Astaxanthin Supplementation Does Not Attenuate Muscle Injury Following Eccentric Exercise in Resistance-Trained Men

Richard J. Bloomer, Andrew Fry, Brian Schilling, Loren Chiu, Naruhiro Hori, and Lawrence Weiss

This investigation was designed to determine the effects of astaxanthin on markers of skeletal muscle injury. Twenty resistance trained men (mean ± standard error of the mean: age, 25.1 ± 1.6 y; height, 1.79 ± 0.02 m; weight, 86.8 ± 4.4 kg) were assigned to either a placebo (1732 mg safflower oil, \( n = 10 \)) or astaxanthin (BioAstin; 1732 mg safflower oil; haematococcus algae extract [contains 4 mg astaxanthin and 480 mg lutein], \( n = 10 \)). Subjects consumed their assigned treatment for 3 wk prior to eccentric exercise (10 sets of 10 repetitions at 85% of one repetition maximum) and through 96 h post-exercise. Muscle soreness, creatine kinase (CK), and muscle performance was measured before and through 96 h post-exercise. A similar response was observed for both treatment groups for all dependent variables, indicating that in resistance trained men, astaxanthin supplementation does not favorably affect indirect markers of skeletal muscle injury following eccentric loading.

**Key Words:** dietary supplements, exercise, muscle force, carotenoids

It is well documented that unaccustomed or strenuous exercise, particularly involving eccentric muscle actions, induces skeletal muscle injury resulting in a variety of signs and symptoms (9, 13). Aside from the direct measure of cytoskeleton disruption, several indirect markers exist to lend information regarding the degree of muscle injury. These include affected limb girth, range of motion, muscle soreness, blood borne variables such as creatine kinase (CK) and various interleukins, as well as markers of muscle performance (13). Due to the great degree of variability between subjects with respect to several of these variables, it has been suggested that the most reliable indirect marker of skeletal muscle injury is that of muscle performance (37).

In an attempt to suppress skeletal muscle injury following acute exercise, a variety of nutritional supplements have been examined. These have included protein and carbohydrate mixtures (39), creatine monohydrate (30), and HMβ (16, 21, 26), all of which have yielded limited benefit. Nutritional supplements that appear to hold promise include the antioxidant vitamins and minerals, in particular...
vitamins C and E (14). While some investigators have reported little or no effect from antioxidant supplementation (4, 8, 38), others have indeed reported beneficial effects (6, 20, 24).

It has been suggested that free radicals might play a role in both the initiation and the progression of muscle fiber injury (5, 18, 23, 33, 35, 40). This could occur as a result of transient periods of ischemia/reperfusion and the generation of xanthine oxidase. In addition, neutrophil respiratory burst activity might give rise to reactive oxygen species (19), either during or following strenuous eccentric exercise. While the role of free radicals in muscle injury remains controversial, the reality remains that numerous products are marketed to the fitness and bodybuilding community claiming to suppress free radical mediated damage and enhance exercise recovery. Most often, these products include the antioxidant vitamins and minerals, either alone or in combination. The theory behind these recommendations is that antioxidant therapy could assist in preventing further fiber injury following the initial mechanical insult, perhaps by minimizing the degrading effects of reactive oxygen species. In relation to this, most investigations have used the water-soluble vitamin C, either alone or in conjunction with the lipid-soluble vitamin E, with reasonable success. Few other antioxidant vitamins or minerals have been used in an attempt to suppress muscle fiber injury following damaging exercise.

The carotenoid astaxanthin has been suggested to exhibit antioxidant properties superior to most other antioxidant agents, and has been suggested to provide cell membrane stability, in addition to acting to suppress inflammation (15, 25). Due to its antioxidant properties, astaxanthin is used extensively in the nutraceutical, cosmetics, food, and feed industries. It was recently reported that astaxanthin can attenuate exercise-induced damage in mouse skeletal and heart muscle, including the associated neutrophil infiltration that might potentiate further injury (1). This was the first investigation to report the effects of astaxanthin in suppressing muscle fiber injury in vivo. To date, no data are available in reference to astaxanthin and human subjects exposed to muscle-injury inducing exercise.

