

Astaxanthin Clinical Trial for
Delayed Onset Muscular Soreness

Report I

Funded by:
Cyanotech, Inc.

Submitted by:
Andrew C. Fry, Ph.D., C.S.C.S.
Associate Professor
Director - Exercise Biochemistry Laboratory
The University of Memphis
Memphis, TN 38152

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Introduction

The phenomenon of muscular soreness is problematic for those individuals participating in intense physical activity. This soreness, which is most obvious 24 - 48 hrs. after the physical activity takes place, is readily apparent in both athletes and non-athletes alike. Due to the time frame of the onset of such soreness, this condition is often termed "delayed onset muscle soreness" (DOMS). In the athletic community, activities such as long distance running and strength training alike can result in excessive muscular soreness that can interfere with the long term training program. Likewise, such soreness is also evident among those exercising for general fitness purposes, and for those participating in therapeutic and rehabilitative exercise programs. Additionally, the problem of muscle soreness can be evident at the work site for jobs requiring high levels of stressful physical activity. It thus becomes rapidly apparent that DOMS is a problem that both athlete and non-athlete alike must deal with for successful performances.

Eccentric muscle activity (i.e., force production while the muscle is lengthening) appears to be most responsible for for DOMS. As such, heavy resistance exercise, which typically includes muscle activity of this type, can result in large levels of DOMS. Those individuals who are not accustomed to vigorous physical activity (i.e., untrained) are most susceptible to DOMS. The initial exposure to such eccentric activity is consistently the most deleterious, with subsequent exposures resulting in DOMS to a lesser degree. Regardless, even among the highly trained individuals, DOMS can consistently occur whenever large volumes of eccentric muscle activity occurs. Although the extent of DOMS is attenuated among the highly trained/conditioned, it is never eliminated, and remains a potential problem. Interestingly, it is the highly trained/conditioned individual who will most often utilize high volumes of eccentric muscle activity, thus being repeatedly exposed to the problems associated with DOMS.

It has been argued that a certain degree of muscle soreness is a necessary part of the tissue remodeling occurring due to a training program. In reality, the debilitating effects of DOMS are not necessary for appropriate muscle adaptation to occur. Appropriate tissue adaptation can occur without excessive muscular soreness, thus resulting in less interference with the total training program.

To date, the primary option for those wishing to avoid the deleterious effects of DOMS has been proper exercise prescription, which is undoubtedly the most important prophylactic measure. Recently, another preventive measure has been suggested. Astaxanthin, an anti-oxidant compound derived from algae extract, has provided promise for the problem of DOMS. In vitro studies have suggested that astaxanthin has membrane stabilizing properties, as well as the ability to modify immune responses to physical insults. Due to these properties, astaxanthin is currently being used for clinical trials among patients suffering from carpal tunnel syndrome. If astaxanthin does indeed provide a stabilizing factor to cellular membranes, it may result in a diminished physiological response to exercise resulting in DOMS. It is well documented that one of the physiological responses to soreness-inducing exercise is disruption of the sarcolemma, initiating a cascade of events resulting in diminished muscle performance. Although individuals who are currently participating in heavy resistance exercise may have adapted somewhat to the stimulus of heavy eccentric muscle actions, these individuals repeatedly must respond to lesser degrees of DOMS that can interfere with the recovery and adaptation process. If astaxanthin proves to be beneficial for recovery from such training, it may augment the positive adaptation process, and result in a greater and/or more rapid training process.

It has also been suggested that since astaxanthin may possess stabilizing properties for the sarcolemma, it is conceivable that circulating levels of blood lipids may be affected by supplementation. Likewise, the immune responses to DOMS and astaxanthin supplementation may be influenced since the immune system may be a critical contributor to the skeletal muscle adaptation/maladaptation processes. As with many supplements, concern for health related responses to the supplement necessitate the monitoring of various physiological systems in response to the exogenous supplement.

Few data are available on the muscle fiber type specificity of the DOMS response. It has been suggested that type I fibers may be less susceptible to DOMS, but it is not known if such a response is related to various physiological markers of health and immune function.

Purposes

The purposes of the present research proposal were;

- 1) to determine the efficacy of astaxanthin as a prophylactic for DOMS resulting from heavy resistance exercise,
- 2) to determine the effect of astaxanthin supplementation on the health and immune related variables, and
- 3) to collect pilot data on the role of muscle fiber type on the effect of astaxanthin supplementation.

This report represents data from the psychological questionnaires, the blood samples, and the muscle biopsies. Report II, to come later, will include muscle performance data and the relationships with muscle fiber characteristics.

