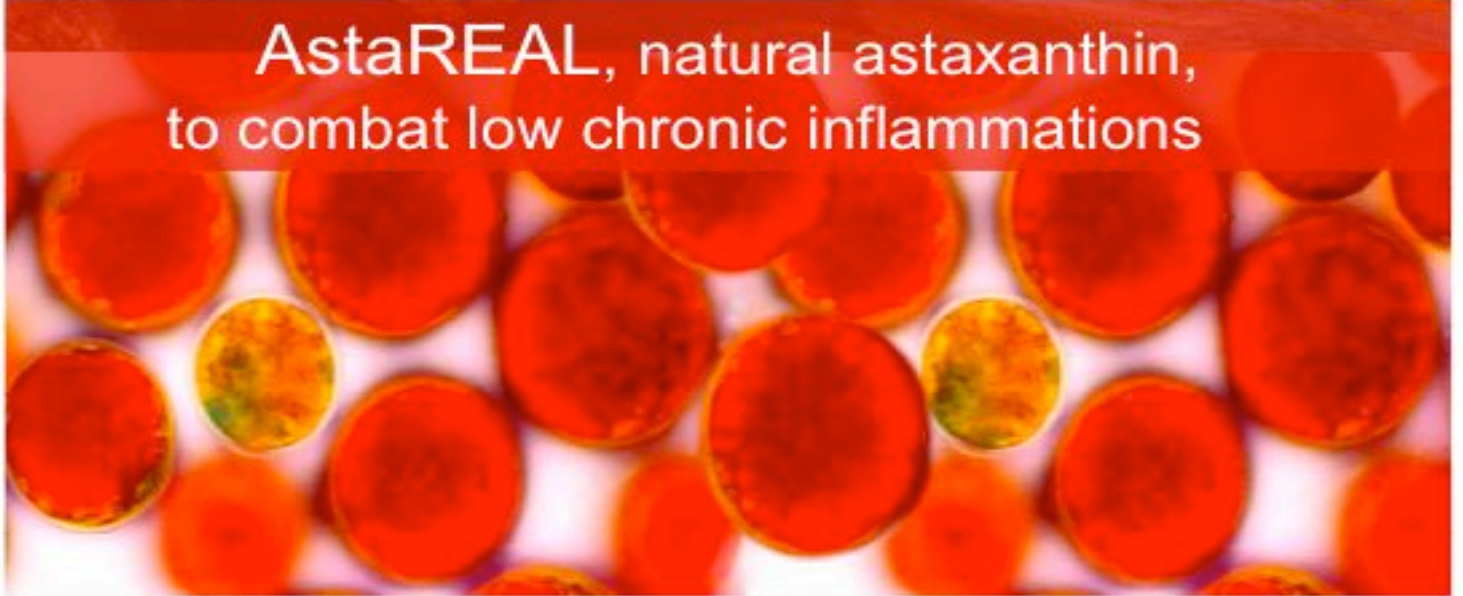




AstaREAL, natural astaxanthin,
to combat low chronic inflammations



 **BIOREAL**
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Introduction

Low chronic inflammation is an underlying cause of many seemingly unrelated diseases as atherosclerosis, diabetes, digestive system diseases and obesity. The inflammation is a process initiated by the immune system as it reacts to injury or infection. The process is generally accompanied by tissue damage associated with oxidation of macromolecules by inflammation-derived free radicals. Recent results indicate that oxidative modulations of lipids normally present in e.g. cellular membranes, contributes to disruption of the tightly controlled balance of immune tolerance and ultimately provokes chronic inflammation (Leitinger 2008).