The purpose of the present research was to determine the effects of astaxanthin supplementation on various indirect markers of muscle injury following eccentric exercise in trained men, with a particular emphasis on muscle performance measures. We hypothesized that astaxanthin supplementation would attenuate the typical increase in muscle soreness and CK, in addition to possibly resulting in a quicker muscle force recovery than is normally observed following damaging eccentric exercise.

**Methods**

**Subjects**

Twenty weight-trained men volunteered to participate as subjects following explanation of all experimental procedures, which were approved by the University of Memphis Human Subjects Committee. This population was chosen since these are the individuals most likely to train with the volume and intensity required to induce muscle injury on a regular basis, and hence, potentially benefit from the use of astaxanthin. In addition, the target market for use of dietary supplements to suppress muscle injury is those who regularly perform resistance exercise. All subjects had been weight training their lower body for a minimum of 12 months
prior to testing and demonstrated a minimum strength of 1.5 times their body weight in the barbell back squat exercise. A medical history and physical activity questionnaire was completed by all subjects to determine eligibility. All subjects were free of orthopedic and metabolic conditions that could have affected the variables of measurement. Subject descriptive characteristics are shown in Table 1.

**Treatment**

Subjects were randomized in double blind manner to either a placebo condition (1732 mg safflower oil, \( n = 10 \)) or astaxanthin (BioAstin; 1732 mg safflower oil; haematococcus algae extract [contains 4 mg astaxanthin and 480 mg lutein], \( n = 10 \)), to be consumed for a 3-wk period prior to the eccentric exercise. Each subject ingested 2 capsules per day to allow for the above dosages, as recommended by the manufacturer (Cyanotech Corp., Kailua-Kona, HI), and as sold on the nutritional supplement market. All subjects received the same dosage which was not determined based on body weight but was similar to, although slightly lower than that previously demonstrated to be beneficial and safe in mice (1) and humans (31). During the 4-d period following the eccentric exercise protocol, all subjects continued to ingest the prescribed astaxanthin or placebo capsules to monitor the recovery phase. Subjects were instructed to maintain their normal diet and exercise habits during the study period and were to refrain from exercise for the 2-d period prior to the eccentric exercise protocol, in addition to the 4 d following the protocol. On study completion, subjects returned their capsule bottles to ensure that the supplements were taken as prescribed.

**Eccentric Exercise**

Following the 3-wk supplementation period, each subject participated in an eccentric resistance exercise session designed to induce muscle injury. A York knee extension machine was modified to permit the tester to lift the resistance so that the subject performed only the eccentric portion of the knee extension exercise. Subjects performed a concentric 1 repetition maximum (1-RM) at maximal velocity and an eccentric 1-RM at an average velocity of 0.52 rad \( \cdot s^{-1} \) (30° \( \cdot s^{-1} \)) to be used as

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Descriptive Characteristics of 20 Resistance-Trained Men</th>
</tr>
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<tbody>
<tr>
<td>Variable</td>
<td>Astaxanthin</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
</tr>
<tr>
<td>Age (y)</td>
<td>24.0 ± 1.1</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.78 ± 0.02</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>84.4 ± 5.2</td>
</tr>
<tr>
<td>Wgt. training experience (y)</td>
<td>3.7 ± 1.3</td>
</tr>
<tr>
<td>Squat 1-RM (kg)*</td>
<td>129.9 ± 8.3</td>
</tr>
<tr>
<td>Wgt. training freq. (× · wk)</td>
<td>2.3 ± 0.3</td>
</tr>
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*With the exception of Squat 1-RM, there were no significant differences between the astaxanthin and placebo groups for any variables (\( P > 0.05 \)).
baseline values. Then, a series of eccentric muscle actions were performed using the dominant leg. Ten sets of 10 eccentric repetitions were performed, starting with 85% of the eccentric 1-RM load. When the subject was unable to maintain the required lowering velocity (0.52 rad · s⁻¹) throughout a given set, the load was decreased by approximately 3.6 kg for the subsequent sets. This occasional failure on the part of the subject to maintain the required lowering velocity resulted in the performance of 7 to 10 repetitions per set, as opposed to the originally planned 10 repetitions. It should be noted that neither the absolute (51 vs. 48 kg) nor the relative (0.57 kg/kg body mass vs. 0.56 kg/kg body mass) workloads per repetition were different between the placebo and astaxanthin conditions, respectively. A timing system was interfaced with the weight machine to insure adherence with the lifting velocity requirements. This protocol has been previously used to induce muscle injury and has been reported in the scientific literature (12).