Subjects

Twenty weight-trained males were recruited as subjects. This population was targeted since these are the individuals most likely to train at the volumes and intensities required to induce DOMS on a regular basis. All subjects had been weight training their legs for at least 1 year. The subjects were randomly assigned in a double-blind fashion to either the astaxanthin supplemented group (n=10) or the placebo supplemented group (n=10). Table 1 lists their descriptive characteristics. There were no significant differences between the astaxanthin or placebo groups for any of these variables ($p > 0.05$).

Table 1 - Descriptive characteristics ($\bar{X} \pm SE$).

<u>Variable</u>	<u>Astaxanthin Group</u>	<u>Placebo Group</u>
n	10	10
Age (yrs)	24.0 \pm 1.1	26.2 \pm 2.0
Height (m)	1.78 \pm 0.02	1.80 \pm 0.02
Body Weight (kg)	84.4 \pm 5.2	89.2 \pm 3.6
Wgt. Trng. Experience (yrs)	3.7 \pm 1.3	5.3 \pm 1.2
Squat 1 RM (kg)	129.9 \pm 8.3	160.4 \pm 13.2
Wgt. Trng. Frequency ($x \cdot wk^{-1}$)	2.3 \pm 0.3	2.0 \pm 0.2

Double-blind Effectiveness - The effectiveness of the double-blind group assignment process was extremely effective as determined by an exit survey. Only 4 of the 20 subjects (20%) correctly identified which supplement they were taking, astaxanthin or the placebo. Only 10% (1/10) of the placebo group correctly identified their group assignment, while only 30% (3/10) of the astaxanthin group correctly identified their group assignment. Overall, 50% of the subjects (10/20) reported they had no idea as to their group assignment.

Design

Each subject orally ingested the supplement (astaxanthin) or a placebo provided by the manufacturers for three weeks. Dosages adhered to the manufacturers recommendations. Following the 3 week supplementation period, each subject participated in a heavy eccentric resistance exercise session designed to induce DOMS. A York knee extension machine was modified to permit performance of only the eccentric portion of the knee extension exercise. After a concentric 1 RM at maximal velocity, and an eccentric 1 RM at $0.52 \text{ rad}\cdot\text{s}^{-1}$ ($30^\circ\cdot\text{s}^{-1}$) were determined for both legs, a series of eccentric lifts were performed by the dominant leg. Ten sets of 7-10 eccentric repetitions were performed, starting with 85% of the eccentric 1 RM load. When the subject was unable to maintain the required lowering velocity ($0.52 \text{ rad}\cdot\text{s}^{-1}$), the load was decreased by approximately 3.6 kg for the subsequent eccentric repetitions. A timing system was interfaced with the weight machine to insure adherence with the lifting velocity requirements. This protocol has been previously used by several laboratories and has been reported in the scientific literature. Pilot work was performed to verify the effectiveness of this protocol for inducing DOMS in a weight trained population.

Tests

In order to closely monitor the health-related responses to astaxanthin supplementation, and the time course of maladaptation to the DOMS, test batteries were performed at the following time points; pre-supplementation (PreSuppl), immediately pre-DOMS session (IPre), immediately post-DOMS session (IPost), 10 hrs, 24 hrs, 48 hrs, 72 hrs, 96 hrs, and 288 hrs (12 d) after the DOMS session. In addition, a 2 familiarization sessions were included during the supplementation phase

to thoroughly introduce each subject to the various performance tests that were performed. To thoroughly study the physiological systems included in this report, the following tests were performed.

Muscular Strength - One repetition maximum (1 RM) concentric strength was determined for the knee extension exercise on eight different occasions. After a standardized warm-up, single repetitions were performed with increasing loads until the subjects were unable to lift the weight. Approximately 1 minute of rest was allowed between each repetition, with a total of 2 - 4 lifts required to reach failure. Test-retest reliability for this test in our laboratory is $r = 0.95$.

Pain Survey - A Likert scale commonly used by the pharmaceutical industry was administered to determine perceptions of soreness. In addition, a previously validated questionnaire was used to monitor perceptions of muscular strength, recovery, knee pain, and lower back pain. Based on inversely-scored multiple items on the questionnaire, Alpha Cronbach reliability coefficients for each of these items was acceptable ($\alpha > 0.70$). Previously used questions regarding muscle soreness and pain were not determined to be valid in the present study ($\alpha < 0.50$) and thus were not included in the analysis. As a result, all soreness scores were derived from the Likert scale assessment.

