The World's Strongest *and Highest Quality* Natural Antioxidant: Astaxanthin

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Introduction

If an antioxidant is powerful but it can't reach the brain and eyes, how beneficial is it? If an antioxidant gets into the brain but can't protect the fat-soluble part of cells, how effective will it be in protecting our body's command center which is comprised approximately 60% of fat? On the other hand, if an antioxidant can reach the brain and eyes, and it can protect the fat-soluble part of cells, how much good can it do if it isn't particularly potent in eliminating damaging free radicals?

For many years, we've heard products being touted as strong antioxidants. Supplements in particular, but also many food and beverage items and even ingredients used in cosmetics put out claims of having antioxidant activity. As a result, consumers have become somewhat numb to companies hyping their products' antioxidant status over the last few decades.

Going back to the questions in the first paragraph above, the answer to each is exactly what any logically thinking person would deduce. To summarize our position on these questions: There is absolutely no doubt that it's very important to consume a strong antioxidant in our diet to help combat the increased levels of oxidation that occur in our bodies in modern life; however, it's equally if not more important to ensure the quality and efficacy of the antioxidant you consume.

Since the 1940's, scientists have known that Astaxanthin is a powerful antioxidant. In the 1990's and since, a multitude of head-to-head antioxidant studies have shown that Astaxanthin is consistently the strongest natural antioxidant yet discovered. Its antioxidant activity in eliminating free radicals and quenching harmful singlet oxygen is usually at least ten times stronger and often hundreds of times stronger than other antioxidants.

But where Astaxanthin truly shines is in its qualitative properties. Astaxanthin has four distinguishing factors that make it the world's highest quality antioxidant, allowing it to far outdistance all the other contenders:

- Astaxanthin can cross the blood-brain and blood-retinal barriers to bring its antioxidant and anti-inflammatory protection to some of our bodies' most vital organs: Our brains and eyes.
- The Astaxanthin molecule's long structure allows it to span the cell membrane and protect both the water-soluble and fat-soluble areas in our cells.
- Astaxanthin never turns into a Pro-Oxidant.
- Lastly, of particular interest to athletes and active people, Astaxanthin can bond with muscle tissue.

While some other antioxidants may share one or two of these properties, we're not aware of any other antioxidant that possesses all four of these vital qualitative properties. Combined with its superior antioxidant strength and its other cell-protective benefits such as broad-spectrum anti-inflammatory action, these qualitative differences have led many scientists and doctors to recommend Natural Astaxanthin as the #1 choice for a preventative dietary supplement. In fact,

the only possible concern in using Astaxanthin exclusively as a supplemental antioxidant is the fact that antioxidants tend to work better in synergy with other antioxidants in our diets. But nature has even taken care of this: Astaxanthin occurs in nature as a "carotenoid complex" of antioxidants in the algae in which it accumulates. When you take a Natural Astaxanthin capsule, you're not getting just Astaxanthin; you're also getting supporting carotenoids lutein, beta-carotene and canthaxanthin which help the Astaxanthin work even better in your body. In fact, you could call algae-based Astaxanthin an "All-in-One Carotenoid Cocktail." We can safely say that if there is only one preventative supplement you take for a longer, healthier life, it should undoubtedly be Natural Astaxanthin!

In this paper we will first examine Astaxanthin's strength in the very best way to analyze antioxidant power: In a series of different comparative studies pitting it against many other highly regarded antioxidants. Next, we'll go into more detail about the four key qualitative differences between Astaxanthin and other antioxidants. Then we'll cite some of the many human clinical trials showing that Natural Astaxanthin is actually reducing oxidation in our bodies. We'll also briefly delve into some of the other facets of cell protection that make Astaxanthin a complete cell protector including its anti-inflammatory effects; its protection of DNA; and its protection of the mitochondria of the cells. Finally, we'll include an important discussion of the vast differences between Natural Astaxanthin from microalgae and Astaxanthin from other sources such as genetically mutated yeast or petrochemicals which is of critical importance for consumers to understand.

The World's Strongest Natural Antioxidant

Most of what we do in our daily life causes oxidation in our bodies. Believe it or not, breathing and digestion cause increases in free radicals, as does the normal functioning of our immune system. When we exert ourselves, huge quantities of singlet oxygen and other harmful free radicals are produced. As a matter of fact, a hard-training athlete's free radical production can be ten times higher than a sedentary person's. The reason behind this is that when we exert ourselves, our bodies burn additional fuel for energy, and during this process an onslaught of singlet oxygen are produced and released throughout the body (Witt, et al, 1992; Dekkers, 1996; Goldfarb, 1999). The levels of free radicals produced by athletes in particular clearly calls for supplementation with a strong antioxidant. Interestingly, athletes who take strong antioxidant supplements like Astaxanthin often say that they can actually feel them working in their bodies; they commonly report longer workouts with faster recoveries that result in improved performance.

The human body is designed to accommodate normal free radical production in two different ways: First of all, we naturally produce antioxidants such as superoxide dismutase in our bodies. This is part of nature's balancing act: Our bodies naturally produce free radicals; but at the same time, our bodies naturally produce antioxidants to eliminate the free radicals before they can do any harm. The second way our bodies combat oxidation is through assimilating antioxidants from our diets and dispersing them throughout our bodies. When we eat an orange for example, we are ingesting antioxidants like Vitamin C and citrus bioflavonoids. And when we eat other colorful vegetables, we are often ingesting even more powerful antioxidants from the same carotenoid family as Astaxanthin. So between self-production of antioxidants by our bodies and antioxidants ingested by our bodies through our diets, you would assume that we are pretty well protected from normal levels of oxidation that occur in the course of daily life from bodily processes and reasonable levels of exertion. But you would be wrong. There are two serious problems that have surfaced in modern life that have rendered nature's antioxidant balance out-of-whack: First of all, the proliferation of packaged foods, modern farming techniques and long-range transportation of produce have led to severe decreases in antioxidants and other fragile phytonutrients in the food we consume. Commercial, non-organic farming depletes the soil of nutrients resulting in less antioxidants, enzymes and nutrients in our diets. Frankly, a fruit or vegetable that is raised on a commercial farm with pesticides and herbicides and then chemically treated to control ripening and maintain appearance, after which it is transported a few thousand miles from where it was grown and put in a store's produce section simply isn't going to be chock full of antioxidants by the time the consumer eats it. Secondly, our world has changed dramatically since our grandparents were young, resulting in unprecedented levels of oxidation occurring in our bodies. There are many new causes of free radicals in modern life including:

- Air and water pollution
- Chemicals in consumer items that we use on a daily basis
- Preservatives, pesticides and additives in our food supply
- Increased levels of stress in modern life
- Increased UV exposure due to the diminishing ozone layer

All of these lead to oxidation levels in our bodies that self-produced and dietary antioxidants simply cannot handle (Harman 1981; Esterbauer et al, 1992; Ames and Shigenaga 1992; Ames, et al, 1993). The result of all of this increased oxidation in our bodies in modern life is higher levels of cancer and heart disease and the numerous other diseases associated with oxidation.

We have arrived at an oxidation imbalance in today's world that necessitates not only eating a healthy diet full of fruits and vegetables (preferably locally-grown, organic fruits and vegetables), but also requires supplementing with a strong, effective antioxidant for maximum protection. And as you'll see as you read this paper, as an antioxidant Natural Astaxanthin has no equal. We recommend taking at least 4mg per day of Natural Astaxanthin as a preventative health measure to ward off oxidative imbalance and the various diseases it can cause which should help us live a long, healthy life.

Astaxanthin is the strongest natural antioxidant that science has found to date. We stress "natural" because there are some synthetic antioxidants that would probably have similar free-radical quenching activity to Astaxanthin. One that comes to mind is ethoxyquin, a synthetic preservative antioxidant used in animal feeds. But as with most synthetically-produced substances, there is a serious downside to ethoxyquin. According to a study done at Nagoya City University Medical School in Japan, ethoxyquin leads to stomach hyperplasia and cytotoxicity. It has also been implicated as a source of cancer of the kidneys and bladder, and can increase the incidence of stomach tumors (Hirose, et al, 1986). So while this synthetic antioxidant is permitted in the USA in food designed for pets and farm animals, we wouldn't recommend giving it to any animals (let alone humans) due to its potential for serious side effects.

Astaxanthin has been tested head-to-head in many experiments on antioxidant strength against several other carotenoids and antioxidants; it has consistently come out as the very strongest of all natural antioxidants in these tests regardless of the type of test. For example, whether examining free radical elimination or singlet oxygen quenching, Astaxanthin's power as an antioxidant comes out far beyond the capacity of other antioxidants. This is really amazing when you think about it, since many of the antioxidants Astaxanthin has been tested against are closely related molecules in the carotenoid family. Yet Astaxanthin usually comes out superior by at least a power of ten. And when compared with vitamin antioxidants such as Vitamin C and Vitamin E, Astaxanthin has been shown to be as high as 550X to 6000X stronger!

