AstaReal® Astaxanthin for Dry Eye Applications
Performance: Prevalence of Dry Eye is Staggering

- The prevalence of Dry Eye Syndrome increases with age and affects 7.8%, or (3.23 million) women and 4.3% or (1.68 million) men aged 50+ in the US\textsuperscript{1,2}.
- Dry eye is more common in people age 50 years or older.\textsuperscript{3}
  - Natural decline in tear production
- Dry Eye 2X more common in Women than in Men\textsuperscript{3}
  - Hormonal changes


THE DRY EYE DISEASE CONVERSATION GAP
Reasons Why Discussing DED Sooner Matters

An estimated 30M

U.S. adults report symptoms consistent with dry eye.*

*Based on a dry eye prevalence of 14.5% from the 2014 BESS (Beaver Dam Eye Study) of self-reported symptoms and the 2014 U.S. Census estimate of adults ages 25 to 84 years.

Survey found that people wait about 2 years between when dry eye symptoms first occur and when they go to an eye doctor even though these symptoms affects their daily life.
How AstaReal® Can Complement Dry Eye Products

1. Antioxidant protection for ocular surface and lens
2. Anti-inflammatory action to support lacrimation
3. Promoting ocular hydration through aquaporin 5
4. Reducing subjective symptoms associated with dry eye
5. Supporting lacrimal glad function
Antioxidant Protection for the Ocular Surface & the Lens
Free Radicals Can Cause Damage to our Eyes

- Excess levels of ocular oxidants can cause damage to the lens and other tissues, and is implicated with pathogenesis of retinopathies including diabetic retinopathy, glaucoma, and senile cataract.

- Oxidants in the eye induce cross-linking, aggregation, fragmentation, and insolubilization of structural proteins, inactivation of enzymes, and lipid peroxidation (LPO) of the membrane-bound polyunsaturated fatty acids (PUFA), leading to impaired cell function, apoptosis, and necrosis\(^1,2,3\).

### AstaReal® Clinical Investment: Antioxidant Capacity

#### Significant Improvement in 100% (6/6) AstaReal® Astaxanthin studies for Antioxidant Capacity

<table>
<thead>
<tr>
<th>Reference</th>
<th>Dose</th>
<th>Intake Period</th>
<th># Participants (Total)</th>
<th>Age</th>
<th>Antioxidant Capacity Result</th>
</tr>
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<tbody>
<tr>
<td>Hashimoto et al. 2016</td>
<td>6mg/day</td>
<td>2 weeks</td>
<td>35</td>
<td>Average 71</td>
<td>Increased superoxide scavenging activity, reduced total hydroperoxides</td>
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<td>Hashimoto et al. 2013</td>
<td>6mg/day</td>
<td>2 weeks</td>
<td>35</td>
<td>Average 71</td>
<td>Increased superoxide scavenging activity, reduced total hydroperoxides</td>
</tr>
<tr>
<td>Hashimoto et al. 2011</td>
<td>6mg/day</td>
<td>2 weeks</td>
<td>35</td>
<td>Average 70.6</td>
<td>Reduced total hydroperoxides</td>
</tr>
<tr>
<td>Hashimoto et al. 2009</td>
<td>6mg/day</td>
<td>2 weeks</td>
<td>35</td>
<td>Average 70</td>
<td>Increased superoxide scavenging activity</td>
</tr>
<tr>
<td>Iwabayashi et al. 2009</td>
<td>12mg/day</td>
<td>8 weeks</td>
<td>20</td>
<td>Average 57</td>
<td>Blood Antioxidant Capacity (BAP) significantly increased (+4.6%, p=0.030).</td>
</tr>
<tr>
<td>Hashimoto et al. 2007</td>
<td>6mg/day</td>
<td>2 weeks</td>
<td>37</td>
<td>Average 72</td>
<td>Lower flare intensity after surgery (light reflecting off proteins in aqueous humor)</td>
</tr>
</tbody>
</table>

Total: 197 participants
Primary Antioxidant Defense in Tears

The ocular surface composed of the *tear film, the cornea, and the aqueous humor* forms the first physical and biochemical barrier of the eye and plays a *pivotal role in combating free radicals*.

The following AstaReal® Astaxanthin human clinical studies show improved antioxidant capacity:

Iwabayashi et al. 2009 showed improved BAP
Hashimoto et al. 2009 showed improved superoxide scavenging activity
Yamada et al. 2010 showed suppression of H2O2 induced ROS
Hashimoto et al. 2011 showed improved superoxide scavenging activity
Djordevic et al. 2012 shows superoxide levels did not increase after exercise in astaxanthin group
Baralic et al. 2012 showed SH groups increased in astaxanthin group
Hashimoto et al. 2013 showed improved superoxide scavenging activity
Yagi et al. 2013 showed reduced urinary 8-OHdG
Baralic et al. 2015 showed improved pro-oxidant to antioxidant balance
Fujino et al. 2016 showed reduced d-ROM
AstaReal® Astaxanthin Supports Antioxidant Protection of the Lens

Ciliary Body:
1. **Ciliary muscles** - smooth muscle bundles that control shape of the lens.
2. **Ciliary processes** – *actively secrete fluid (aqueous humor)* that fills anterior of the eye, supplying nutrients and oxygen to avascular lens and cornea, and carries away metabolic waste. Also controls pressure within the eye.
3. **Ciliary zonules** – suspensory ligaments extend ciliary processes to lens to hold lens in upright position.