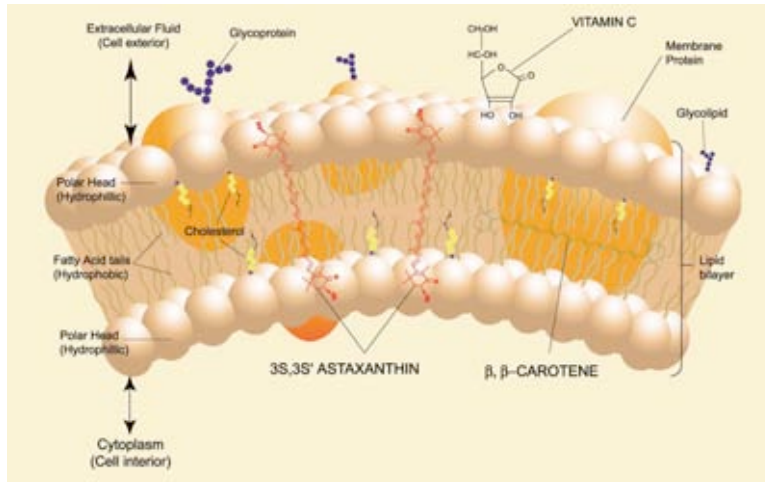
Astaxanthin is a natural lipid soluble antioxidant that is deposited in cellular membrane and has been shown to have anti-inflammatory effects both in in vitro and in vivo studies. The aim of this article is to present results of studies with astaxanthin in connection to inflammation in order to give an insight how supplementation with astaxanthin might be beneficial in the combat of low chronic inflammations.

Astaxanthin – How it works

Astaxanthin is a lipid soluble carotenoid antioxidant. It is found naturally in e.g. fish, crustaceans and birds. It gives the pink colour to the flesh of wild salmon and astaxanthin is often occurring together with omega-3 lipids in nature. Upon oral administration astaxanthin can be found in all organs of the body (Petri et al 2007). At the cellular level astaxanthin accumulates in the membrane fractions like the cell membrane and in the membranes of the mitochondria. Astaxanthin has a unique structure that enables the molecule to span the double layer membrane and thereby exposing itself both to the interior as well as the outside of the cell, figure 1.

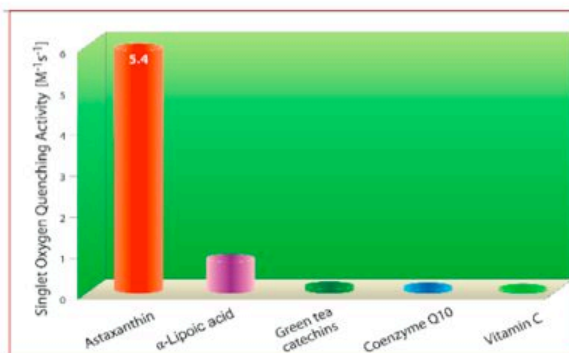
The antioxidant activity of astaxanthin is greater than that of many other well known antioxidants like β -carotene or alfa-tocopherol. Reports on the antioxidant property of astaxanthin includes quenching and scavenging of reactive oxygen species such as singlet oxygen, superoxide radicals and lipid peroxy radicals (Miki 1991, FukuSawa 1998, Nagub 2000). Figure 2 shows the ability of astaxanthin to efficiently quench singlet oxygen *in vitro* compared to some other antioxidants.

The superior antioxidant effect of astaxanthin to other antioxidants is also shown on its effect to protect cultured fibroblast against exposure to singlet oxygen, see figure 3.

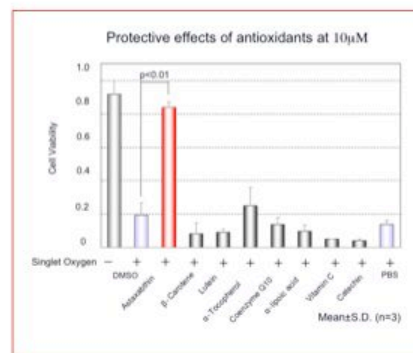


Figur 1. Astaxanthin spans through the cell membrane.

An example of the *in vivo* effect of astaxanthin as antioxidant was seen on its effect to improve functionality of human spermatocytes. Male infertility is often connected to increased frequency of oxidised lipids in the cell membrane of the sperms, which decrease their ability to fuse with the egg cell. In a double-blind, randomised and placebo controlled trial on men with decreased fertility AstaREAL supplementation resulted in a pregnancy rate of 23.1% during the trial period of three months compared to 3.6% in the placebo group. The astaxanthin treatment did not result in increased number of sperms but the functionality was improved which was also seen as improved motility and decreased amount of free radicals in semen (Comhaire et al 2005).



Figur 2. Singlet oxygen quenching rate constants of different antioxidants. Nishida et al., 2007.