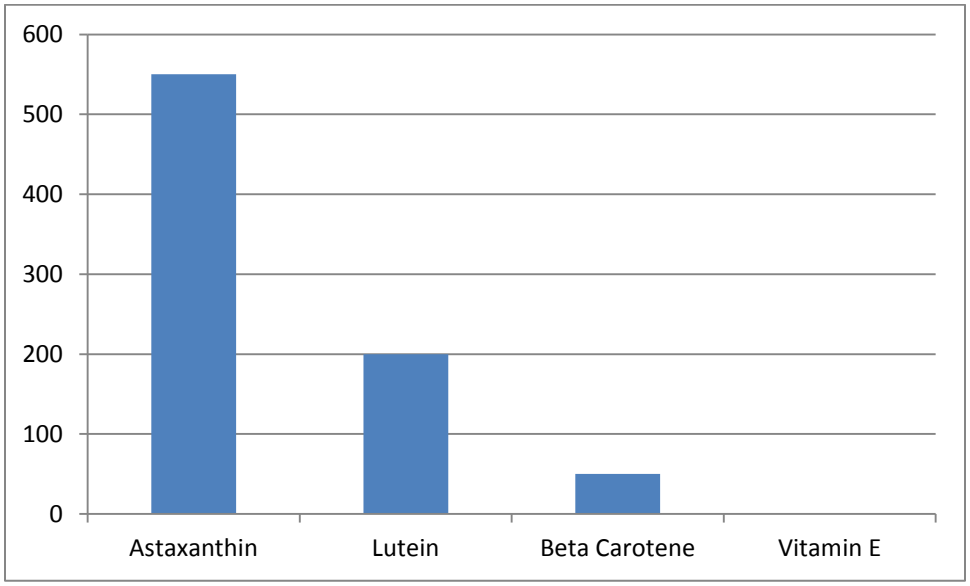
As far back as the 1940's, scientists had discovered the antioxidant abilities of carotenoids and had isolated Astaxanthin as being extremely potent. Research in France in 1946 found that Astaxanthin and beta-carotene were both powerful antioxidants, with Astaxanthin being the stronger of the two (Herisset, A., 1946). By the 1990's, Astaxanthin's powerful antioxidant activity was becoming widely accepted. A paper published in Japan in 1991 set the platform for the flurry of research that would follow:

> "Astaxanthin, one of the dominant carotenoids in marine animals, showed both a strong quenching effect against singlet oxygen and a strong scavenging effect against free radicals. These effects are considered to be defense mechanisms in the animals for attacking these active oxygen species. The activities of Astaxanthin are approximately

> 10 times stronger than those of other carotenoids that were tested, namely zeaxanthin, lutein, tunaxanthin, canthaxanthin and beta-carotene, and 100 times greater than a-tocopherol. Astaxanthin also showed strong activity as an inhibitor of lipid peroxidation mediated by these active forms of oxygen. From these results, Astaxanthin has the properties of a 'Super Vitamin E'" (Miki, et al, 1991).

Dr. Miki must have been extremely impressed to call Astaxanthin a "Super Vitamin E." During that period in the early 1990's, Vitamin E was considered by many to be the most beneficial nutrient for both topical application and internal consumption. However, in finding that Astaxanthin was 10 times stronger as an antioxidant than its carotenoid cousins and 100 times stronger than Vitamin E, he must have felt that it deserved such a venerable title.

Many other experiments have been done since Dr. Miki's, all with the same results—Astaxanthin remains the most powerful natural antioxidant found to date. The first study we'll examine was also done in the 1990's and also in Japan. This study focused on singlet oxygen quenching. It pitted Astaxanthin against several other antioxidants including carotenoids such as lutein and beta carotene, and it also tested Astaxanthin against Vitamin E. The results were heavily favored toward Astaxanthin; lutein got within the same realm as Astaxanthin in this particular test, but beta carotene and particularly Vitamin E were far weaker than Astaxanthin.



Singlet Oxygen Elimination (Shimidzu, Goto, Miki, 1996)

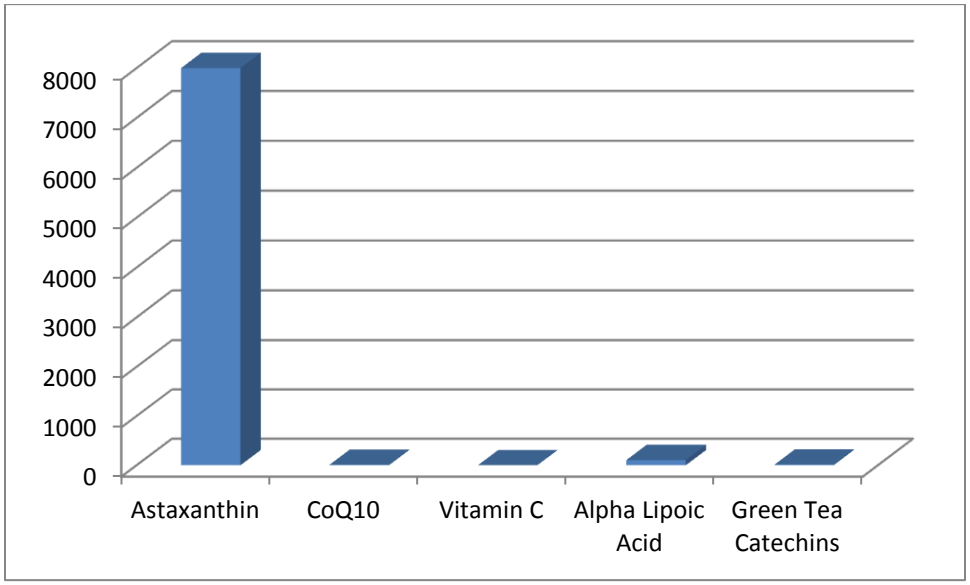
In singlet oxygen elimination, results of this study found Astaxanthin to be:

- 550 times stronger than Vitamin E
- 11 times stronger than beta-carotene
- 2.75 times stronger than lutein (Shimidzu, et al, 1996)

After this study was published, a few early pioneering companies began producing Natural Astaxanthin commercially since the results were so striking. As we mentioned earlier, Vitamin E was widely regarded as one of the very best supplements to take and topical antioxidants to apply in the 1990's. Of course, when an antioxidant was shown in a published study to be 550 times stronger than the preferred antioxidant of that time, it really turned heads.

One of the authors of this study was Dr. Miki, the original researcher who did the oft-times quoted study from 1991 showing Astaxanthin to be phenomenally stronger than other antioxidants and calling it a "Super Vitamin E." As a great fan of Astaxanthin, Dr. Miki participated in another study of Astaxanthin's strength against singlet oxygen many years later in 2007. This time they pitted Astaxanthin against a completely different set of antioxidants. The antioxidants evaluated in this study were Coenzyme Q10, green tea catechins, alpha lipoic acid and Vitamin C. The main difference between this study and Dr. Miki's earlier work is that the results were even more slanted in Astaxanthin's favor.

Many people consider CoQ10 an excellent antioxidant. And among vitamins, Vitamin C is also fairly highly regarded as an antioxidant. Yet when tested against Astaxanthin for their ability to eliminate singlet oxygen, Astaxanthin wasn't just superior—it was phenomenally more potent.



Singlet Oxygen Quenching (Nishida, Yamashita, Miki, 2007)

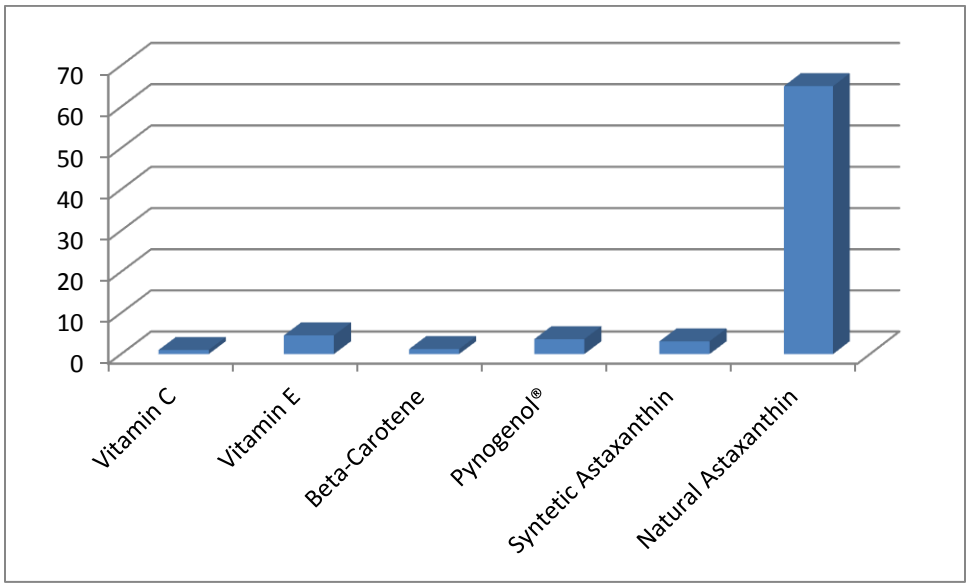
As you can see from the chart above, none of the other antioxidants was even remotely close to Astaxanthin's capacity to eliminate harmful singlet oxygen. The closest of the four was alpha lipoic acid, yet Astaxanthin was still 75 times more potent. Results showed that Astaxanthin is:

- 8000 times more potent than Vitamin C
- 800 times more potent than CoQ10
- 550 times more potent than Green Tea Catechins
- 75 times more potent than Alpha Lipoic Acid (Nishida, et al, 2007)

Another particularly interesting comparative study was done at Creighton University by a leading antioxidant and nutritional supplement researcher, Debasis Bagchi, PhD. Professor Bagchi is very well respected in his field with almost 300 publications including several books and hundreds of peer-reviewed studies. Incredibly, Dr. Bagchi's work has been cited by his colleagues over 12,000 times!

When comparing antioxidants, it is very important to analyze them head-to-head and to test them in different experiments. A single test of, for example, Astaxanthin versus Vitamin E as a singlet oxygen eliminator is not a comprehensive view of the two different molecules' antioxidant capacity. Singlet oxygen are without a doubt extremely harmful to our cells over time, but they are just one of many different types of oxidants that wreak havoc in our bodies. The research in the 1990's focused primarily on Astaxanthin as a singlet oxygen eliminator, so Dr. Bagchi decided to look at Astaxanthin from a different angle: He tested Astaxanthin head-to-head against other well-known antioxidants by measuring their ability to scavenge free radicals in a very well designed experiment.

While this research was originally done in 2001, Dr. Bagchi and his co-author Dr. Gerald Cysewski had great foresight and decided to test both Natural Astaxanthin and Synthetic Astaxanthin in this study even though Synthetic Astaxanthin was not available at the time as a human nutritional supplement. They pitted the natural and synthetic versions of Astaxanthin against Vitamin E, Vitamin C, beta-carotene, and they also included the trademarked supplement Pycnogenol® in the mix as it was claiming to be an extremely powerful antioxidant in its marketing literature. Although this was a completely different way to measure antioxidant strength from the earlier Miki studies, and this set of antioxidants included two completely new molecules—Synthetic Astaxanthin and Pycnogenol®—Natural Astaxanthin again came out the undisputed champion with antioxidant strength ranging from 14X greater than Vitamin E to 65X greater than Vitamin C.



