Astaxanthin supplementation attenuates disuse muscle atrophy and myonuclear apoptosis in rat skeletal muscle

Toshinori Yoshihara¹, Takao Sugiura², Hisashi Naito¹

¹Graduate School of Health and Sports Science, Juntendo University, 1-1 Hirakagakuen-dai, Inzai, Chiba, 270-1695 Japan
²Faculty of Education, Yamaguchi University, 1677-1 Yoshida, Yamaguchi, Yamaguchi, 753-8513 Japan

Correspondence: Toshinori Yoshihara
E-mail: t-yoshih@juntendo.ac.jp
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Prolonged skeletal muscle disuse results in muscle atrophy. Currently, no therapeutic treatment is available for the prevention of this problem. Nonetheless, growing evidence suggests that prevention of disuse-induced oxidative stress in inactive muscle fibers can delay inactivity-induced muscle atrophy. We recently tested the hypothesis that dietary supplementation with the antioxidant astaxanthin (AX) might protect against disuse muscle atrophy, in part, by preventing myonuclear apoptosis. Seven-day unloading resulted in reduced soleus muscle weight and myofiber cross-sectional area. However, this decline was suppressed by AX supplementation. Further, AX prevented disuse-induced increase in the number of terminal deoxynucleotidyl transferase dUTP nick end labeling-positive nuclei. Our data showed that AX supplementation before and during hindlimb unloading attenuated soleus muscle atrophy, in part, by suppressing myonuclear apoptosis.

Keywords: antioxidant; apoptotic myonuclei; disuse muscular atrophy

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Prolonged periods of skeletal muscle inactivity (e.g., immobilization, chronic bed rest, or spaceflight) result in a significant decrease in the cross-sectional area of individual myofibers and production of muscle force[^11]. Skeletal muscle mass is maintained by a balance between protein synthesis and degradation[^2, 3], which are regulated via multiple signaling pathways[^4-6]. However, the molecular mechanisms underlying these processes are not completely understood.

Numerous studies have shown that increased production of reactive oxygen species (ROS) in skeletal muscle could be important as signaling molecules contributing to disuse muscle atrophy by promoting protease activation and depressing protein synthesis[^5, 7]. Therefore, antioxidant supplementation has been investigated as a potential therapeutic countermeasure to protect against disuse skeletal muscle atrophy[^8]. Evidence suggests that disuse muscle atrophy induced by immobilization and hindlimb unloading can be partially prevented by treatment with vitamin E supplementation[^9, 10]. In addition, Azzi et al.[^11] reported that vitamin E mediates cell signaling and regulates the expression of several genes. In contrast, Koesterer et al.[^12] and Ikemoto et al.[^13] indicated that vitamin E supplementation failed to suppress skeletal muscle atrophy in rat hindlimb unloading model. Therefore, the effectiveness of antioxidants in disuse muscle atrophy and the underlying...
mechanisms remain unclear. Thus, applying a more potent antioxidant formulation to prevent disuse muscle atrophy might be necessary.

Recently, astaxanthin (AX) was found to be an effective treatment against disuse-induced muscle atrophy. AX is a red carotenoid pigment that is found in some marine animals such as fish and shrimp, plants, and algae \cite{14-16}. It has potent antioxidant and anti-inflammatory properties, with an antioxidant capacity that is 100 times greater than that of alpha-tocopherol (i.e., vitamin E) \cite{17, 18}. Kanazashi et al. \cite{19, 20} reported that high-dose (100 mg/kg/day) AX administered orally during hindlimb unloading reduced capillary regression and enhanced intermittent reloading-induced protection against muscle atrophy. However, the preventive effects of AX supplementation alone in disuse muscle atrophy remain unclear. To our knowledge, our recent study first showed that dietary AX supplementation before and