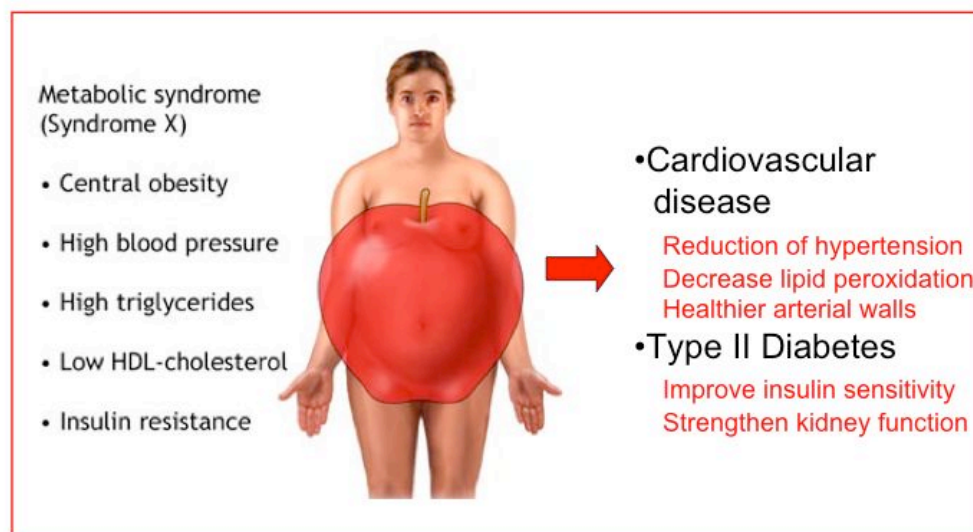


Figur 3. Protective effects of antioxidants on fibroblast against exposure to singlet Oxygen. Tominaga et al., 2009.

Astaxanthin has shown anti-inflammatory effects in several *in vitro* and *in vivo* studies like inhibitory effects of NK-kB (Lee et al 2003) and COX-2 (Choi et al 2008) and balancing the Th1/Th2-response during ongoing infection (Bennedsen et al 1999). The anti-inflammatory effects of astaxanthin is also likely to be its protection of membrane components against oxidation that would otherwise activate the NF-kb and subsequently triggering the pro-inflammatory response.

Metabolic syndrome

Metabolic syndrome is defined as a life-style disease consisting of clusters of multiple metabolic abnormalities and cardiovascular risk factors including hypertension, obesity, hyperlipidemia and insulin resistance. The syndrome has been linked to increased risk of developing Type 2 Diabetes and cardiovascular diseases (CVD). Emerging reports indicate that oxidative stress is an underlying theme that exacerbates inflammation and the development of those health problems. Study results indicate that astaxanthin may have a great potential in the prevention of metabolic syndrome and diseases linked to it.



Figur 4. Factors involved in metabolic syndrome and the potential role of astaxanthin in prevention of diseases connected to metabolic syndrome.

Vascular health

Atherosclerotic plaques are initially developed by lipoproteins, LDL, entering into the intima of the arterial wall. The oxidised lipoproteins attract monocytes. They accumulate the oxidised lipoproteins and turn into macrophages which release inflammatory cytokines by activation of NF-kB. The inflammatory reaction generates free radicals and auto-oxidation of lipoproteins begins. As

the inflammatory reaction proceeds in the arterial wall a plaque consisting of oxidised lipoproteins and foam cells build up. If the plaque ruptures it can cause thrombosis as stroke or heart attack.

Astaxanthin supplementation demonstrated the ability to reduce the oxidation of LDL. In a human trial, the peroxidation of LDL was reduced dose dependently during two weeks of supplementation. A protective effect was seen even at a dose of 1.8 mg astaxanthin/day (Iwamoto et al 2000). This finding has further been supported by another double-blind, placebo controlled study in humans including 40 healthy volunteers that were supplemented with astaxanthin during 8 weeks (Karppi et al 2007). The astaxanthin supplementation significantly reduced oxidation of the most easily oxidised fatty acids in the plasma. Those two studies clearly indicate that astaxanthin can reduce the oxidation of lipids in human plasma.

Studies have also shown that astaxanthin can perform anti-inflammatory effect in the arterial wall and thereby prevent the occurrence of ruptured plaques that can cause thrombosis. Astaxanthin supplementation to rabbits that spontaneously develop atherosclerosis resulted in reduced inflammatory reaction measured as less invading macrophages in the arterial wall. The supplementation also stabilised the plaques and reduced the release of proteolytic enzymes resulting in less ruptured plaques than in the control group (Li et al 2004).