Free Radical Elimination (Capelli, Bagchi, Cysewski, 2013)

The results showed that, in free radical quenching, Natural Astaxanthin is:

- 14X stronger than Vitamin E
- 18X stronger than Pycnogenol®
- 21X stronger than Synthetic Astaxanthin
- 54X stronger than beta-carotene
- 65X stronger than Vitamin C (Capelli, et al, 2013)

This university-based research led by one of the world's leading experts in the antioxidant field did three very important things:

- ✓ It proved the consistency of Astaxanthin's superior antioxidant strength regardless of how it is analyzed.
- ✓ It quantitatively proved that Astaxanthin is much stronger than other antioxidants that were claiming to be extremely powerful at that time such as Pycnogenol®.
- ✓ It showed how incredibly different *Natural* Astaxanthin is from *Synthetic* Astaxanthin.

We see by the relationship between Astaxanthin and Vitamin E in the studies cited above how important it is to use more than one method of measuring antioxidant strength. In the singlet oxygen experiments in the 1990's, Astaxanthin was proven to be 550X stronger than Vitamin E. Yet, when Dr. Bagchi tested the two as free radical scavengers in 2001, Astaxanthin was shown to be 14X stronger. While 14X is still quite impressive, it is a far cry from 550X. So the question comes up as to which number is accurate? The answer is that both of these numbers are accurate, and Astaxanthin is 14 times better than Vitamin E in eliminating free radicals and 550 times better than Vitamin E in specifically eliminating singlet oxygen. It would be impossible to accurately give an exact number when comparing the two in "antioxidant strength," but if we had to, Dr. Miki's original estimate of 100X back in 1991would probably be just about right. Which may be why Dr. Miki simplified things and started calling Astaxanthin a "Super Vitamin E."

There have been dozens of other publications validating Astaxanthin's antioxidant activity, ranging from comparative in-vitro work to animal trials on a variety of health conditions to full-on placebo-controlled human clinical trials. While a serious review of all of these studies would be far beyond the scope of this paper, we will review some of the human studies in a later chapter and we'll briefly discuss some of the most interesting of the comparatives studies below. (For discussion of the trials on various health conditions for which Astaxanthin has shown potential benefits, please refer to our series of white papers on each specific health condition by contacting the authors at support@bggworld.com.) The first three studies cited below are of particular interest because they once again tested Natural Astaxanthin against Synthetic Astaxanthin and show tremendous differences:

- Natural Astaxanthin is superior to Synthetic Astaxanthin in prolonging the life of investigational worms by reducing reactive oxygen species more effectively (Liu, et al, 2016).
- Natural Astaxanthin's intracellular antioxidant activity is approximately 90X stronger than Synthetic Astaxanthin's (Regnier, et al, 2015).

- Astaxanthin from *H. pluvialis* microalgae is superior to Synthetic Astaxanthin as an antioxidant and Natural Astaxanthin shows stronger protective properties in the livers of rats (Rao, et al, 2013).
- Astaxanthin is more effective than other carotenoids as a neuroprotectant in rats due to its superior reactive oxygen species scavenging activities (Chang, et al, 2013).
- Astaxanthin is stronger as an antioxidant than lutein and beta-carotene (Sangeetha and Baskaran, 2010).
- Astaxanthin is more effective than other carotenoids as an antioxidant with higher electron transfer activity (Han, et al, 2009).
- Astaxanthin is the most potent antioxidant amongst several carotenoids tested (Naguib, Y., 2000).
- Astaxanthin is more stable than zeaxanthin, canthaxanthin and beta-carotene during lipid peroxidation (Jorgensen and Skibsted, 1993).
- Astaxanthin is a potent antioxidant in a membrane model, much more so than beta-carotene (Palozza and Krinksky, 1992).
- Astaxanthin is a more effective antioxidant than beta-carotene through its stabilization of trapped radicals (Terao, J., 1989).

The World's *Highest Quality* Antioxidant

Astaxanthin is not only an incredibly powerful antioxidant, it is also a unique antioxidant in terms of how it works in our bodies. There are four distinct ways we can see its superior qualitative properties. While each of these independently would be a critical differentiator from other antioxidants in terms of health value and efficacy, the four of these taken together form a critical mass of evidence of Astaxanthin's superior qualitative antioxidant properties. Each of these on its own is extremely impressive, and while hard to pick the most important or least, below we list these qualitative differences in the order of their relative importance in our opinion:

1. **Spans the cell membrane to protect the entire cell**: A general rule of antioxidants is: "Lipid soluble antioxidants protect the lipid (oil) soluble part of our cells, and water soluble antioxidants protect the water soluble part of our cells." So when we ingest Vitamin C which is water soluble, its antioxidant properties are useful in one part of our cells, and when we ingest Vitamin E which is oil soluble, its antioxidant properties are useful in the remaining part of our cells. The length and shape of the Astaxanthin molecule allow it to span the cell membrane and have one end of the molecule in the lipid soluble part of the cell and the other end of the molecule in the water soluble part of the cell. This gives Astaxanthin the distinctive characteristic of being able to protect the entire cell. And Astaxanthin has been found capable of traveling throughout the entire body, into the bloodstream, muscle tissue, skin, as well as various critical organs (Capelli and Cysewski, 2014). This double feature of being able to get throughout the body and being able to protect the entire cell makes Astaxanthin a super-effective antioxidant and anti-inflammatory for humans.

2. **Never a Pro-Oxidant**: A lot of very good antioxidants can, under certain conditions, turn into oxidants and start harming our cells. This is what happened in the famous "Finnish Smokers Study" on beta-carotene published in the prestigious "New England Journal of Medicine" in 1994. This study tested consumption of synthetic beta-carotene, which (like Synthetic Astaxanthin) is completely different from the natural form. Heavy smokers (who were smoking on average three packs of cigarettes each day) were supplemented with synthetic beta-carotene and found after time to have a slightly higher (although statistically insignificant) incidence of cancer. This was amazing to all involved since dozens of epidemiological studies as well as pre-clinical research had previously shown that natural beta-carotene has cancer-preventative properties (Moorhead, et al, 2005). What was happening was that the beta-carotene was turning into a pro-oxidant in the smokers' bodies because smoking depleted their Vitamin C levels. In the absence of Vitamin C, the beta-carotene molecules had no supporting antioxidants to pass off the supercharged free radicals caused by smoking, so the beta-carotene molecules "changed teams" and became oxidants. This caused additional cellular damage, which in turn increased the incidence of cancer (Heinonen and Albanes, 1994). "Without Vitamin C, beta-carotene can catch the destructive energy of a free radical and itself become a damaging molecule. In this situation, beta-carotene has

entered a 'pro-oxidant' state. If Vitamin C is available this pro-oxidant state will quickly be converted back to an antioxidant state without damage to cells" (Malila, et al, 2006).

Many other excellent antioxidants besides beta-carotene can become pro-oxidants under certain conditions. For example, well-known vitamin antioxidants such as Vitamins C & E, zinc, and even carotenoid antioxidants such as lycopene and zeaxanthin can all become pro-oxidants (Martin, et al, 1999). Fortunately, Astaxanthin can never become a pro-oxidant and cause damage to our cells (Beutner, et al, 2000).

3. **Crosses the blood-brain barrier and blood-retinal barrier**: A lot of very good antioxidants cannot help protect our eyes and brains. Even carotenoid antioxidants that are closely related to Astaxanthin such as beta-carotene and lycopene cannot get through these barriers that are present to protect our most vital organs from foreign matter and contaminants. Since our brains are the control center for everything we think and do, an antioxidant that cannot protect the brain seems to be of little value to us. Fortunately, Astaxanthin can get through the blood-brain barrier to protect our brains. When it reaches our brains, it can then travel through the blood-retinal barrier to help protect our eyes. Some of the earliest research on Astaxanthin back in the 1940's and 1950's showed Astaxanthin's ability to get into the brains and eyes of rats (Grangaud, 1951; Massonet, 1958); meanwhile, many human clinical studies have been completed over the last several years to confirm Astaxanthin's diverse health benefits for the eyes and brain (Capelli and Cysewski, 2014). And once present in the eyes and brain, it is not only Astaxanthin's antioxidant activity that is working prophylactically, but also its broad spectrum anti-inflammatory properties are providing additional protection to these vital organs. This one-two punch against oxidation and inflammation is exactly what brains and eyes need to stay healthy and function well.

4. **Bonds with muscle tissue**: Astaxanthin can get throughout the entire body and into all the critical organs. It can also bond with muscle tissue to protect muscles from increased levels of oxidation and inflammation and keep the muscles functioning smoothly. One important result from this qualitative property of Astaxanthin is faster recovery from training and improved performance for athletes.

If Astaxanthin only had one distinct advantage over other antioxidants, it would be unjustified to call it the "World's Highest Quality Antioxidant;" however, with four important, documented advantages over more commonplace antioxidants, we feel that it's perfectly warranted and Astaxanthin has earned this venerable title. Coupled with its broad spectrum anti-inflammatory properties and ability to protect DNA and the cells' mitochondria, it becomes clear that Astaxanthin is unquestionably the most useful antioxidant to consume as a dietary supplement and is particularly recommended for everyone over the age of 40 as a preventative anti-aging supplement.

Human Clinical Research Demonstrates Astaxanthin's Antioxidant Activity

There have been approximately one hundred human clinical trials showing a variety of health benefits for Natural Astaxanthin. Along with its anti-inflammatory properties, many of these studies cite Astaxanthin's antioxidant activity as mechanisms of action for its various health benefits ranging from eye and brain health, cardiovascular support, skin health and UV protection, joint and tendon support, immune system modulation and benefits for athletes and active people, among others. Let's look at some of the studies that clearly outline antioxidant activity as a key mechanism for Astaxanthin's clinical success:

- ✓ Soccer is the world's sport—the most closely followed competitive sport in most countries around the globe. A series of studies in Europe focused on the effects of Astaxanthin supplementation on young elite soccer players. The most recent study was randomized and placebo-controlled; it spanned 90 days of supplementation at a dosage of 4mg per day. Plasma of the athletes was tested and improved results were found in the Astaxanthin group including reduction in inflammation, improvement in muscle recuperation, and better immune system function. The scientists concluded that Astaxanthin "attenuates muscle damage, thus preventing inflammation induced by rigorous physical training." They hypothesized that the mechanism of action may be that Astaxanthin "protects the cell membranes against free radicals generated during heavy exercise, thus preserving the functionality of muscle cells" (Baralic, et al, 2015).