AstaReal® Astaxanthin Supports Antioxidant Capacity of the Aqueous Humor for Lens Health.
Anti-inflammatory Action to Support Lacrimation
Dry Eye Stress Induces Inflammation Response

1. Hyperosmolarity → signals production of proinflammatory cytokines

2. Activation of innate and adaptive immune responses

3. Further ocular surface damage
Innate immune responses to a variety of environmental stresses stem from ROS-induced cytosolic NLRP3 inflammasome activation.

- Increased ROS is associated with ocular surface epithelial damage and can induce lacrimal gland dysfunction and dry eye.
- An imbalance between a radical scavenging system and ROS generation in the tears of patients with DES may cause ocular surface injury.
- Antioxidants can limit the harmful effect of ROS
Inflammation Studies with Astaxanthin

Astaxanthin has been shown to downregulate NF-κB mediated inflammatory response.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Dose</th>
<th>Intake Period</th>
<th>Model</th>
<th>Age</th>
<th>Inflammation Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeh et al. 2016</td>
<td>0.6mg/kg or 3mg/kg</td>
<td>8 weeks (intragastric feeding)</td>
<td>Wistar rats</td>
<td>6-8 weeks old</td>
<td>Reduced oxidative stress and inflammatory mediators and NF-κB. Increased antioxidant enzyme levels.</td>
</tr>
<tr>
<td>Lennikov et al. 2012</td>
<td>1, 0.1, and 0.01 mg/ml</td>
<td>Single dose (15μl eye drop)</td>
<td>C57BL/6 mice</td>
<td>6-8 weeks old</td>
<td>Improved survival, corneal thickness (p&lt;0.01), and reduced NF-κB (p&lt;0.05) and oxidative stress in UVB-irradiated corneas.</td>
</tr>
<tr>
<td>Izumi-Nagai et al. 2008</td>
<td>1, 10, or 100 mg/kg</td>
<td>3 days (intraperitoneal injections)</td>
<td>C57BL/6J mice</td>
<td>6-8 weeks old</td>
<td>Suppression of NF-κB, inflammatory markers, macrophage infiltration, and choroidal neovascularization development.</td>
</tr>
<tr>
<td>Hashimoto et al. 2007</td>
<td>6mg/day</td>
<td>6 days</td>
<td>37 patients undergoing cataract surgery</td>
<td>Average 71</td>
<td>3 days after surgery, flare intensity was significantly lower (00.8± 3.03 vs. 13.6±5.57 photon count/msec, p&lt;0.01).</td>
</tr>
<tr>
<td>Suzuki et al. 2006</td>
<td>1, 10, or 100 mg/kg</td>
<td>Single dose (intravenous)</td>
<td>Lewis rats</td>
<td>8 weeks old</td>
<td>Reduced LPS-induced accumulation of protein, NO, TNF-α, PGE2 in aqueous humor, inhibition of NF-κB.</td>
</tr>
<tr>
<td>Ohgami et al. 2003</td>
<td>1, 10, or 100 mg/kg</td>
<td>Single dose (intravenous)</td>
<td>Lewis rats</td>
<td>8 weeks old</td>
<td>Suppression of endotoxin-induced uveitis, reduced activity of iNOS, reduced NO, TNF-α, PGE2.</td>
</tr>
</tbody>
</table>
Salivation as a Model for Lacrimation
Astaxanthin Increases Aquaporin 5 Expression in Aging Mice

AQP-5 (water transporter) is present in the salivary and lacrimal glands, the tracheal gills, the eyes, and the lungs and plays a role in the generation of saliva, tears, and pulmonary secretions.

Greater AQP-5 Expression in AX Group Compared to Control

![Control Group](image1.png) ![Astaxanthin Group](image2.png)

**Fig. 9.** Immunohistochemically stained image of aquaporin (AQP)-5-positive domain in the mandibular gland (A) Control group. (B) Astaxanthin (AX) group. Mandibular gland of AX group shows numerous AQP-5-positive cells while that of control group shows few.

**Fig. 10.** Positive area of aquaporin (AQP)-5. Positive domain of AQP-5 in astaxanthin (AX) group covered a larger area than that of control group.

