

The astaxanthin-induced improvement in lipid metabolism during exercise is mediated by a PGC-1 α increase in skeletal muscle

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Astaxanthin, a xanthophyll carotenoid, accelerates lipid utilization during aerobic exercise, although the underlying mechanism is unclear. The present study investigated the effect of astaxanthin intake on lipid metabolism associated with peroxisome proliferator-activated receptor- γ coactivator-1 α (PGC-1 α) in mice. Mice were divided into 4 groups: sedentary, sedentary and astaxanthin-treated, exercised, and exercised and astaxanthin-treated. After 2 weeks of treatment, the exercise groups performed treadmill running at 25 m/min for 30 min. Immediately after running, intermuscular pH was measured in hind limb muscles, and blood was collected for measurements. Proteins were extracted from the muscle samples and PGC-1 α and its downstream proteins were measured by western blotting. Levels of plasma fatty acids were significantly decreased after exercise in the astaxanthin-fed mice compared with those fed a normal diet. Intermuscular pH was significantly decreased by exercise, and this decrease was inhibited by intake of astaxanthin. Levels of PGC-1 α and its downstream proteins were significantly elevated in astaxanthin-fed mice compared with mice fed a normal diet. Astaxanthin intake resulted in a PGC-1 α elevation in skeletal muscle, which can lead to acceleration of lipid utilization through activation of mitochondrial aerobic metabolism.

Key Words: astaxanthin, skeletal muscle, lipid metabolism, running exercise, PGC-1 α

Astaxanthin is a naturally occurring xanthophyll carotenoid found in lobsters by Kuhn *et al.*⁽¹⁾ and was shown to be present in algae, fish, and crustacean. It is reported to have high antioxidant activity, which is 100–1,000-fold higher than that of other antioxidant phytochemicals.^(2,3) The muscles of fish such as salmon contain high levels of astaxanthin, which may contribute to their physical stamina. Astaxanthin also accumulates in mammalian tissues including skeletal muscle after oral administration and can attenuate the oxidative damage induced by acute exercise in mice and humans.^(4,5)

Our group and others have shown that astaxanthin decreases muscle fatigue and increases endurance during aerobic exercise in mice and humans.^(6–8) When the energy expended during exercise is derived from lipids, a large amount of energy can be continuously obtained via aerobic metabolism. On the other hand, when the energy source is carbohydrate-based, muscular pH will decrease due to increased lactic acid production, which may lead to impaired muscle contraction. Therefore, increased lipid utilization in the mitochondria of skeletal muscle cells is associated with aerobic endurance. Previous studies have shown that astaxanthin accelerates lipid utilization during aerobic exercise,^(6,7)

although the underlying mechanism is unclear.

The number of mitochondria as well as mitochondrial function influences the utilization of fatty acids in muscle cells. In the past decade, peroxisome proliferator-activated receptor- γ coactivator-1 α (PGC-1 α) has emerged as a key transcriptional co-activator, and has provided a mechanistic insight into nuclear regulatory pathways in the biogenesis of mitochondria in skeletal muscle.^(9–11) PGC-1 α contents are changed by physical activity level, metabolic disorders, and aging, which associated with metabolic capacity.^(12,13) PGC-1 α interacts with nuclear receptors and transcription factors to activate the transcription of lipid metabolic genes, and its activity is responsive to aerobic metabolic events.^(10,11) Therefore, we hypothesized that intake of astaxanthin may improve aerobic metabolism of mitochondria associated with elevated levels of PGC-1 α . The purpose of this study is to examine the effects of astaxanthin intake on lipid metabolism and muscle fatigue in exercise in relation to PGC-1 α and mitochondrial metabolic proteins.

Materials and Methods

Animals and experimental design. This study complied with the guidelines of the Japanese Council on Animal Care and was approved by the Committee for Animal Research at the Kyoto Prefectural University of Medicine. ICR mice (7 weeks old) (Clea Japan, Inc., Osaka, Japan) were acclimatized for 1 week in an air-conditioned (22 \pm 2°C) room on a 12-h light/dark cycle (lights on from 7:30 to 19:30). Mice were divided into 4 groups containing 8 animals each: rested control, astaxanthin-fed rested control, exercised, and astaxanthin-fed exercised mice. Astaxanthin-enriched diets were provided by supplementing with *Haematococcus pluvialis* microalgal extract containing astaxanthin (0.02% w/w) (Fuji Chemical Industry, Toyama, Japan). After 2 weeks, the exercise groups were accustomed to treadmill running at a speed of 25 m/min for 30 min. After an initial 5-min warm up, the running speed was gradually increased to 25 m/min. Immediately after treadmill running, intermuscular pH were measured under anesthesia, and subsequently hind limb muscles and blood were collected for biochemical measurements.