**Dependent Variables**

Pre-exercise and at 0, 10, 24, 48, 72, and 96 h post-eccentric exercise, the following dependent variables were measured and compared between conditions: muscle soreness, CK, 1-RM concentric strength, mean isometric force (MIF), and mean dynamic force (MDF) across the loading spectrum (90%, 65%, and 40% concentric 1-RM). A focus was placed on the various measures of muscle performance, as these have been suggested to best represent muscle fiber injury (37).

**Tests**

Muscle soreness was measured in the dominant leg during knee extension using a 10 cm visual analog scale as previously described (10). Approximately 5 mL of blood was taken from an antecubital vein by Vacutainer and immediately processed for CK using standard procedures (Quest Laboratories).

One repetition maximum (1-RM) concentric strength was determined in the knee extension exercise using a Body-Solid knee extension machine (Body-Solid, Inc., Forest Park, IL). After a standardized warm-up, single repetitions were performed with increasing loads until the subjects were unable to lift the weight. Approximately 1 min of rest was allowed between each repetition, with a total of 2 to 4 lifts required to reach 1-RM. Intraclass correlation coefficients for strength tests in our laboratory have consistently been 0.95 or greater.

The isometric knee extensor action was performed using a modified York barbell (York, PA) knee extension/flexion machine. The cable length was adjusted so that the knee was at 90° flexion. A load cell (model MLP-500, Transducer Techniques, Temecula, CA) was attached in-line with the machine cable and attached to the frame. The signal was sent through a signal conditioner (model TMO-2, Transducer Techniques), which serves as a power source for the load cell and amplifies the output signal. The signal conditioner was calibrated prior to each trial. From the signal conditioner, a 0 to 5 VDC electrical output was channeled through an analog-to-digital board interfaced with a computer. Data were sampled at 1000 Hz using the analog module of the Ariel Performance Analysis System (APAS version 9.50; Ariel Dynamics, San Diego, CA) and filtered using a fourth-order recursive Butterworth.
The isometric protocol consisted of two 4 to 5 s unilateral quadriceps contrac-
tions. All trials were performed with the affected leg. Prior to each trial, subjects were instructed to contract as hard and fast as possible on a verbal command signal. Subjects were instructed to stop contracting immediately upon verbal command. A 5-s pause was given before a second contraction was performed. The best contraction, based on the highest mean rate of force development was used for analysis. Intraclass correlation coefficients for performance variables have routinely been determined in our lab to be over the minimal acceptable level of 0.7 (3).

Mean dynamic force was determined in the knee extension exercise using a Body-Solid knee extension machine interfaced with a Fitrodyne dynamometer (Fitronic, Bratislava, Slovakia). Loads of 90%, 65%, and 40% concentric 1-RM were used to provide measures across the loading spectrum. The knee extension machine was modified to permit each subject to extend the knee with maximum acceleration until the knee was almost fully extended (approximately 170° flexion).

Prior to the eccentric exercise protocol, two familiarization sessions were included during the supplementation phase to thoroughly introduce each subject to the various performance tests described above.

Statistical Analyses

The data obtained for all dependent variables were analyzed using a 2 × 7 repeated measures ANOVA. Significant interactions and main effects were further analyzed using a priori contrasts. Data were analyzed using commercially available software (SPSS, Inc., Chicago, IL). Alpha was maintained at $P < 0.05$ for all comparisons. The data are presented as means ± standard error of the mean.

