 10.3171/2014.2.jns13730