- ✓ The same group of researchers from Europe who did the above study did two preliminary human clinical trials before embarking on their landmark study. The first of these preliminary studies tested whether Astaxanthin can reduce free radical production after intense two-hour long exercise. The treatment group took Astaxanthin for 90 days. As expected, the results were promising for Astaxanthin. The conclusion stated, "Supplementation with Astaxanthin could prevent exercise induced free radical production and depletion of non-enzymatic antioxidant defense in young soccer players" (Djordjevic, et al, 2012). Another preliminary clinical trial done by this same research team further validated the results of the first preliminary trial: Astaxanthin supplementation again led to improvement in oxidative status in young soccer players (Baralic, et al, 2013). The implications of this series of studies are indeed very promising for athletes and active people whose bodies generate huge amounts of free radicals when working, training and competing.

- ✓ A very different clinical trial looked at the effects of Natural Astaxanthin on smokers' oxidative status. This study was done on 78 people who smoked over twenty cigarettes per day. They were separated into four groups: A placebo group of 39 people along with three treatment groups who received different daily dosages of Astaxanthin: 5mg per day, 20mg per day and 40mg per day over the course of three weeks. Three different oxidative stress biomarkers as well as total antioxidant capacity were tested at the

beginning of the study and after each full week of supplementation. The results were positive for each of the four markers tested at every dosage level. The conclusion stated, "The results suggest that Astaxanthin supplementation might prevent oxidative damage in smokers by suppressing lipid peroxidation and stimulating the activity of the antioxidant system" (Kim, et al, 2011). This study is an excellent complement to the series of studies on soccer players we cited above; we see that Astaxanthin can help not only healthy, active people with oxidation issues, but it may also benefit smokers by decreasing their susceptibility to the oxidative ravages of tobacco.

- ✓ There have been several human clinical trials showing potential cardiovascular health benefits from Natural Astaxanthin supplementation and attributing the effects to Astaxanthin's antioxidant activity. One of these was done in Scandinavia on healthy, non-smoking men. This randomized, double-blind study took young men aged 19 – 33 and gave them either 8mg of Natural Astaxanthin per day or placebo for three months. The object of this experiment was to see if Astaxanthin has an effect on lipid peroxidation. The Astaxanthin group experienced significantly reduced 12- and 15-hydroxy fatty acids while the placebo group had no changes. "Supplementation with Astaxanthin may decrease in vivo oxidation of fatty acids in men" concluded the researchers (Karppi, et al, 2007). An earlier study found related results. These researchers from Japan first tested Astaxanthin's effects on the oxidation of LDL cholesterol in-vitro, and found that it dose-dependently prolongs LDL oxidation lag time. Then they performed a clinical trial in healthy volunteers. They tested four different dosages of 1.8mg per day, 3.6mg per day, 14.4mg per day and 21.6mg per day over the course of two weeks. The oxidation lag time of LDL increased at all doses. Surprisingly, the best result was found at 14.4mg per day rather than the upper dosage of 21.6mg per day. The conclusion of this paper stated that this study provides evidence that Astaxanthin inhibits LDL oxidation, and that it may contribute to the prevention of atherosclerosis (Iwamoto, et al, 2000). In a third study which was done in Korea, overweight and obese subjects were given placebo or Astaxanthin for a twelve week period. This double-blind, randomized study found that Astaxanthin supplementation resulted in decreased LDL cholesterol and apolipoprotein B. Total antioxidant capacity increased, while two oxidative markers malondialdehyde and isoprostane decreased significantly (Choi, et al, 2011a). The same group of researchers published another study the same year, also on overweight and obese subjects. This study only lasted three weeks, and only measured oxidative stress markers. They tested two different dosages of 5mg per day and 20mg per day and found that all four oxidative stress markers tested improved significantly at both dosages over the course of the three week supplementation (Choi, et al, 2011b).

- ✓ Oxidation also adversely affects fertility in men by damaging sperm. A very interesting clinical trial took thirty couples that could not conceive a child. The women in these couples had no demonstrable cause of infertility, while the men were diagnosed as having poor sperm quality. This was also a double-blind, randomized trial; the treatment group received 16mg per day of Natural Astaxanthin over a three month period. Amazingly, over half of the couples got pregnant after just three months of Astaxanthin supplementation for the infertile men! Measurement of reactive oxygen species as well

as the hormone Inhibin B decreased significantly in the treatment group, while sperm linear velocity increased (Comhaire, et al, 2005).

- ✓ For the final clinical trial we'll cite, let's look at a very different condition than most people think of when they consider the damage that oxidation causes in our bodies. This study examined patients suffering from an autoimmune disease called Sjogren's syndrome. This affliction has the very unpleasant effect of decreasing salivary secretions and causing sufferers to experience dry mouths. In this publication, the authors from Tsurumi University (a famous dental school in Japan) first tested the scavenging ability of Astaxanthin against reactive oxygen species in-vitro using a human salivary epithelial cell line. Next, they did an animal trial on mice who were subjected to irradiation-induced salivary gland dysfunction. And finally, they did a human clinical trial on patients suffering from Sjogren's syndrome as well as healthy subjects.

  The in-vitro study showed that Astaxanthin partially suppresses reactive oxygen species in the human salivary gland cells when they were exposed to hydrogen peroxide. With the mice that were exposed to radiation which created salivary gland dysfunction, Astaxanthin helped the mice to maintain normal salivary secretions. And finally, in both of the human subject groups (patients with Sjogren's syndrome as well as normal subjects), Astaxanthin appeared to increase salivary output, while the level of an oxidative stress marker, hexanoyl-lysine was reduced after supplementation with Astaxanthin. The researchers concluded "These results suggest that Astaxanthin might act as a reactive oxygen species scavenger, providing benefits to Sjogren's syndrome patients with impaired salivary secretion" (Yamada, et al, 2010).

Again, this is just a short sampling of the scores of human clinical trials on Astaxanthin, many of which attribute the health benefit attained in the study to an antioxidant mechanism. While we've only listed ten clinical trials, by the diversity of results our Readers can appreciate the far-ranging potential of Astaxanthin as a preventative supplement as well as some of Astaxanthin's potential therapeutic applications.

The Ultimate Cell Protector

The most obvious ways that Astaxanthin protects the cells in our bodies are through its antioxidant and anti-inflammatory activity. These two properties are, in fact, at the very root of all the health benefits that Astaxanthin bestows on consumers. But there are also many studies on two very important components of our cells showing that Astaxanthin has a protective effect: These two components are DNA and the mitochondria. Let's briefly look at Astaxanthin's anti-inflammatory properties and then examine how it protects our DNA and mitochondria.

A Safe and Natural Broad-Spectrum Anti-Inflammatory: It is difficult to say whether Astaxanthin's anti-inflammatory properties or its antioxidant power are more important with regards to cellular health; both create benefits to our cells that can help prevent many maladies and keep ours cells healthy and vital. Fortunately, Astaxanthin is quite capable in both respects, leading to a variety of distinct advantages for anyone looking for a preventative supplement for general health.

Astaxanthin works as an anti-inflammatory through multiple pathways. The various mechanisms of action for Astaxanthin as an anti-inflammatory have been demonstrated in several studies (Lee, et al, 2003; Ohgami, et al, 2003; Choi, et al, 2008; Kishimoto, et al, 2010). This research has consistently shown that Astaxanthin works on a variety of different causes of inflammation. In fact, there is evidence that it works on eight different inflammatory markers, but that it works in a gentle, broad-spectrum manner. This is in distinct contrast to anti-inflammatory drugs such as Celebrex® and Vioxx® as well as over-the-counter anti-inflammatories such as Non-Steroidal Anti-Inflammatory Drugs (NSAIDs including Tylenol®, Motrin®, Alleve®, etc.) and aspirin, all of which target a single inflammatory marker, but in an intense manner. Inflammatory markers gently reduced by Astaxanthin include:

- Prostaglandin E-2
- Interleukin 1b
- Interleukin 6
- Tumor Necrosis Factor-A
- Nitric Oxide
- Nuclear Factor Kappa B
- Cox 1 enzyme
- Cox 2 enzyme (Lee, et al, 2003; Ohgami, et al, 2003; Choi, et al, 2008; Kishimoto, et al, 2010)

Natural Astaxanthin has never been reported to have any side effect or contraindication in hundreds of medical research studies as well as over 15 years of commercial consumer use. Additionally, there are numerous safety studies such as acute toxicity and chronic toxicity studies validating Natural Astaxanthin's safety (Capelli and Cysewski, 2014). Meanwhile, prescription anti-inflammatory drugs as well as over-the-counter anti-inflammatories all have serious side effects. Over-the-counter anti-inflammatory NSAIDs such as Tylenol, Motrin and Aleve can all

cause serious liver problems, while aspirin can harm the stomach lining and cause ulcers. The prescription drugs such as Vioxx and Celebrex are even more dangerous; Vioxx was taken off the market several years ago after causing an increase in heart disease and premature death in many consumers, while Celebrex remains on the market albeit with extensive warnings about its potential for adverse cardiovascular events.

Natural Astaxanthin is completely different from these other drugs. It takes significantly longer to work, but it has no side effects. The prescription and over-the-counter drugs can work the same day to combat pain, while Astaxanthin usually takes at least two and up to six or eight weeks to show its full effects; but once it starts working, users report that Natural Astaxanthin has the same positive effects on painful inflammatory conditions as the anti-inflammatory drugs, but without any side effects.