Blood analysis. Blood lactate and glucose were measured (Lactate Pro, GluTest; Arkray, Inc., Kyoto, Japan), and then blood samples were centrifuged at 3,500 rpm for 15 min at 4°C to collect plasma. Plasma nonesterified fatty acid (NEFA) was measured using a NEFA-C test assay kit (WAKO, Osaka, Japan).

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Western blotting. Protein was extracted from muscle tissues using a lysis buffer (Sigma, St. Louise, MO). Equal amounts of protein in the lysates were separated by 10% SDS-PAGE, and proteins were then transferred onto nitrocellulose membranes. The blots were incubated with primary antibodies against PGC-1 α (Chemicon International, Temecula, CA), cytochrome c (Cell Signaling Technology, MA), and fibronectin type III domain containing 5 (FNDC5) (Phoenix Pharmaceuticals, CA), and reaction products visualized using a horseradish peroxidase (HRP)-conjugated secondary antibody (GE Healthcare, Buckinghamshire, UK) and enhanced chemiluminescence (ECL Prime, GE Healthcare). Band densities were measured using Image J software (NIH, Research Service Branch).

pH measurements. pH levels in the interstitial fluid of exercised muscle tissue were measured using a glass microelectrode under anesthesia. The microprobe was inserted into the interstitium between the gastrocnemius and tibialis anterior muscles.

Statistical analysis. All data are reported as the mean \pm SE. Differences between groups were evaluated using a 2-way ANOVA or student's *t* test. If ANOVA indicated a significance difference, Tukey-Kramer test was used to determine the significance of differences between means, and $p < 0.05$ was considered to indicate statistical significance.

Results

Body weight and blood parameters. Body weight and blood biochemical parameters are shown in Table 1. There were no significant differences in body weight, plasma lactate, or blood glucose levels between control and astaxanthin-fed groups. In contrast, plasma NEFA after exercise was significantly decreased

by intake of astaxanthin compared with intake of normal diet. The reduced tendency of plasma NEFA by astaxanthin was also found sedentary condition.

Intermuscular pH. Most of the lactate anion is released into the circulation or metabolized as an energy substrate immediately after generation through glycolysis.⁽¹⁴⁾ In contrast, protons, which are lactate by-products, are positively buffered in muscle cells and blood. However, it barely buffered in the interstitial fluid;⁽¹⁵⁾ thus, we measured intermuscular pH immediately after exercise. pH levels were significantly reduced by running exercise, although this reduction was inhibited by the addition of astaxanthin to the diet (Fig. 1).

PGC-1 α and downstream proteins. PGC-1 α was significantly elevated by intake of astaxanthin for 2 weeks (Fig. 2). In addition, cytochrome c and FNDC5, proteins downstream of PGC-1 α , were also significantly higher in the astaxanthin-fed group than in the normal diet group (Fig. 3A and B).

Discussion

The present study revealed the following: 1) astaxanthin elevated levels of PGC-1 α and its downstream proteins in skeletal muscle, 2) astaxanthin induced the decrease in plasma fatty acids during exercise, and 3) astaxanthin prevented reduction in intermuscular pH due to exercise. Previously, our group and others have shown that astaxanthin accelerates the utilization of lipids as an energy substrate during aerobic exercise.⁽⁶⁻⁸⁾ Although potential mechanisms regarding the effect of astaxanthin have been suggested, the detailed mechanism remains unclear. The present study is the first to demonstrate that astaxanthin elevates the expression of PGC-1 α and its downstream proteins, which are involved in the activation of mitochondrial biogenesis, leading to the acceleration

Table 1. Body weight and blood metabolic parameters

	Sedentary		Exercise	
	Normal	Astaxanthin	Normal	Astaxanthin
Body weight (g)	41.4 \pm 0.9	41.9 \pm 1.1	41.3 \pm 0.8	41.3 \pm 0.6
Blood glucose (mg/dL)	118 \pm 5	109 \pm 7	112 \pm 3	121 \pm 2
Plasma NEFA (mEq/L)	1073 \pm 47	891 \pm 43	1151 \pm 61	905 \pm 41 [#]
Blood lactate (mM)	—	—	2.1 \pm 0.3	2.1 \pm 0.1

Values are the mean \pm SE. Normal; normal-fed group, astaxanthin; astaxanthin-fed group. [#] $p < 0.05$ vs Normal.