Results

All subjects successfully completed testing with 100% compliance. With the exception of squat 1-RM being higher for the placebo condition ($P < 0.05$), subject characteristics were not statistically different between conditions.

Muscle Soreness

In both conditions, muscle soreness was increased following exercise, with no between-condition differences noted ($P > 0.05$). Subjects reported increased muscle soreness immediately post-exercise that remained elevated above pre-exercise through 96 h post-exercise in both conditions ($P < 0.05$, Figure 1).

Creatine Kinase

Creatine kinase was elevated above pre-exercise in both conditions as early as 10 h post-exercise and remained elevated through 96 h post-exercise ($P < 0.05$). The greatest elevation was noted for the astaxanthin condition at 72 and 96 h post-exercise, attributed to the large increase in 3 subjects within this condition. No differences were noted between conditions at any time ($P > 0.05$, Figure 2).
Figure 1—Condition by Time for Muscle Soreness

Note. Values are means ± standard error of the mean. *Different from pre-exercise ($P < 0.05$).

Figure 2—Condition by Time for Creatine Kinase

Note. Values are means ± standard error of the mean. *Different from pre-exercise ($P < 0.05$).
Measures of Muscle Performance

The eccentric exercise resulted in significantly attenuated 1-RM concentric strength from 0 h post-exercise through 48 h post-exercise in both conditions \((P < 0.05, \text{ Figure 3})\). The 1-RM concentric strength decreased between 8 to 20% for both conditions and no between-condition differences were noted \((P > 0.05)\).

MIF was lower in both conditions at 24 and 48 h post-exercise compared to pre-exercise \((P < 0.05)\) and was not different between conditions at any time \((P > 0.05, \text{ Figure 4})\). Peak isometric force exhibited the same results, and did not provide further insight (data not shown).

With respect to MDF, values were below pre-exercise across the loading spectrum (at 90%, 65%, and 40% 1-RM) in both conditions. MDF, whether expressed in absolute terms (Figure 5) or as a percent of pre-exercise values, was lower in the astaxanthin condition compared to the placebo condition from 10 h through 72 h post-exercise at all loads \((P < 0.05)\).

Discussion

The main findings of this investigation indicate that astaxanthin as supplemented in the present study: 1) had no favorable effect on muscle soreness, CK, concentric 1-RM, or MIF; and 2) resulted in a greater decrease in MDF from 10 through 72 h post-exercise compared to the placebo condition. It should be noted that this is the first investigation in humans to study the effects of the carotenoid astaxanthin.

![Figure 3](image-url)  
**Figure 3**—Condition by Time for 1-RM Concentric Strength  
*Note.* Values are means ± standard error of the mean. *Different from pre-exercise \((P < 0.05)\).*
Figure 4—Condition by Time for Mean Isometric Force
*Note. Values are means ± standard error of the mean. *Different from pre-exercise (P < 0.05).

Figure 5—Condition by Time for Mean Dynamic Force
*Note. Values are means ± standard error of the mean. *Lower for astaxanthin condition compared to placebo condition for all percentages of 1-RM (P < 0.05).
on markers of skeletal muscle injury. Based on these findings, it appears as though astaxanthin supplementation does not favorably affect indirect markers of muscle injury in resistance-trained men.

Subjective muscle soreness was unaffected by the astaxanthin supplementation, and was elevated as early as immediately post-exercise in both conditions. Previous studies have reported similar elevations following high force eccentric exercise, with a blunted response in subjects supplemented with vitamin C, either alone or in combination with vitamin E (6, 20). It was believed that the antioxidant properties of astaxanthin would have aided in suppressing inflammation and the associated release of reactive oxygen species, which is often linked to muscle soreness. This is because reactive oxygen species control transcription factors (e.g., nuclear factor-κB) that give rise to inflammatory cytokines, leading to inflammation (36). It was hypothesized that astaxanthin would work to suppress inflammation, possibly by interfering with the transcription factors that promote the inflammatory response. With decreased inflammation, muscle soreness could have been favorably affected. We noted, however, no difference between conditions for muscle soreness, suggesting that if inflammation was suppressed, the degree of suppression was not sufficient to alter muscle soreness.