In addition to several human clinical trials, two consumer surveys corroborate Natural Astaxanthin's ability to combat painful inflammatory conditions. In fact, one of these consumer surveys asked users to compare Natural Astaxanthin's anti-inflammatory effects to prescription and OTC anti-inflammatories and found that Natural Astaxanthin has similar results to those non-natural drugs:

- A survey of people with joint, tendon or muscle pain found that:
    - 84% had positive results from using Natural Astaxanthin
    - 83% experienced less pain
    - 60% had increased mobility
    - When asked how Natural Astaxanthin's effects compared to other anti-inflammatories found in the drug store:
        - 75% said that Natural Astaxanthin works the same or better than over-the-counter pain medications such as aspirin, Tylenol, Aleve or Motrin
        - 64% said that Natural Astaxanthin works the same or better than prescription anti-inflammatories such as Celebrex or Vioxx (Capelli, et al, 2008).
- In a consumer survey of 247 Natural Astaxanthin users, "over 80% of those reporting back pain and symptoms from osteoarthritis or rheumatoid arthritis reported an improvement from Astaxanthin supplementation. Astaxanthin supplementation was also reported to improve symptoms of asthma and enlarged prostate. All of these conditions have an inflammation component which is closely tied to oxidative damage" (Guerin, et al, 2002).

To summarize, it appears from these consumer surveys that Natural Astaxanthin works about as well as prescription and OTC anti-inflammatories. It does, however, take considerably longer to work. But the critical distinction is that Natural Astaxanthin has never been reported to have any side effects or contraindications—it is completely safe and natural—while OTC pain pills and prescription anti-inflammatories all have serious side effects under certain conditions, some that can end up killing you (Capelli and Cysewski, 2014). So the crucial decision is left up to the consumer: Do you want fast results that may end up seriously hurting you, or would you rather wait about a month for the same results and be safe and healthy?

Astaxanthin's Anti-Inflammatory Mechanisms of Action: Back in 2003, scientists working concurrently but independently in Japan and Korea were honing in on Astaxanthin's broad-spectrum mechanisms of action for combatting inflammation. Although they were not corresponding or sharing information, and even though they used very different paths to get there, both groups of researchers arrived at similar conclusions. This was the start, but other studies since then have further substantiated the early findings. Below is a summary of some of the most significant research in this area:

1. First Study Proving Mechanism of Action: Researchers at Japan's Hokkaido Graduate School of Medicine were the first to prove Astaxanthin's multiple mechanisms for controlling inflammation. They did their research in test tubes and also in rats, focusing on the rats' eyes. They found that Astaxanthin reduced three key causes of inflammation: Nitric oxide (NO), tumor necrosis factor alpha (TNF-a) and prostaglandin E-2 (PGE-2) (Ohgami, et al, 2003).
2. Second Mechanism of Action Study: Later the same year, Korean researchers working independently found similar results to the Ohgami study in vitro and ex-vivo. In harmony with the Ohgami results, they found that Astaxanthin suppresses the inflammatory mediators nitric oxide, prostaglandin E-2 and tumor necrosis factor alpha. But they also demonstrated Astaxanthin's positive effects on three other inflammatory markers: Interleukin 1B (IL-1b), COX-2 enzyme and nuclear factor kappa-B (Lee, et al 2003).
3. Further Validation: Several years later, scientists from Korea University further validated the earlier results finding broad-spectrum anti-inflammatory activity (Choi, et al, 2008).
4. "Remarkable" Results: Japanese researchers referred to Astaxanthin's anti-inflammatory activity as "remarkable" and found a statistically significant reduction in six different inflammatory markers tested (Kishimoto, et al, 2010).
5. Inhibition of Mast Cells: Mast cells are the key initiators of inflammation. Research at Kyoto University showed an inhibitory effect of Astaxanthin in rats' mast cells (Sakai, et al, 2009).
6. In the most recent study in this area, Astaxanthin was found to be effective at protecting against UV-induced inflammation in a broad-spectrum manner. In fact, cell death that is frequently caused by UV exposure was significantly decreased in the Astaxanthin-treated cells (Yoshihisa, et al, 2014).

There have been several human clinical trials showing that Astaxanthin reduces pain in joints, tendons and muscles in groups of patients suffering from chronic inflammatory conditions as well as in healthy men doing intense exercise. For more information on this research, please contact the authors at support@bggworld.com.

Prevention of DNA Damage: DNA is the main constituent of chromosomes in all living organisms. It carries genetic information which makes us who we are. Damage to DNA has profound implications to our cells; in fact, when DNA is damaged, it can have grave effects up to and including development of a cancerous cell line (Moorhead, et al, 2005). A supplement that

can help prevent DNA damage is certainly a powerful preventive medicine to help keep us living long, healthy lives.

For our discussion of how Natural Astaxanthin can prevent DNA damage, we'll look at research from two distinguished carotenoid researchers from Washington State University, Dr. Boon Chew and Dr. Jean Soon Park. They hold a patent on preventing DNA damage from oxidation through the use of Astaxanthin. As amazing as this may seem, the findings from their research show that using only 2mg of Natural Astaxanthin each day over four weeks can reduce DNA damage by approximately 40% (Chew and Park, 2006). This is really a phenomenal result—in only one month and at an incredibly low dosage level of only 2mg per day, Natural Astaxanthin can effectively prevent DNA damage by close to half!

In addition to their patent, Drs. Park and Chew published a randomized, double-blind, placebo-controlled human clinical trial in 2010 that outlines the effects of 2mg of Natural Astaxanthin supplementation on several parameters including inflammation, immune response as well as DNA damage. Improvements in numerous indicators were noted including inflammatory markers such as C-reactive protein, DNA damage, and a variety of immune markers. This study concluded that "dietary Astaxanthin decreases a DNA damage biomarker and acute phase protein, and enhances immune response in young healthy females" (Park, et al, 2010).

While the research of Drs. Chew and Park is excellent and clearly demonstrates Astaxanthin's prevention of DNA damage, one wonders how much damage could be prevented over a longer period of time than just one month as Astaxanthin accumulates throughout the cells in our bodies. And it would be very interesting to see how Astaxanthin performs on DNA damage at a dosage level toward the 12mg per day upper end of the recommended range. We suspect that the effect would be much more pronounced than the 40% result that was found at 2mg per day for one month.

Supporting Pre-Clinical DNA Research: In addition to the human research done by Drs. Chew and Park, a flurry of pre-clinical studies has been performed related to Astaxanthin's effect on DNA damage over the last ten years. Here are some of the most exciting ones:

- Astaxanthin shows a neuroprotective effect in rat retinal cells and aids against oxidative stress, glutamate stress and DNA damage (Yamagishi and Aihara, 2014)
- Astaxanthin enhances a DNA repair enzyme and is a novel candidate for cancer prevention (Kavitha, et al, 2013)
- Astaxanthin improves oxidative stress markers and an indicator of oxidative DNA damage in mouse cells and may be developed as an antioxidant drug to treat diabetic retinopathy (Dong, et al, 2013)
- Astaxanthin modulates age-associated mitochondrial dysfunction in dogs which is attributed to alleviating oxidative damage to cellular DNA and protein (Park, et al, 2013)
- Astaxanthin reduces DNA damage in rat liver cells (Turkez, et al, 2014)
- Astaxanthin may protect against oxidative impairment and DNA damage (Zhao, et al, 2011)

- Astaxanthin heightens the immune response and reduces DNA damage and inflammation in dogs (Chew, et al, 2011)
- Astaxanthin improves oxidative stress and DNA damage in rats (Tripathi and Jena 2010)
- Astaxanthin protects retinal cells against oxidative stress and reduces an indicator of DNA damage in mice (Nakajima, et al, 2008)
- Astaxanthin inhibits cytotoxic and genotoxic effects and restores DNA damage in mouse cells (Tripathi and Jena, 2008)
- Astaxanthin protects against DNA damage in human neuroblastoma cells (Santocono, et al, 2007)
- Astaxanthin reduces DNA damage in UVA-irradiated cells (Santocono, et al, 2006)

Astaxanthin's Effects on the Mitochondria: Mitochondria are commonly known as the "powerhouse of the cell." To put it simply, mitochondria are responsible for energy production in our cells. There is already a great deal of pre-clinical research on how Astaxanthin can protect and benefit the mitochondria, which is probably why it is so effective at increasing energy levels and boosting strength and endurance in human clinical trials. We'll review some of the most relevant of these studies here:

- In a study done at University of Pittsburgh's School of Medicine, Astaxanthin protected against mitochondrial dysfunction and reactive oxygen species in a mouse model of Parkinson's disease and also in-vitro (Lee, et al, 2011).
- In perhaps the earliest study on Astaxanthin's effects on the mitochondria, Japanese researchers at Kochi Medical School found that Astaxanthin protects the mitochondria of rats better than a-tocopherol (Kurashige, et al, 1990).
- In another study done at Washington State University under the auspices of the famous carotenoid researchers Boon Chew, PhD and Jean Soon Park, PhD, Astaxanthin prevented age-related mitochondrial dysfunction in dogs (Park, et al, 2013).
- Astaxanthin extended the lifespan of C. elegans (a model organism used in longevity studies) by protecting the mitochondria and the nucleus of the cells (Yazaki, et al, 2011).
- Astaxanthin was found capable of protecting the mitochondrial membrane and preventing DNA damage and cell-death in-vitro in a university study done in Taiwan (Chan, et al, 2009).
- Cells subjected to heat stress in-vitro were protected by Astaxanthin, which the researchers attributed to Astaxanthin's positive effect on the mitochondria (Kuroki, et al, 2013).
- In different studies on Astaxanthin's effects on the mitochondria, it was found to be effective in benefiting various organs in different ways. The organs positively affected include:
    - Liver (Ma, et al, 2011; Song, et al, 2011).
    - Kidneys (Manabe, et al, 2008)
    - Heart (Nakao, et al, 2010)
    - Brain and central nervous system (Liu and Osawa, 2009; Liu, et al, 2009; Lu, et al, 2010)

- The last study we'll cite showed that Astaxanthin can protect mitochondria that are subjected to oxidative stress. This study's abstract summarizes our discussion in this section very well:

  > "Mitochondria combine the production of energy with an efficient chain of reduction-oxidation (redox) reactions but also with the unavoidable production of reactive oxygen species. Oxidative stress leading to mitochondrial dysfunction is a critical factor in many diseases, such as cancer and neurodegeneration and lifestyle-related diseases. Effective antioxidants thus offer great therapeutic promise…Astaxanthin at nanomolar concentrations was effective in maintaining mitochondria in a reduced state. Additionally, Astaxanthin improved the ability of mitochondria to remain in a reduced state under oxidative challenge. Taken together, these results suggest that Astaxanthin is effective in improving mitochondrial function through retaining mitochondria in a reduced state" (Wolf, et al, 2009).