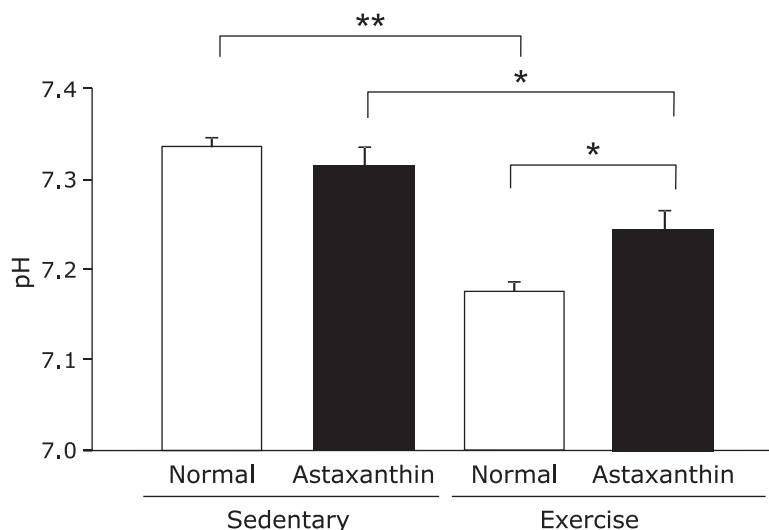


Fig. 1. The effect of astaxanthin on intermuscular pH in sedentary and exercised mice. Values are the mean \pm SE. Normal, normal-fed group; astaxanthin, astaxanthin-fed group. * $p < 0.05$, ** $p < 0.001$.

of fat utilization during exercise through aerobic metabolism in mitochondria.

Levels of circulating NEFA are regulated by a balance between catabolic processes in adipose tissue and fatty acid substrate utilization by the skeletal muscle. Circulating catecholamines such as adrenalin and noradrenalin are increased in response to exercise and stimulate lipolysis of triglycerides in adipose tissues, which causes elevation of circulating fatty acids. In contrast, muscle contraction increases uptake of fatty acids from the circulation into muscle cells, which leads to a decrease in circulating fatty acids. Therefore, we suggest that the reduction of NEFA in astaxanthin-fed group was due to an increase in utilization in muscle when compared to release from adipose tissue. Although plasma NEFA was less in astaxanthin diet group compared with in normal diet group in both rest and exercise conditions, it was more remarkable in the exercise condition. Previously, we have shown by respiratory metabolic analysis, that astaxanthin treatment increased utilization of lipids during exercise as an energy substrate compared with a normal diet,⁽⁶⁾ which supports this hypothesis.

Astaxanthin also inhibited the decrease in intermuscular pH.

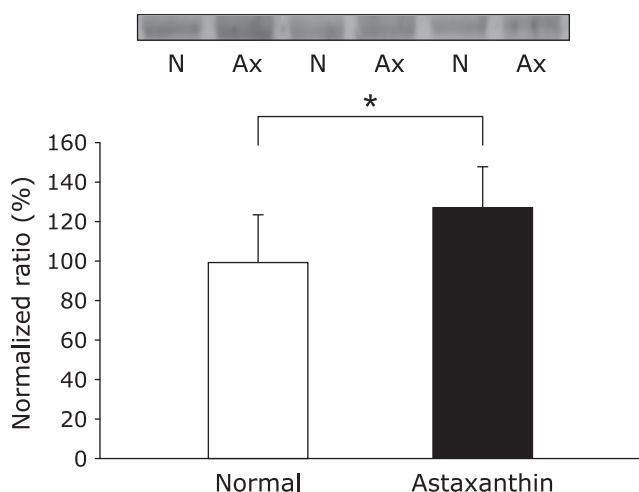


Fig. 2. The effect of astaxanthin on PGC-1 α levels in skeletal muscle. Values are the mean \pm SE. Normal (N), normal-fed group; astaxanthin (Ax), astaxanthin-fed group. * $p < 0.05$.