**Astaxanthin Improves Salivation and BAP in Aging Mice**

**Fig. 2.** Comparison of saliva amounts between astaxanthin (AX) and control groups. Saliva flow of AX group was significantly higher than that of control group (p<0.05).

**Fig. 4.** Comparison of biological antioxidative potential (BAP) values of astaxanthin (AX) and control groups. BAP value of AX group 72 weeks after AX administration was significantly higher than that of control group.

AstaReal
The Natural Astaxanthin of Choice
Sjogren's syndrome (pronounced SHOW-grins; also spelled Sjögren's) is an autoimmune disease

- Attacks and destroys glands responsible for keeping the eyes, mouth and other parts of the body moist and lubricated.
- Chronic dry eye is one of the major symptoms of Sjogren's syndrome
  - decreased secretion of tears by the lacrimal (tear-producing) glands
  - loss of tears due to excess evaporation.
- Sjogren’s syndrome affects as many as 4 million Americans, and ~90% are women.

ROS elicit a “danger signal” that activates the hypersensitive immune response of SS patients resulting in dysfunction and destruction of salivary and lacrimal glands.

https://www.allaboutvision.com/conditions/sjogrens-syndrome.htm
50 mg/day AstaReal® Astaxanthin administered intravenously before irradiation resulted in the suppression of salivary impairment compared to control \((p<0.05)\).

Astaxanthin did not have a therapeutic effect when administered after irradiation.

Oxidative Stress Markers In Sjörgen’s Syndrome Group

Oxidative stress marker 8-hydroxy-2'-deoxyguanosine (8-OHdG) and the lipid peroxidation marker hexanoyl-lysine (HEL) were significantly elevated in Sjörgen’s Syndrome group.

A

\[ p = 0.008 \]

B

\[ p = 0.005 \]

12 individuals were divided into two groups supplementing with 12mg/day AstaReal® for 2 weeks.

- Sjögren's syndrome patients with dry mouth (n=6, average age 65)
- Healthy volunteers (n=6, average age 29)

**Astaxanthin Significantly Decreased Lipid Peroxidation in Sjorgen’s Syndrome Group**

8-OHdG and HEL measured from saliva samples

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Astaxanthin Supports Healthy Saliva Production in Healthy Individuals

Saliva output improved in healthy group but not in Sjorgen’s Syndrome Group. This is consistent with mouse results showing astaxanthin was preventative but not therapeutic.

Reducing Subjective Symptoms Associated with Dry Eye
Astaxanthin Improves Subjective Symptoms Associated with Dry Eye

Signs and symptoms, which usually affect both eyes, may include:

- A stinging, burning or scratchy sensation in your eyes
- Stringy mucus in or around your eyes
- **Sensitivity to light**
- **Eye redness**
- A sensation of having something in your eyes
- Difficulty wearing contact lenses
- Difficulty with nighttime driving
- Watery eyes, which is the body’s response to the irritation of dry eyes
- **Blurred vision or eye fatigue**

https://www.mayoclinic.org/diseases-conditions/dry-eyes/symptoms-causes/syc-20371863

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<tr>
<th>Reference</th>
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<th># Participants (Total)</th>
<th>Age</th>
<th>Dry Eye Result</th>
</tr>
</thead>
</table>
| Kajita et al. 2009 | 6mg/day| 4 weeks       | 22                     | 45-65 (average 54)   | 15% reported improvement in “lacrimation”  
19% reported improvement in “eye redness”  
46% reported improvement in “ocular pain” |
## AstaReal® Clinical Investment: Eye Strain