Algae-Based Astaxanthin is Far Superior to Synthetic and *Phaffia* Yeast Astaxanthin

It is extremely important to understand the vast differences between Natural Astaxanthin from microalgae and its distant cousins which are produced from genetically mutated *Phaffia* yeast or from petrochemicals. This is becoming a critical topic for people in the supplement industry as well as consumers since synthetically-produced Astaxanthin is now being promoted as "Nature Identical." Synthetic Astaxanthin (which is synthesized from petrochemicals in an elaborate process) has been used for over thirty years in the animal feed industry, primarily to pigment the flesh of farm-raised salmon. Yet it was only introduced as a human nutritional supplement in 2013 after several famous doctors and opinion leaders started publicizing what an excellent supplement Natural Astaxanthin is and its popularity quickly escalated.

Phaffia-derived Astaxanthin (whose official nomenclature was changed recently to *Xanthophyllomyces dendrorhous* but is still commonly referred to as "*Phaffia*") is a species of yeast which in nature produces small amounts of Astaxanthin. Companies involved in the commercial production of *Phaffia* have genetically manipulated this species to produce exponentially more Astaxanthin. While a full review of the vast differences between these molecules would be too comprehensive for this paper, it is important that our Readers understand that these are three completely distinct molecules. In fact, other than sharing the same chemical formula, all three are almost exact opposites in all other respects.

The regulatory status of both *Phaffia*-derived and Synthetic Astaxanthin is in question in many countries. For example, in the USA, Synthetic Astaxanthin has never undergone a New Dietary Ingredient petition with the US Food and Drug Administration, while algae-based Astaxanthin has been allowed by FDA at ongoing doses of 12mg per day and for thirty day periods at 24mg per day. Astaxanthin from *Phaffia* is allowed by the US FDA, but with restrictions against long term use; against the use in children; and perhaps most significantly, at dosage levels of only 2 mg per day. Generally, a 2 mg dosage of algae-based Astaxanthin has only been shown sufficient in human clinical research in the area of immunomodulation (Park, et al, 2010), one of many potential physiological benefits of Astaxanthin. Incredibly, for products that are being offered as health supplements, the published literature does not contain human clinical research on safety or any health benefits from either Synthetic or *Phaffia*-derived Astaxanthin.

Natural Astaxanthin from Algae is 20 to 90 Times Stronger as an Antioxidant than Synthetic Astaxanthin: Of paramount importance, a critical finding of a landmark series of head-to-head antioxidant experiments is the clear superiority of Natural Astaxanthin to Synthetic Astaxanthin in antioxidant strength. In both university research at Creighton University under the auspices of acclaimed antioxidant researcher Debasis Bagchi, PhD as well as in independent laboratory testing at Brunswick Laboratories, Natural Astaxanthin extracted from microalgae was found to be a minimum of 20X stronger in antioxidant activity than Synthetic Astaxanthin produced from petrochemicals (Capelli, et al, 2013).

Further corroborating this landmark research, a study published by French university professors in conjunction with a leading medical doctor in 2015 again showed how much stronger Natural Astaxanthin from *Haematococcus* microalgae is than Synthetic Astaxanthin. They tested two forms of Natural Astaxanthin against Synthetic in two different models, the Trolox equivalent antioxidant capacity assay and an evaluation on HUVEC (human umbilical vein endothelial cells). The two natural forms tested were algae extracts produced by supercritical CO2 extraction and by solvent extraction, both of which are commercially available for use in food supplements. The results for both of the extracts with Natural Astaxanthin were outstanding—90X stronger than Synthetic Astaxanthin—and with absolutely no sign of toxicity. "The intracellular antioxidant activity in natural extracts was approximately 90 times higher than Synthetic Astaxanthin…Therefore, these results revealed the therapeutic potential of the natural extracts in vascular human cell protection against oxidative stress without toxicity, which could be exploited in the prevention and/or treatment of cardiovascular diseases" (Regnier, et al, 2015).

Animal Research Shows Huge Differences in Efficacy Between Algae-Based Astaxanthin and *Phaffia*-Derived or Synthetic Astaxanthin: The vast differences in antioxidant activity result in different functionality between Natural and Synthetic Astaxanthin. In addition, functional differences between Natural Astaxanthin and *Phaffia*-derived Astaxanthin are also apparent, although we are still awaiting the first publication of tests of antioxidant strength between the two. Pre-clinical research in this area is beginning to emerge as well; in fact, five indicative studies have already clearly defined Natural Astaxanthin's functional superiority in animal trials. In the first, Natural Astaxanthin was again shown to have superior antioxidant activity as compared to Synthetic Astaxanthin when fed to rats. Rats were given either Natural Astaxanthin from *Haematococcus* microalgae or Synthetic Astaxanthin for fourteen days, and then enzymes indicative of liver protection were measured along with the antioxidant enzymes catalase, superoxide dismutase and glutathione. Natural Astaxanthin showed "better hepatoprotection and antioxidant activity, therefore it can be used in pharmaceutical and nutraceutical applications" (Rao, et al, 2013).

The second study was even more interesting because it tested all three forms in an animal trial—algae-based, *Phaffia*-derived, and Synthetic Astaxanthin. This study was published as a joint project between the Department of Food Science at University of Massachusetts and the Department of Food Science at South China Agricultural University. The authors had no conflicts of interest (meaning that they were not employed or associated with any company involved in the Astaxanthin business, making them completely independent and unbiased). They experimented with a worm that is commonly used as a model organism for longevity and antioxidant testing called *Caenorhabditis elegans*. This worm is very appropriate to use in this type of experiment for two key reasons: First of all, it has 60% to 80% of the human gene homologues making experiments with this worm very indicative of potential results in humans (Kaletta and Hengartner, 2006). Secondly, this worm has a three week lifespan, allowing for rapid testing and results.

The worms were separated into four groups: A control group, a group treated with Natural Astaxanthin from *Haematococcus* microalgae, a group treated with Astaxanthin from the

mutated yeast *Phaffia*, and finally, a group treated with Synthetic Astaxanthin made from petrochemicals. Each of these distant Astaxanthin cousins has different stereoisomeric forms (which in plain English means that they are shaped differently): The form in Natural Astaxanthin from microalgae is 3S, 3'S; the form in mutated *Phaffia* yeast is 3R, 3'R; and finally, the form in Synthetic Astaxanthin is a statistical mixture of 25% each of the forms in microalgae and *Phaffia* yeast along with the predominant form 3R,3'S (which is called "meso" and accounts for 50% of the total). The authors of this study stated, "Astaxanthin is a potent antioxidant with different stereoisomers in nature. However, there have been no reports about the functional activities of different stereoisomers so far. Our present finding shows there is a significant difference between Astaxanthin stereoisomers against oxidative damage, which is useful for rational utilization of Astaxanthin in functional food."

Worms underwent oxidative stress for 24 hours. The way that the scientists created oxidative stress was by introducing paraquat (a toxic, fast-acting herbicide) to the worms. The worms were tracked for five days after exposure to paraquat; amazingly, by the fifth day, Natural Astaxanthin had kept approximately 50% more of the worms alive versus control. In fact, all forms of Astaxanthin made the worms live significantly longer, with Natural Astaxanthin from *Haematococcus* microalgae-treated worms living the longest of all four groups. Additional tests were done on different oxidative parameters with all Astaxanthin groups showing improvements. Results indicated:

- Antioxidant enzyme superoxide dismutase (SOD) in the Natural Astaxanthin group was approximately 50% higher than control.
- Catalase in the Natural Astaxanthin group was approximately 90% higher than control.
- The Natural Astaxanthin group was higher than the *Phaffia* Astaxanthin and Synthetic Astaxanthin groups in both SOD and Catalase.
- Relative fluorescence intensity (which indicates the accumulation of reactive oxygen species) was much lower in all Astaxanthin groups, again with N-AX being the best performer.
    - The change versus control for Natural Astaxanthin was approximately 33% more than Phaffia.
    - The change versus control for Natural Astaxanthin was almost double Synthetic Astaxanthin.
- Green-fluorescent protein (GFP) production is induced by oxidative stress and is used to measure SOD-3 production levels. SOD-3 can convert the major isoform of mitochondrial SOD, an important antioxidant enzyme (Libina, et al, 2003). "We found that SOD-3 GFP expression among Astaxanthin treatment groups was markedly higher than that of the control, and the SOD-3 GFP expression in the Natural Astaxanthin-treated group was significantly higher than the *Phaffia* and Synthetic Astaxanthin-treated groups during the first to third day period ($P < 0.05$). This result was consistent with the effects of Astaxanthin stereoisomers on the reactive oxygen species level where Natural Astaxanthin exhibited higher antioxidative activity than the other two stereoisomers."

"In conclusion, our results demonstrated that Astaxanthin could increase oxidative resistance, decrease levels of reactive oxygen species, increase enzyme activity of SOD and Catalase, and

enhance expression of SOD-3 in *C. elegans*. These effects may contribute to the observed survival increase in the worms under oxidative stress" (Liu, et al, 2016).

This study is excellent proof-in-action of how different Synthetic and *Phaffia*-derived Astaxanthin are from Natural Astaxanthin from microalgae. Coupled with the rodent study we cited above where Natural Astaxanthin was superior to Synthetic in protecting the liver, we're starting to see conclusive evidence in animals of how the vast differences between these disparate forms of Astaxanthin can affect the health benefits that the animals experience.