During muscle contraction, lactic acid, a major source of protons, is produced rapidly through increased glycolytic metabolism, which causes the pH reduction and inhibits muscle contraction. Because energy consumed in muscle during exercise is mainly supplied by carbohydrates and lipids, astaxanthin-induced lipid utilization can decrease energy obtained from carbohydrates, which may lead to the decrease in lactate/proton production. Indeed, our previous study showed that deposition of muscle glycogen, a major carbohydrate utilized during muscle contraction, during exercise was prevented by intake of astaxanthin,⁽⁶⁾ which suggests that glycolytic metabolism is prevented by astaxanthin intake. In contrast, blood lactate was unchanged between normal-fed and astaxanthin-fed groups. Lactate is almost completely dissociated from proton immediately after the generation and carried by the blood to other tissues for oxidative metabolism or gluconeogenesis.⁽¹⁴⁾ Therefore, lactate anion itself is not a metabolic preventative factor, but rather it is utilized as an energy substrate. Circulating lactate levels are regulated by balance between release into blood and clearance. In contrast, protons generated from cytosolic lactic acid are immediately buffered in the cell or exported to the interstitial fluid and further transported to the blood. The buffering capacity is relatively high in the cytosol and in blood, whereas capacity is low in interstitial fluid as buffering factors such as proteins are limited.^(15,16) Therefore, interstitial fluid pH in muscle tissues can drastically change in response to muscle contraction and can also be a marker of acid-base conditions in muscle tissue. Taken together, astaxanthin improves metabolic acidosis through the activation of lipid metabolism.

PGC-1 α has been shown to be a central member in a family of transcriptional co-activators involved in aerobic metabolism. Activation of PGC-1 α alters the metabolic phenotype through interaction with nuclear respiratory factor and peroxisome proliferator-activated receptor α ,⁽⁹⁻¹¹⁾ which leads to increased mitochondrial biogenesis and activity. Improved understanding of PGC-1 α activation has implications beyond the improvement of athletic performance,^(12,17) such as the possibility of providing targets for the treatment of muscle weakness in the elderly, in obesity, and in other diseases such as mitochondrial myopathies and diabetes.^(9,18,19) Therefore, elevation of PGC-1 α by astaxanthin may induce the acceleration of lipid metabolism during exercise. Indeed, cytochrome c, a component of the mitochondrial electron transport chain and a major PGC-1 α -inducible protein, was also upregulated by astaxanthin. In addition, we found an increase in FNDC5, another PGC-1 α -inducible protein. FNDC5 is a mem-

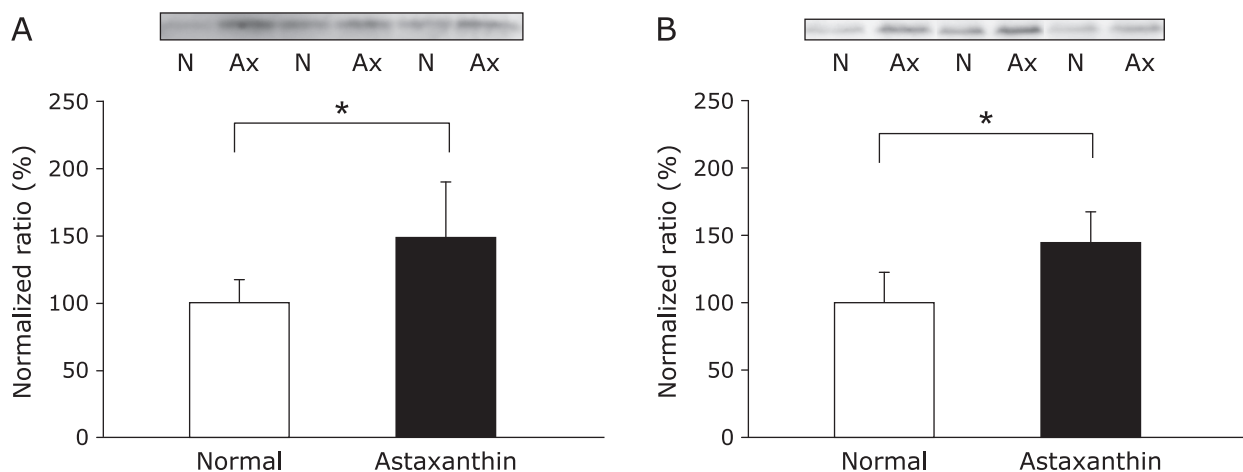


Fig. 3. The effect of astaxanthin on cytochrome c (A) and FNDC5 (B) levels in skeletal muscle. Values are the mean \pm SE. Normal (N), normal-fed group; astaxanthin (Ax), astaxanthin-fed group. * $p < 0.05$.

brane protein that is cleaved and secreted into the circulation as the newly identified myokine, irisin.⁽¹⁷⁾ It has been shown that FNDC5 induces browning of subcutaneous fat through irisin and mediates metabolic improvement. Therefore, astaxanthin may also activate energy metabolism in adipose tissues. Future studies should study the metabolic effect of astaxanthin not only in skeletal muscle, but also in other tissues. In addition, the regulatory mechanism of PGC-1 α elevation induced by astaxanthin is unclear. Other antioxidants including vitamin C and E do not elevate PGC-1 α in skeletal muscle;^(18,19) thus, this may be a specific action of astaxanthin, in addition to its antioxidant properties. The respective properties of each antioxidant should be studied individually, not by the absolute antioxidative capacity in the future.