<table>
<thead>
<tr>
<th>Reference</th>
<th>Dose</th>
<th>Intake Period</th>
<th># Participants (Total)</th>
<th>Age</th>
<th>Accommodation result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nagaki et al. 2010</td>
<td>9mg/day</td>
<td>4 weeks</td>
<td>82</td>
<td>30-45 (average 39)</td>
<td>“eyestrain” (p&lt;0.05 @4wk), “hazy vision” (p&lt;0.05 @2wk, p&lt;0.01 @4wk), “flickering images” (p&lt;0.05 @4wk), “my shoulders/back feel stiff” (p&lt;0.05 @2wk and @4wk) significantly improved in AX vs. control.</td>
</tr>
<tr>
<td>Iwasaki et al. 2006</td>
<td>6mg/day</td>
<td>2 weeks</td>
<td>10</td>
<td>18-21 (average 20.5)</td>
<td>No survey items had significant difference between the two groups for Pre values, Post values and Rest values.</td>
</tr>
<tr>
<td>Nagaki et al. 2005</td>
<td>6mg/day</td>
<td>4 weeks</td>
<td>36</td>
<td>Average 41</td>
<td>Improvement tendency in “My eyes tire easily,” a significant improvement in “My eyes get red easily” and an improvement tendency in “My eyes can’t focus properly” in AX vs. control.</td>
</tr>
<tr>
<td>Nagaki et al. 2006</td>
<td>6mg/day</td>
<td>4 weeks</td>
<td>48</td>
<td>30-45 (average 37)</td>
<td>Significant improvement in “dimness of sight” and “stiff shoulders and back,” and an improvement tendency for “heavy head” in AX vs. placebo.</td>
</tr>
<tr>
<td>Nitta et al. 2005</td>
<td>6 and 12mg/day</td>
<td>4 weeks</td>
<td>30</td>
<td>23-49 (average 35)</td>
<td>No significant differences in AX vs. placebo. After 4 weeks AX 6mg/day group showed significant improvement compared to 0 weeks (which were not observed in placebo group) for: “My eyes hurt,” p&lt;0.05; “My eyesight is dim,” p&lt;0.01; “My eyes get red easily,” p&lt;0.05; “My shoulders and waist are stiff,” p&lt;0.01; “I get irritated easily,” p&lt;0.05; “My head becomes heavy easily,” p&lt;0.05; “The insides of my eyes are painful,” p&lt;0.05; “My eyes get bleary,” p&lt;0.01; “My eyelids twitch,” p&lt;0.05. After 4 weeks AX 12mg/day group showed significant improvement compared to 0 weeks (which were not observed in placebo group) for: “The insides of my eyes are painful,” p&lt;0.05; “My eyes get bleary,” p&lt;0.05; “Light is too bright” p&lt;0.05.</td>
</tr>
<tr>
<td>Shiratori et al. 2005</td>
<td>6mg/day</td>
<td>4 weeks</td>
<td>39</td>
<td>20-60 (average 30)</td>
<td>“blear-eye feeling” and “tendency of irritation” significantly improved in AX vs. placebo (p&lt;0.05)</td>
</tr>
<tr>
<td>Takahashi et al. 2005</td>
<td>6mg/day</td>
<td>2 weeks</td>
<td>9</td>
<td>30-42</td>
<td>A 0 weeks, four subjects stated their eyes had become tired because of VDT work, but after 2 weeks AX, this had decreased to two subjects. Following 20min rest after VDT work, at 0 weeks no subjects reported improvement in fatigue as the result of rest, but after 2 weeks AX, two subjects responded their eyes had recovered.</td>
</tr>
<tr>
<td>Iwabayashi et al. 2009</td>
<td>12mg/day</td>
<td>8 weeks</td>
<td>20</td>
<td>Average 57</td>
<td>Significant improvement in “tired eyes” “stiff shoulders” after 8 weeks compared to 0 weeks (p&lt;0.05).</td>
</tr>
<tr>
<td>Nagaki et al. 2002</td>
<td>5mg/day</td>
<td>4 weeks</td>
<td>39</td>
<td>Average 48</td>
<td>At beginning of study all participants complained of eye strain. After 4 weeks 7 of 13 VDT workers at 0mg/day AX no longer reported eye strain, while 12 of 13 VDT workers at 5mg/day AX no longer reported eye strain. Between group difference was statistically significant (p&lt;0.05).</td>
</tr>
</tbody>
</table>
Supporting Lacrimal Glad Function
Dry Eye Studies with Astaxanthin

VDT-related Dry Eye is associated with tear film instability despite a normal lacrimal secretion, also known as short tear break-up time (BUT) Dry Eye. It is also associated with **ciliary stress, ciliary spasms, and eye fatigue** that is measured as and increase in accommodative microfluctuations (high frequency component HFC; between 1.0 and 2.3 Hz)\(^1\).

### Improvement in 33% (2/3) studies for Dry Eye

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<th>Reference</th>
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<th>Dry Eye Result</th>
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</thead>
<tbody>
<tr>
<td>Huang et al. 2016</td>
<td>4mg/day plus other ingredients</td>
<td>8 weeks</td>
<td>43</td>
<td>20-75</td>
<td>Tear film breakup time and Schirmer’s test without topical anesthesia (reflex tears from the main lacrimal gland) significantly improved (p&lt;0.05). Tear ROS decreased after treatment (p&lt;0.05). Subjective improvement of dry eye (p&lt;0.05).</td>
</tr>
<tr>
<td>Keio Univ. Ophthalmology. Unpublished.</td>
<td>0.02% mixed in feed</td>
<td>4 months</td>
<td>N/A</td>
<td>db/db obese mouse</td>
<td>Significant effect of the amounts of lacrimal fluid and lacrimal secretion per lacrimal weight in 4 months.</td>
</tr>
<tr>
<td>Kajita et al. 2009</td>
<td>6mg/day</td>
<td>4 weeks</td>
<td>22</td>
<td>45-65 (average 54)</td>
<td>15% reported improvement in “lacration” 19% reported improvement in “eye redness” 46% reported improvement in “ocular pain”</td>
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Thank You!
Questions?