There is one additional animal trial that tested Astaxanthin from algae against both Astaxanthin from *Phaffia* and from petrochemicals. This study was done in 2005 at a university in Japan on the effects of Astaxanthin on gastric ulcers in rats. The results of this study showed that algae-based Astaxanthin may protect rats from ulcers. This is not particularly earth-shattering news, as it's been known for years that Astaxanthin has potential to prevent ulcers, particularly ulcers caused by the bacterium *H. plyori* (Bennedsen, et al, 1999; Wang, et al, 2000; Akyon, et al 2002) and ulcers caused by damaging substances such as alcohol (Kim, et al, 2005a; Kamath, et al, 2008), naproxen (Kim, et al, 2005b) and acetic acid (Yang, et al, 2009). (Relevant to our present review, it is interesting to note that some of these studies called out antioxidation as the mechanism of action for how Astaxanthin prevented gastric damage and ulcers.)

What is of top interest here is that this publication showed significantly better potential for Astaxanthin from algae to prevent gastric ulcers than Astaxanthin from the mutated yeast *Phaffia Rhodozyma* and Astaxanthin synthesized from petrochemicals. Rats were stressed by putting them into chest-level water for 24 hours after having fasted for 24 hours. This study tested the three forms of Astaxanthin as well as beta-carotene and Vitamin C. All the rats given carotenoids including all three forms of Astaxanthin as well as beta-carotene before being stressed were appreciably protected against the formation of gastric ulcers as compared to rats in the control group and the group given Vitamin C. But the rats given algae-based Astaxanthin did the best of all: "Ulcer indexes in particular were smaller with the rat group fed Astaxanthin extracted from *Haematococcus* than the other groups" (Nishikawa, et al, 2005).

In 2008, a similar study was done on the effects of Astaxanthin on ulcers in rats. This study tested Synthetic against algae-based Astaxanthin, but did not include *Phaffia*-derived Astaxanthin. They used ethanol to induce ulcers and found that pre-treatment with Natural Astaxanthin outperformed Synthetic Astaxanthin in inhibiting the formation of ulcers. Synthetic Astaxanthin did not show any inhibition all, while amazingly, Natural Astaxanthin showed inhibition of ulcers at a level that is better than the ulcer drug omeprazole (which is sold under the brand name Prilosec®). Natural Astaxanthin showed a "dose-dependent gastroprotective effect on acute, ethanol-induced gastric lesions in rats…Presence of Astaxanthin esters in *Haematococcus pluvialis* has an added advantage that, generally carotenoids, although potential antioxidants, may lack such properties in vivo because of the pro-oxidant effect. Esterified Astaxanthin shows comparatively better stability than free Astaxanthin, and hence it may exhibit more health beneficial effects than free Astaxanthin" (Kanath, et al, 2008).

The first study of differences in animals between different forms of Astaxanthin was done in 1998. This study focused on a species of shrimp called *Penaeus monodon* which is known as the

"giant tiger prawn." This study was done at a university in Thailand in support of the large shrimp-farming industry in that country. They did a series of tests on three different larval and post-larval stages during the shrimp's life cycle. They separated the shrimp into four different groups:

- One treatment group was fed a commercial diet augmented with algae-based Astaxanthin.
- The second treatment group was fed a commercial diet augmented with Synthetic Astaxanthin.
- One control group was fed the same commercial diet without any addition of Astaxanthin
- A different control group was fed a natural diet that the shrimp would normally eat in the wild.

Believe it or not, in all three larval stages, shrimp fed algae-based Astaxanthin survived at higher rates than shrimp fed Synthetic Astaxanthin. Fifteen days after the post larval stage, shrimp fed algae-based Astaxanthin were showing better survival rates than all three other groups (amazingly, even better than the shrimp fed the natural diet). In addition, tests of low water salinity were done to examine the different groups' tolerance levels, and the shrimp fed the algae-based Astaxanthin diet again outperformed all others. Shrimp from all three other groups died faster than the algae-Astaxanthin fed shrimp when subjected to low salinity. Similarly, there were statistically significant differences in growth rates as well; shrimp fed the algae-based Astaxanthin and the natural diet grew faster than shrimp fed Synthetic Astaxanthin or the commercial diet without Astaxanthin.

It's very interesting to note that the shrimp fed Synthetic Astaxanthin were the lowest performers. For example, in one larval stage, all three other groups outlived the shrimp fed Synthetic Astaxanthin. This clearly shows that Synthetic Astaxanthin is not "Nature Identical." Not only couldn't Synthetic perform as well as Natural Astaxanthin in this study, it didn't even do as well as fish fed a diet without any Astaxanthin!

> "From our results, the highest survival rate of zoea and mysis [two larval stages] was obtained with shrimp fed algae-based Astaxanthin, followed by the natural diet, the commercial diet without Astaxanthin and the Synthetic Astaxanthin diet in descending order. This indicated that shrimp larvae accept Natural better than Synthetic Astaxanthin…The postlarva after fifteen days fed natural diets containing Natural Astaxanthin were larger than those fed diets containing Synthetic Astaxanthin or no Astaxanthin. The best postlarval growth was in the group fed algae-based Astaxanthin and was significantly better than that for the groups fed Synthetic Astaxanthin. This indicated that Astaxanthin from *Haematococcus pluvialis* (mostly in esterified form) performs significantly better than free, Synthetic Astaxanthin…Determination of 50% cumulative mortality upon low salinity challenge showed that larvae fed algae-based Astaxanthin endured better than larvae fed the natural diet, the Synthetic Astaxanthin diet and the commercial diet without Astaxanthin."

Among many fascinating aspects of this study, one of the most is that they examined shrimp in both a healthy environment, plus shrimp subjected to stress by being put in a low salinity environment. In both cases, Natural Astaxanthin helped the shrimp survive much better when

compared to Synthetic Astaxanthin. This is a powerful first study on this topic, and the fact that no other animal research like this was done until many years later is troubling; if there had been multiple studies done over the years, perhaps synthetically-produced Astaxanthin would not have been released for human use.

The final statement of this study really sums it up nicely: "Although the mechanism by which Astaxanthin improved the response to stress cannot be explained, the information that Natural Astaxanthin (from *Haematococcus pluvialis*) is more efficacious than Synthetic Astaxanthin for growth, survival and stress resistance of shrimp larvae should be useful for further research on shrimp larval nutrition" (Darachai, et al, 1998). We might add that, while the authors of this study were focused on shrimp in support of the Thai shrimp farming industry, the implications of this study and the four more recent studies showing the clear superiority of Natural Astaxanthin from microalgae over other forms should be strongly considered for all living organisms (including humans), not just shrimp.

Survival rates, resistance to stress, protection of the liver, prevention of gastric ulcers, even growth rates and longevity—all of these health concerns show better results when animals are fed Natural Astaxanthin from *Haematococcus* microalgae than when animals are fed Astaxanthin from other forms or control diets without Astaxanthin. After examining these five comparative animal studies, a key question remains: Does this research relate to the use of Astaxanthin in humans? We don't know, as we're not aware of a single clinical trial showing <u>any</u> health benefit in humans for Astaxanthin from mutated *Phaffia* yeast or Synthetic Astaxanthin from petrochemicals. And even more frightening, we're not aware of any safety studies in humans for *Phaffia* or Synthetic Astaxanthin. Without clinical trials showing health benefits and extensive safety research in humans for these other forms, we highly recommend that consumers avoid being the guinea pigs on these products. In fact, it's hard to imagine why anyone would experiment on themselves by taking these untested forms since Astaxanthin from microalgae (which has approximately 100 clinical trials showing various health benefits and extensive safety data) is widely available.

<u>Safety of *Phaffia*-Derived and Synthetic Astaxanthin is a Troubling Question Mark</u>: It's particularly important to understand the safety concerns with Synthetic and *Phaffia*-derived Astaxanthin. As discussed above, the US FDA is so concerned with the mutated yeast form that they do not recommend it for long-term use or for children, and only allow it to be used at a level of 2mg per day. The safety concerns with Synthetic Astaxanthin are even more severe. And Astaxanthin is not the only nutrient that comes in both synthetic and natural forms where there are serious safety concerns with the synthetic form. In fact, even with molecules in the carotenoid family that are closely related to Astaxanthin, synthetic forms have been found to be a grave concern. The reason that synthetic nutrients may have compromised safety is not yet understood by scientists. One theory is that a molecule that has been synthesized may not contain all physiologically active components that nature created in the natural version. A good example of this is with Vitamin E; the synthetic version is solely dl-alpha tocopherol. But in nature, the Vitamin E complex contains several different tocopherols and tocotrienols; and in fact, the tocotrienol constituents yield outstanding health benefits while the tocopherol constituents are not nearly as active. For example, tocotrienols have been shown active in

preventing neurodegeneration (Sen, et al, 2006), protecting the liver (Magosso, et al, 2013) and kidneys (Haghhighat, et al, 2014), and even in preventing hair loss (Beoy, et al 2010).

Now let's look at two carotenoids closely related to Astaxanthin that are available synthetically. We'll start with the most famous member of the carotenoid family which is also the most researched: Beta-carotene. There are hundreds of published studies showing potential benefits for beta-carotene for immunity as well as prevention of cancer (Moorhead, et al, 2005). But it appears that synthetic beta-carotene does not absorb well; in fact, one study indicated that natural beta-carotene absorbs ten times better than its synthetic cousin in rats and chickens (Ben-Amotz, et al, 1989). And absorption is only one of the concerns with synthetic beta-carotene; even more importantly, there are valid concerns with both its safety and efficacy. With regards to antioxidant potential, synthetic versus natural beta-carotene mimics the results with Astaxanthin. Synthetic beta-carotene is primarily the trans-form, while natural beta-carotene contains large amounts of the cis-form. The 9-cis beta-carotene form, which is found in high amounts in natural beta-carotene, is a more efficient lipophilic antioxidant than the synthetic trans-form. The stereochemistry of this carotenoid (similar to the situation with Astaxanthin) is important in antioxidant potential as well as absorption and transport (Ben-Amotz, et al, 1996).