In conclusion, we show that intake of astaxanthin for 2 weeks elevated levels of PGC-1 α and downstream proteins in skeletal muscle. Astaxanthin also induced the decrease in plasma fatty acids and prevented the exercise-induced reduction in intermuscular pH. These observations suggest that astaxanthin induces lipid metabolism in skeletal muscle during exercise through the elevation of PGC-1 α .

References

- Kuhn R, Soerensen NA. The coloring matters of the lobster (*Astacus gammaurus* L.). *Z Angew Chem* 1938; **51**: 465–466.
- Miki W. Biological functions and activities of animal carotenoids. *Pure Appl Chem* 1991; **63**: 141–146.
- Naguib Y. Antioxidant activities of astaxanthin and related carotenoids. *J Agric Chem* 2000; **48**: 1150–1154.
- Aoi W, Naito Y, Sakuma K, *et al.* Astaxanthin limits exercise-induced skeletal and cardiac muscle damage in mice. *Antioxid Redox Signal* 2003; **5**: 139–144.
- Naito Y, Uchiyama K, Aoi W, *et al.* Prevention of diabetic nephropathy by treatment with astaxanthin in diabetic db/db mice. *Biofactors* 2004; **20**: 49–59.
- Aoi W, Naito Y, Takanami Y, *et al.* Astaxanthin improves muscle lipid metabolism in exercise via inhibitory effect of oxidative CPT 1 modification. *Biochem Biophys Res Commun* 2007; **366**: 892–897.
- Ikeuchi M, Koyama T, Takahashi J, Yazawa K. Effects of astaxanthin supplementation on exercise-induced fatigue in mice. *Biol Pharm Bull* 2006; **29**: 2106–2110.
- Earnest CP, Lupo M, White KM, Church TS. Effect of astaxanthin on cycling time trial performance. *Int J Sports Med* 2011; **32**: 882–888.
- Wu Z, Puigserver P, Andersson U, *et al.* Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* 1999; **98**: 115–124.
- Olesen J, Kiilerich K, Pilegaard H. PGC-1 α -mediated adaptations in skeletal muscle. *Pflugers Arch* 2010; **460**: 153–162.
- Patti ME, Butte AJ, Crunkhorn S, *et al.* Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1. *Proc Natl Acad Sci USA* 2003; **100**: 8466–8471.
- Calvo JA, Daniels TG, Wang X, *et al.* Muscle-specific expression of PPAR- γ coactivator-1 α improves exercise performance and increases peak oxygen uptake. *J Appl Physiol (1985)* 2008; **104**: 1304–1312.
- Wenz T, Rossi SG, Rotundo RL, Spiegelman BM, Moraes CT. Increased muscle PGC-1 α expression protects from sarcopenia and metabolic disease during aging. *Proc Natl Acad Sci U S A* 2009; **106**: 20405–20410.
- Brooks GA. The lactate shuttle during exercise and recovery. *Med Sci Sports Exerc* 1986; **18**: 360–368.
- Robergs RA, Ghiasvand F, Parker D. Biochemistry of exercise-induced metabolic acidosis. *Am J Physiol Regul Integr Comp Physiol* 2004; **287**: R502–R516.
- Steinhagen C, Hirche HJ, Nestle HW, Bovenkamp U, Hosselmann I. The interstitial pH of the working gastrocnemius muscle of the dog. *Pflugers Arch* 1976; **367**: 151–156.
- Boström P, Wu J, Jedrychowski MP, *et al.* A PGC- α -dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 2012; **481**: 463–468.
- Ristow M, Zarse K, Oberbach A, *et al.* Antioxidants prevent health-promoting effects of physical exercise in humans. *Proc Natl Acad Sci U S A* 2009; **106**: 8665–8670.
- Gomez-Cabrera MC, Domenech E, Romagnoli M, *et al.* Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance. *Am J Clin Nutr* 2008; **87**: 142–149.

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Abbreviations

- FNDC5 fibronectin type III domain containing 5
 NEFA nonesterified fatty acid
 PGC-1 α peroxisome proliferator-activated receptor- γ coactivator-1 α

Conflict of Interest

No potential conflicts of interest were disclosed.