Hypertension is one of the conditions linked to metabolic syndrome and it is also a risk factor for CVD. In studies by Hussein *et al.*, (2005a, 2005b, 2006) it was shown in a mouse model of hypertension that supplementing astaxanthin to the animals significantly reduced the blood pressure compared to the control group. It was found that in the supplemented group the arterial wall was more elastic and the lumen area greater resulting in less resistance. Furthermore, nitric oxide dependent relaxation and sensitivity to constriction mechanisms were improved. These findings most likely contributed to the positive effect on the blood pressure.

These recent findings show that astaxanthin may contribute to the prevention of atherosclerosis and hypertension. Consequently, improvements of overall vascular health can be expected.

Type 2 Diabetes

Insulin resistance is another central component to the cluster of metabolic syndrome. Research revealed a strong link between foods with high glycemic index and prevalence of type 2 diabetes. Excess blood glucose needs to be converted by insulin, produced by the pancreas, into glycogen stores. However, when glycogen stores are full, glucose is converted into fat. Overtime, the body's cells may eventually become desensitized to insulin making it necessary to produce more insulin to achieve the same affect. Eventually, the body loses its ability to control high blood glucose levels (hyperglycemia) that could result in toxic conditions and promote further complications such as kidney failure. It is also thought that high glucose levels induce oxidative stress which triggers a low but chronic inflammatory reaction that by time damage the insulin

producing cells in the pancreas. Chronic high glucose levels could also lead to the pathogenesis of diabetic nephropathy (kidney damage).

Researchers included natural astaxanthin to the diet of type 2 diabetic mice models in controlled studies. They found the following significant results: i) reduction of fasting glucose levels; ii) preservation of insulin levels; and iii) better control in glucose tolerance⁷. The authors concluded that natural astaxanthin may help preserve the pancreas function and insulin sensitivity. Naito *et al.*, 2004, demonstrated additional protective effects of astaxanthin against the progression of kidney damage in type 2 diabetic mice. Significant improvements in the symptoms of renal insufficiency, which normally appear at 16 weeks of age, were detected by analysis of urine and the mesangial area in the kidney glomerulus. The treated mice had 67% less urinary albumin loss, 50% less DNA damage and showed significant preservation of the mesangial area.

Recent studies have revealed that the protective mechanism of astaxanthin in nephropathy includes protection of the mitochondria against oxidative stress due to by high glucose concentrations and by inhibiting the pro-inflammatory response caused by NF- κ B activation (Naito et al 2006, Manabe et al 2008).

These preliminary studies conclude that natural astaxanthin may help manage pre-diabetic conditions, Type 2 diabetic control and delay progressive renal damage.

Obesity

Weight management generally involves two things: i) ingesting less calories and ii) burning more calories. A sensible dietary choice helps with the former and new data suggests that natural astaxanthin may help with the latter in a variety of ways. The first benefit is improvement of lipid metabolism and the second is boosting muscle endurance. The combination of these two effects could mean shedding extra body fat, avoiding rebounds and enjoying a more rewarding exercise experience.

Ikeuchi *et al.*, 2007, demonstrated that even with a high fat diet (40% of daily intake as fat) the weight gain was suppressed in a dose dependent manner with natural astaxanthin. The Japanese researchers noted several significant reductions such as total body weight (15% less), liver weight, adipose tissue (34% less), liver triglycerides (58% less), plasma triglyceride, and total cholesterol in a controlled animal study that lasted 60 days.

Natural astaxanthin in combination with exercise had the greatest effect than with exercise or supplementation alone (Ikeuchi et al 2006, Aoi et al 2008). The working theory for body fat reduction is the improvement of lipid metabolism in muscle and synergy with exercise. The underlying mechanism of the effects of astaxanthin seems again to be protection of components in the mitochondria from oxidative stress. Astaxanthin protects enzymes located in the membranes of the mitochondria against oxidation. Oxidative stress generated as a by-product during energy generation can impair lipid metabolism. One of these

enzymes is CPT 1. It imports lipids into the mitochondria to be used as fuel for generating energy (Aoi et al 2008). Another mitochondrial enzyme is 3-HAD, which is involved in the metabolism of fatty acids. There are also reports of increased utilization of fatty acids as the primary energy source after respiratory exchange analysis (Ikeuchi et al 2006, Aoi et al 2008). Such indications suggest that natural astaxanthin in combination with exercise promotes lipid metabolism or “fat-burning”.