Now for the top issue: Safety. A famous study done in Finland in the 1990's tested synthetic beta-carotene on heavy tobacco smokers. The results of this study contradicted dozens of pre-clinical trials and epidemiological studies that indicate natural beta-carotene has cancer-preventative properties (Moorhead, et al, 2005). The results of this large-scale study showed a slight increase in cancer among the subjects supplementing long-range with synthetic beta-carotene (Heinonen and Albanes, 1994). Imagine how shocked people were who were taking beta-carotene as a cancer-preventative supplement when the newspaper headlines read "Beta-carotene increases the risk of cancer." However, subsequent research that compared natural beta-carotene extracted from *Dunaliella salina* microalgae with synthetic beta-carotene indicated that it's the synthetic form which may be involved in the formation of cancer. In fact, the study concluded that natural beta-carotene could be valuable in tumor prevention and supplementary treatment (Xue, et al, 1998). Even though natural and synthetic beta-carotene have the same chemical formula (just like the case with the different forms of Astaxanthin), they are different in every other way. Natural beta-carotene absorbs better, has stronger antioxidant activity and may prevent cancer; meanwhile synthetic beta-carotene may actually cause cancer.

Another synthetic carotenoid was actually taken off the market because of serious health concerns. Similar to Synthetic Astaxanthin, synthetically-produced canthaxanthin has been sold for many years for inclusion in animal feeds. But for a short time in the 1980's, synthetic canthaxanthin was sold for human use as an internal tanning pill; people who took high doses of this product got a tan without going out in the sun. (Sources of natural canthaxanthin are very scarce, so there are no commercially available products featuring the natural form.)

Canthaxanthin is much more closely related to Astaxanthin than beta-carotene since it's in the same subgroup of carotenoids as Astaxanthin called "xanthophylls." Xanthophylls differ structurally from the other carotenoid subgroup called "carotenes" (of which beta-carotene and lycopene are the most famous members) since they have hydroxyl groups attached to the molecules. (A hydroxyl groups is a hydrogen atom covalently bonded with an oxygen atom.)

After consumers started using the synthetic tanning pills with canthaxanthin, an unforeseen side effect appeared: Golden crystals formed in consumers' retinas, and synthetic canthaxanthin was immediately removed from the supplement market. In addition, regulators around the world began limiting or prohibiting the use of synthetic canthaxanthin in animals feeds due to this serous safety concern (European Commission, 2002; Australia New Zealand Food Standards Code, 2011). The crystallization in the retinas eventually disappeared, but it is extremely disconcerting how long it took for complete reversal. Follow up research published in 2011 found that complete disappearance of the golden crystals took approximately twenty years (Hueber, et al, 2011).

With other synthetic carotenoids increasing the incidence of cancer and causing crystallization in the retina, we were very surprised when Synthetic Astaxanthin was introduced to the human supplement market without doing long-range safety studies in humans. In addition, questions about efficacy of Synthetic Astaxanthin remain unanswered despite multiple antioxidant tests showing 20X to 90X inferior antioxidant activity and multiple animal trials showing poor performance as compared to Natural Astaxanthin. And the ultimate insult is that, despite these profound differences, Synthetic Astaxanthin is being marketed as "Nature Identical," which couldn't be further from the truth. The conclusion of the peer-reviewed Creighton University study testing Synthetic versus Natural Astaxanthin clearly summarized the case against Synthetic:

> "For these reasons, the authors recommend against the use of Synthetic Astaxanthin in human nutraceutical supplements until extensive, long-range safety parameters are established and human clinical trials showing health benefits are conducted. In the event that Synthetic Astaxanthin attains these two milestones, due to the extensive differences between the two molecules, it should be distinctly labeled as "Synthetic Astaxanthin" on consumer product labels, and dosage levels should be approximately 20X to 30X higher than those of Natural Astaxanthin in order to obtain similar antioxidant activity" (Capelli, et al, 2013).

List of the Vast Differences Between Algae-Based Astaxanthin and Other Forms: It's not only differences in antioxidant potential and real-world health benefits that we described in the paragraphs above that separate these distant cousins; as we mentioned in the beginning of this discussion, in every way other than the chemical formula they share, Natural Astaxanthin from algae is as different as night and day from the two other forms of Astaxanthin. Briefly, the primary differences between the forms of Astaxanthin are:

- **Shape**: As we pointed out above, the Natural Astaxanthin molecule's stereochemistry is unique (it is shaped differently than the Synthetic and the *Phaffia* Astaxanthin molecules).
- **Esterification**: Natural Astaxanthin is 95% esterified (it has a fatty acid molecule attached to either one or both ends of the Astaxanthin molecule). Synthetic and *Phaffia* Astaxanthin are exclusively "free" Astaxanthin and do not have fatty acid molecules attached.

- **Synergy**: Natural Astaxanthin from *Haematococcus pluvialis* microalgae comes complexed in nature with supporting carotenoids: There are consistently small amounts of other antioxidant carotenoids such as lutein, beta-carotene and canthaxanthin ranging from 3% - 15% of the total carotenoid fraction which work in unison with Astaxanthin to provide a synergistic effect when ingested. Synthetic and *Phaffia* Astaxanthin do not contain supporting carotenoids.
- **Source**: Synthetic Astaxanthin is synthesized from petrochemicals in an elaborate process. *Phaffia* Astaxanthin is produced from genetically-manipulated yeast. Natural Astaxanthin is extracted from natural *Haematococcus pluvialis* microalgae.
- **Safety**: Natural Astaxanthin has an extensive portfolio of human safety studies and a history of close to twenty years of safe use as a commercially-sold nutritional supplement. Synthetic Astaxanthin has never been directly tested in humans for safety. (This is an overriding concern due to serious safety issues with related synthetic carotenoids beta-carotene and canthaxanthin.) Meanwhile, the US FDA is so concerned with *Phaffia* Astaxanthin from mutated yeast that they do not recommend it for long-term use or for children, and they only allow it to be used at a level of 2mg per day.
- **Efficacy**: Amazingly and perhaps most importantly, Synthetic and *Phaffia* Astaxanthin have <u>never</u> been shown to have any health benefit in human clinical research. They are completely untested and there is a possibility that they do not have any health benefit at all, even at high doses. Meanwhile, Natural Astaxanthin has been shown to have diverse health benefits in approximately 100 different positive human clinical trials.
- **Antioxidant Strength**: Natural Astaxanthin is at minimum 20X to as much as 90X stronger than Synthetic Astaxanthin as an antioxidant. There have not yet been head-to-head antioxidant comparisons between Natural and *Phaffia* Astaxanthin; however, we expect vast differences in antioxidant disparity between these molecules as well due to *Phaffia's* chemical similarities to Synthetic.
- **Dosage**: In the event that Synthetic Astaxanthin is ultimately proven safe for long-range human consumption, dosages would logically be a minimum of 20 times greater than corresponding dosages of Natural Astaxanthin due to its vastly inferior antioxidant profile. This high dosage requirement would most likely put Synthetic Astaxanthin out of reach economically for most consumers (Capelli, et al, 2013). And while the difference in antioxidant strength between *Phaffia* and Natural Astaxanthin remains unproven to date, due to safety concerns, *Phaffia* Astaxanthin is only allowed at 2mg per day dosage in USA rendering it virtually useless for human consumption.

<u>Summary</u>: With this brief analysis of these three distant Astaxanthin cousins, we quickly see that Synthetic and *Phaffia*–derived Astaxanthin are entirely different from and far inferior to Natural Astaxanthin from algae:

- Chemically they are completely different in every way except for sharing the same chemical formula.
- Both *Phaffia* and Synthetic have been shown to be substandard in pre-clinical animal studies.
- Synthetic Astaxanthin is comparatively very weak as an antioxidant.

- Neither *Phaffia* nor Synthetic Astaxanthin has ever been clinically validated to have any health benefit in humans.
- And most frightening of all: Neither *Phaffia* nor Synthetic Astaxanthin has ever been tested for safety in humans.
- Regardless of how Synthetic and *Phaffia* Astaxanthin are marketed, it is clear that they are absolutely not "Nature Identical."

For all of these reasons, we highly recommend that consumers ensure that their Astaxanthin is the natural form extracted from *Haematococcus pluvialis* microalgae.

Conclusion

Astaxanthin is the strongest natural antioxidant. There have been scores of studies demonstrating Astaxanthin's enormous antioxidant potential. These studies run the full gamut from in-vitro experiments to comparative antioxidant studies to pre-clinical animal trials to double-blind, placebo-controlled human clinical trials. Even against closely related molecules in the same family of carotenoids, Astaxanthin consistently tests many times higher in singlet oxygen elimination and overall free radical scavenging (usually by at least a factor of 10X!). In addition, we've seen how Natural Astaxanthin possesses four defined attributes that distinguish it as the highest quality antioxidant:

- Unlike many other antioxidants (even other carotenoids like lycopene and beta-carotene), Astaxanthin can cross the protective blood-brain barrier and bring its antioxidant and anti-inflammatory protection to our brains. Once in our brains, it can then cross the blood-retinal barrier to help protect another vital organ, our eyes.
- Another key difference is that the Astaxanthin molecule is very long; this allows it to span the cell membrane. It can have one end of the molecule in the fat soluble part of the cell and the other end of the molecule in the water soluble part of the cell, thereby protecting the entire cell.
- Of particular interest to athletes and active people, Astaxanthin can bond with muscle tissue. This is very important to help eliminate the onslaught of free radicals in muscle tissue that is generated by high intensity activities.
- Lastly, unlike many otherwise good antioxidants, Astaxanthin can never turn into a "Pro-Oxidant" and start generating additional harmful free radicals in our bodies.

Remarkably, Astaxanthin is not only the best antioxidant we've found so far in nature; it also has other related cell-protective properties. For one, it's a safe and natural broad-spectrum anti-inflammatory. Additionally, it's been shown at a dosage as low as 2mg per day to be able to drastically reduce damage to DNA. And finally, it has demonstrated in many studies the potential to protect the energy-producing mitochondria of our cells.

The end result of Astaxanthin's antioxidant, anti-inflammatory and cell-protective properties are a variety of clinically validated health benefits including:

- Eye & Brain Health
- Cardiovascular Health
- Joint & Tendon Health
- Skin Health & UV Protection
- Energy & Athletic Performance
- Immune System Modulation

It is for these reasons that we highly recommend the preventative use of a minimum 4mg of Natural Astaxanthin from microalgae per day.

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