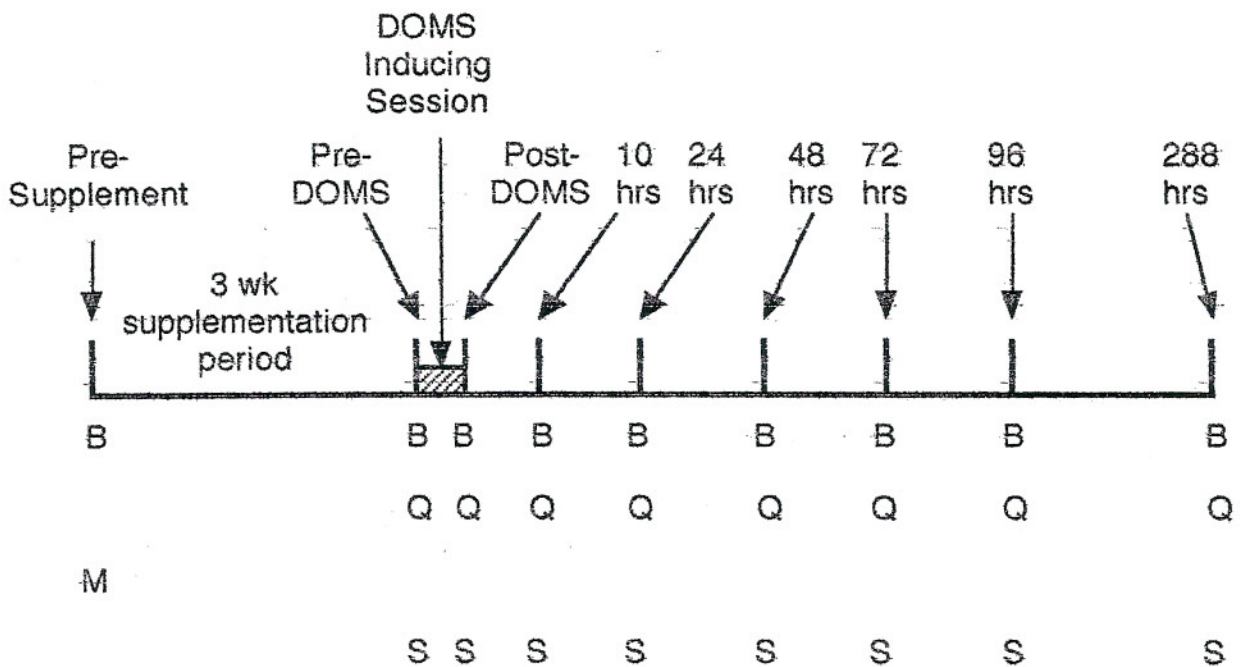
Blood Analyses - Using standard venipuncture techniques, 10 mL of whole blood was sampled at PreSuppl, Ipre, Ipost, 10 hrs, 24 hrs, 48 hrs, 72 hrs, 96 hrs, and 288 hrs (12 d) after the DOMS session. The resulting blood was analyzed for CK activity as a marker of muscle damage/disruption. These samples were also analyzed via a Chem 31 & CBC with differentials blood panel from Quest Laboratories.

Muscle Biopsies - Although not a direct part of this research proposal, there were a number of subjects who were completing a weight training study including post-training muscle biopsies from the vastus lateralis m. We recruited nine of these individuals for the present study (astaxanthin group, $n=4$; placebo group, $n=5$). In this manner, muscle fiber characteristics prior to

the DOMS protocol are known for a sub-set of subjects. Variables that were measured for these subjects include % fiber type, fiber cross-sectional area, % fiber type area, and % myosin heavy chain content. By assessing these variables, it is possible collect preliminary data to determine if the cellular and molecular characteristics of skeletal muscle influence the responsiveness to either the astaxanthin supplementation or the DOMS-inducing exercise session.

Test Time Line

Figure 1 -



B = blood sample Q = questionnaire M = muscle biopsy S = 1-RM strength

Statistical Analyses

All data are expressed as mean \pm SE. Descriptive characteristics were compared using Students independent t-tests. Questionnaire data and blood variables (both absolute and % change values) were analyzed with 2-way (group x test) repeated measures ANOVAs or Students independent t-tests. Validity of the questionnaire was determined via Cronbach Alpha coefficients. Skeletal muscle characteristics were analyzed via a 2-way (group x type or isoform) ANOVA. Pearson product-moment correlation coefficients were calculated to determine relationships between muscle characteristics and perceptions of soreness. Significance was set at $p < 0.05$ for all analyses.

Knee Soreness - Since the knee joints are essential to the movement pattern used in the present study (i.e., knee extension), it was essential to monitor pain/discomfort in these joints. The difference in the response pattern between the groups was not expected since it was theorized that the most likely site of physiological action would be at the sarcolemma. Further study would be necessary to determine if astaxanthin serves in a protective mechanism at the involved joints.

Figure 7 - Perceptions of knee soreness ($X \pm SE$) taken from a 5 point Likert scale. A significant interaction was observed, with the placebo group reporting significantly increased knee pain from immediately-post to 48 hrs, although no difference was observed between groups at any time. The astaxanthin supplemented group exhibited no change in knee soreness. * sig. different from Pre value ($p < 0.05$).