Many investigators have reported a blunted CK response following eccentric or eccentric/concentric exercise protocols when subjects have been supplemented with the lipid-soluble vitamin E (6, 24, 34). It is believed that vitamin E has membrane stabilizing properties that protect the sarcolemma from disruption, leading to less leakage of intracellular proteins into circulation. It has been suggested that astaxanthin could have a similar effect in providing membrane stability, while having antioxidant properties superior to those of beta carotene in vitro (27, 32). Furthermore, it has recently been reported that astaxanthin can attenuate the CK increase in exercising mice (1). Despite these findings, data from the present investigation using resistance-trained humans as subjects are in opposition, indicating no membrane stabilizing effect of astaxanthin, evidenced by the increase in CK. It is possible that use of astaxanthin at the dosage of 4 mg/d actually promoted an increase in membrane permeability, which would help explain the higher CK levels in the astaxanthin condition.

It should be noted that not all studies using lipid-soluble antioxidants have resulted in blunted CK in the circulation following eccentric exercise (8, 28, 38). Discrepancies are likely related to the exercise protocols used, test subjects, dosage and timing of antioxidant administration, and the variability in CK across subjects. Due to these problems, it was our intent to also focus on measures of muscle performance, which appear to be much more reliable indices of skeletal muscle injury (37).

Neither 1-RM concentric strength nor MIF were affected by astaxanthin supplementation, while MDF was negatively impacted by supplementation. In all subjects, strength values were suppressed following the eccentric exercise protocol. Mean dynamic force at every testing load (90%, 65%, and 40% 1-RM) was suppressed to a greater extent in the astaxanthin condition compared to the placebo from 10 through 72 h post-exercise. Although explanations regarding this further decrease in MDF are currently unavailable, it might be linked mechanistically to a possible suppression in inflammation and tissue healing which could negatively influence recovery characteristics. The potential role of anti-inflammatory agents...
to prolong muscle force recovery has been reviewed extensively elsewhere (11). It is possible that astaxanthin might be working in a similar manner. The practical implications of such a difference in recovery warrants further investigation.

Few studies have reported the impact of antioxidant supplements on muscle performance variables following eccentric resistance exercise, noting little difference in maximal isometric force following vitamin E supplementation (4) and vitamin E/vitamin C/selenium supplementation (6). It is likely that markers of muscle strength are affected by factors unrelated to reactive oxygen species. This is underscored by the observation that much of the muscle injury incurred as a result of eccentric exercise is mechanical (13) and thus immediate, as observed in the present study. As such, it is likely that high force, high volume eccentric exercise protocols used in previous investigations damage muscle to such a significant extent that any potential effect of antioxidant supplementation could be overwhelmed by the initial mechanical trauma. It is therefore probable that despite pre-treatment with various antioxidant agents, muscle damage resulting in a loss of force will still occur.

The decline in muscle force capabilities observed in the hours and days after eccentric exercise might be explained primarily by impairment in the excitation–contraction coupling process (7, 17). Moreover, factors discussed earlier such as focal skeletal fiber injury (22, 29), inflammation, loss of calcium regulation in the muscle (2), and a potential redistribution in sarcomere lengths (7) have been suggested as contributory factors to the decline in force following eccentric muscle actions.

In summary, the use of the carotenoid astaxanthin before and during the days following eccentric exercise was associated with no beneficial effects in resistance-trained men. This assumes that astaxanthin was taken up into the skeletal muscle, something that was not verified in this investigation. Based on these data, recommendations for the use of astaxanthin for purposes of attenuating markers of skeletal muscle injury are not justified. Because young, resistance-trained men were used as subjects in this investigation, it is uncertain as to whether these findings can be extended to individuals who are untrained, or those who participate in events other than high force resistance exercise (e.g., high volume aerobic exercise).

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References