Figure 1. Potential underlying mechanism of astaxanthin supplementation-mediated attenuation of disuse muscle atrophy. Inhibition of myonuclear apoptosis and ubiquitin-proteasome proteolytic pathway by astaxanthin supplementation during disuse muscle atrophy. Astaxanthin supplementation attenuates skeletal muscle atrophy by suppressing apoptotic and proteolytic systems.
during hindlimb unloading attenuates soleus muscle atrophy [21]. Interestingly, we also found that unloading-induced apoptotic myonuclei and protein ubiquitination are suppressed by AX (Fig. 1). Previous studies have shown that skeletal muscle atrophy is associated with increased apoptosis [6, 22, 23], and our data revealed that the number of terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive nuclei in the soleus muscle increases significantly with disuse. Many of these protective effects can be likely attributed to the action of AX as a scavenger of free radicals and oxidants. Ikeda et al. [24] have shown that AX treatment abolishes 6-hydroxydopamine-induced ROS generation and p38 activation, including the release of cytochrome c, and the cleavage of caspase-9 and caspase-3 in SH-SY5Y cells. Moreover, we found that AX protects the soleus muscle against unloading-induced oxidative stress, as evidenced by the suppression of superoxide dismutase (SOD) 1 (Cu, Zn-SOD) [25, 26] and the increase in caspase-3 (Fig. 1) [21]. Thus, AX might reduce the disuse-induced production of superoxide anions and hydrogen peroxide (as catalyzed by SOD1) and the subsequent activation of caspases in the atrophied soleus muscle during hindlimb unloading.

AX supplementation prevented the increase in ubiquinated protein content in the atrophied soleus muscle [21]. Ubiquitination of myofibrillar proteins reflects enhancement of muscle protein degradation via the ubiquitin-proteasome pathway; this causes a remarkable decrease in the muscle weight and cross-sectional area [27]. Our data indicate that AX supplementation might be effective in reducing myofibrillar protein ubiquitination induced by oxidative stress, and thus afford protection against increased muscle protein degradation via the ubiquitin-proteasome system [28, 29].

Our data also showed that AX supplementation before (2 weeks) and during unloading increased heat shock protein (HSP) 72 expression in the atrophied soleus muscle (Fig. 1) [21]. HSPs have been shown to play a role in the suppression of cytochrome c release and upstream processes of caspase-3 activation in vitro (U937/HSP70 cells) [30]. In addition, Lee et al. [31] reported that AX treatment increased HSP72 expression and was associated with protective effects against cerebral ischemia in SH-SY5Y cells. Although the cellular mechanisms underlying AX-induced suppression of muscle atrophy remains unclear, these results suggest that AX supplementation is useful in attenuating the loss of muscle mass and size induced by disuse in vivo.

Importantly, we found that relatively low-dose dietary supplementation of AX (30.3 ± 0.1 mg/kg/day; total intake 606.5 ± 20.3 mg/kg) can attenuate unloading-induced soleus muscle atrophy [21]. A previous study applied 700 and 1400 mg/kg AX (50 mg/kg, within a 6 h interval between 2 doses; total dose of 100 mg/kg/day). However, in that study, AX administration alone failed to suppress soleus muscle atrophy [19, 20]. This difference might be attributed to the fact that apoptosis is a very early event (occurring within 2 days after unloading) in disuse muscle atrophy [32, 33]. These findings indicate that atrophy suppression occurring in the early phase of apoptotic and proteolytic activation might be important in effectively attenuating disuse-induced muscle atrophy. Thus, enhancing the pre-antioxidant capacity might be necessary for the prevention of muscle atrophy, even with low-dose treatment, rather than applying a high dose of AX during hindlimb unloading.

In summary, our data showed that dietary supplementation of the antioxidant AX before and during hindlimb unloading attenuates soleus muscle atrophy. This AX supplementation-induced protection against unloading-induced atrophy might be attributed to the decreased apoptotic myonuclei and protein ubiquitination (Fig. 1). However, specific mechanisms responsible for this protection remain unclear. Further studies are warranted to improve our understanding of the mechanism(s) associated with the protective effect of AX administration for disuse-induced muscle atrophy.

Conflicting interests

The authors have declared that no conflict of interests exist.

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Author contributions

T.Y. and T.S. performed the experiments, analyzed the results, and drafted the manuscript. T.S. participated in the design of the study and performed the statistical analysis. T.S. and H.N. conceived and designed the experiment and helped in drafting the manuscript. All authors read and approved the final manuscript.
Abbreviations

AX: astaxanthin; TUNEL: terminal deoxynucleotidyl transferase dUTP nick end labeling; ROS: reactive oxygen species; SOD: suppression of superoxide dismutase.

References