Muscle

Free radicals are generated in our muscles and the amount increase radically during exercise and heavy physical activity. Those free radicals can directly damage the muscle cells and also trigger a inflammation reaction which we experience as stiffness and muscle pain.

Natural astaxanthin can increase muscle performance and boost endurance levels . The mechanism is not fully understood but this benefit is supported by several reports (Malmsten et al 2008). The first is protection of skeletal muscle cell membrane from ROS damage during strenuous physical activity (Figure 5). After strenuous exercise astaxanthin reduced peroxidation damage of heart and leg muscle cells, reduced DNA damage, and lowered inflammatory markers (Aoi et al 2003). This means less muscle soreness and shorter recovery times between exercise sessions. Secondly, natural astaxanthin improves the blood rheology which means more oxygen and fuel reaches the muscles and better removal of waste (Miyawaki et al 2005). The underlying benefits could explain why there is significantly lower lactic acid build-up and increased endurance levels in animals and humans during swimming or running (Sawaki et al 2002, Ikeuchi et al 2006).

Endurance benefits will make physical activity more enjoyable which is perhaps the most important factor to tackle metabolic syndrome.



Gastric health

The bacteria *Helicobacter pylori* can cause ulcer and stomach cancer. *H. pylori* infection has been associated with generation of free radicals, which leads to oxidative stress in the gastric mucosa (Naito et al., 2002). *H. pylori* induces infiltration and activation of neutrophils, which produces inflammatory mediators that include free radicals. These mediators contribute to oxidative stress on the gastric epithelium in the immediate vicinity. Studies in *H. pylori* infected mice indicate that astaxanthin reduced oxidative stress and subsequent effects on neutrophilic leukocytes and activated macrophages recruitment in the gastric mucosa (Bennedsen et al., 1999). Testing *H. pylori*-infected animals, treatment with astaxanthin was shown to reduce gastric inflammation and the bacterial load and modulating cytokine release by splenocytes by down regulating the Th1 response caused by the bacteria in favour of a normalised Th1/Th2 response (Bennedsen et al., 1999). This over active Th1 response is regulated by activation of NF-kB (Mohamed et al., 2006). Activation of NF-kB by reactive oxygen species in both *in vitro* and *in vivo* have been shown to be inhibited by astaxanthin (Lee et al., 2003). Astaxanthin has furthermore been shown to protect gastric mucosa from ulceration by its antioxidant properties in animal models (Kim et al. 2005a, b; Nishikawa et al 2005). Oxidative stress in the esophagus is also important in the development of gastroesophageal reflux disease (Oh et al., 2001; Wetscher et al., 1995).

Astaxanthin treatment of *H. pylori* positive dyspeptic patients in an open study resulted in reduced symptoms in all patients and reduction of gastric inflammation in 6 out of 10 patients (Lignell et al., 1999). The reduction in reflux symptom was most marked. Greater reduction of reflux syndrome was also obtained recently in a double-blind, randomised placebo-controlled study. The response was more pronounced in *H. pylori*-infected patients (Kupcinskis et al 2008).

The results show that astaxanthin is usable to alleviate dyspeptic symptoms and it also indicate that astaxanthin has a role in controlling infections of *H. pylori* and to keep the immune system in balance.

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AstaREAL – natural astaxanthin

The source of astaxanthin used in the clinical trials referred to in this paper is AstaREAL. Natural astaxanthin produced by cultivation of the unicellular alga *Haematococcus pluvialis* cultivated under strict control at BioReal's facility in Gustavsberg, Sweden.

AstaREAL is offered in different forms to suit different applications;
AstaREAL A1010, powder, homogenised and dried biomass containing 5% astaxanthin.

AstaREAL L10, oleoresin, supercritical extract of the biomass containing 10% astaxanthin.

AstaREAL P2AF, powder, encapsulated oleoresin containing 1.8% astaxanthin.

AstaREAL is approved for use in food supplements in Europe, USA, Japan and most other countries.

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