

Effects of Astaxanthin Supplementation on Exercise-Induced Fatigue in Mice

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The present study was designed to determine the effect of astaxanthin on endurance capacity in male mice aged 4 weeks. Mice were given orally either vehicle or astaxanthin (1.2, 6, or 30 mg/kg body weight) by stomach intubation for 5 weeks. The astaxanthin group showed a significant increase in swimming time to exhaustion as compared to the control group. Blood lactate concentration in the astaxanthin groups was significantly lower than in the control group. In the control group, plasma non-esterified fatty acid (NEFA) and plasma glucose were decreased by swimming exercise, but in the astaxanthin group, NEFA and plasma glucose were significantly higher than in the control group. Astaxanthin treatment also significantly decreased fat accumulation. These results suggest that improvement in swimming endurance by the administration of astaxanthin is caused by an increase in utilization of fatty acids as an energy source.

Key words astaxanthin; fatigue; antioxidant; exercise; endurance capacity

Exercise-induced fatigue has been attributed to the following factors. First, myoglobin and an energy metabolic system coenzyme leak out into the blood from cells and tissues damaged by exercise, and destruction of red blood cells occurs. Second, exercise promotes consumption of energy sources such as glycogen by mobilizing internal energy metabolism to the maximum and using and depleting the energy source. Third, through these processes, exercise causes the production and accumulation of products of metabolism, such as lactic acid, in the body.^{1–3)} Therefore, recovery from exercise fatigue requires repair of the damage that has occurred in the body. Specifically, resynthesis of the leaked cell and tissue components and consumed energy sources is needed, as are decomposition and removal of accumulated byproducts of metabolism.

During *in vivo* screening for endurance capacity and anti-fatigue foods, astaxanthin was found to have a potent enhancing effect on endurance capacity in mice.

Astaxanthin, a red carotenoid pigment, is a biological antioxidant that occurs naturally in a wide variety of living organisms. It has many highly potent pharmacological activities, such as antioxidative activity,^{4–7)} anti-tumor and anti-cancer effects,⁸⁾ immunomodulating actions,^{9,10)} and anti-diabetic⁹⁾ and anti-inflammatory actions.^{10,11)}

The chronic effects of astaxanthin on endurance capacity have not previously been demonstrated. In the present study, we investigated endurance capacity by administering astaxanthin to mice and then subjecting the animals to exercise in the form of swimming.

MATERIALS AND METHODS

Astaxanthin The astaxanthin used in this study was AstaREAL 50F, supplied by Fuji Chemical Industry Co., Ltd., Toyama, Japan.

Animals Four-week-old male ddY mice (SLC, Japan) were used. They were housed in standard cages (21.5×32×14 cm, 5 mice/cage) under controlled conditions of temperature (24±1 °C), humidity (50±2%), and lighting (lights on

from 08:00 to 20:00). They were provided a normal diet (MR stock, NIHON NOUSAN, Japan) and water *ad libitum*.

Swimming Exercise Test Protocol Experiment 1: The mice were allowed to adapt to the laboratory housing for at least 1 week. Forty mice were divided into four groups (*n*=10 per group). The mice were given either vehicle (olive oil) or astaxanthin in doses of 1.2, 6, or 30 mg/kg body weight by stomach intubation at 10:00 5 d a week for 5 weeks. Samples were administered in a volume of 200 μ l. The mice were submitted to weekly swimming exercise supporting constant loads (lead fish sinkers, attached to the tail) corresponding to 10% of their body weight. The mice were assessed to be fatigued when they failed to rise to the surface of the water to breathe within 5 s.¹²⁾ The swimming exercise was carried out in a tank (28×46×29 cm), filled with water to 26 cm depth and maintained at a temperature of 30±1 °C. To avoid circadian variations in physical activity, swimming exercise was performed between 11:00 and 17:00, a period during which minimal variation of endurance capacity has been confirmed in rats.¹³⁾

Experiment 2: The mice were given either vehicle (olive oil), or astaxanthin in doses of 1.2, 6, or 30 mg/kg body weight (*n*=10 per group) by stomach intubation 5 days a week for 3 weeks. Each of the mice had a weight attached (5% body weight) to the tail for the duration of the swim-to-exhaustion exercise. The mice were assessed to be fatigued when they failed to rise to the surface of the water to breathe within 5 s.¹²⁾

Experiment 3: The protocol was the same as above except that the mice were made to swim for a predetermined length of time (15 min) supporting loads corresponding to 5% of their body weight.¹²⁾ Blood samples for lactate, glucose, and non-esterified fatty acid (NEFA) determinations were collected 7 times from the tail before the beginning and at 5-min intervals during swimming exercise, as well as 10, 30, and 60 min after exercise. To avoid blood dilution with residual water at the tail of the animal, the mice were quickly dried with a towel immediately before blood collection. The mice were immediately returned to the tank after blood sampling.

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Lactic acid concentration was determined with a Kyowa Medex commercial kit (Determiner LA, Tokyo, Japan). NEFA was measured by the acyl-CoA synthetase and acyl-CoA oxidase enzyme method with a commercial kit (NEFA C-test Wako, Wako Pure Chemical Industries, Osaka Japan). Glucose was assayed by a combination of mutase and glucose oxidase with a commercial kit (Glucose CII test Wako).

The following week, these groups were further subdivided into non-exercise and exercise groups. Exercise groups were made to swim for 15 min supporting loads corresponding to 5% of their body weight, and immediately after swimming were killed by dislocation of the neck. Blood samples for creatine kinase (CK) activity were taken from the heart. Plasma samples were refrigerated until assay, and CK activity was measured using a commercial kit (CPKII test Wako). Liver and muscle samples from mice in both groups were removed and stored at -20°C , and glycogen content was determined using the method of Lo *et al.*¹⁴ Briefly, portions of the muscle and liver were put into a tube containing 1.5 ml of 30% KOH saturated with Na_2SO_4 and immersed in a boiling water bath for 30 min before glycogen was assayed using a commercial kit (Glucose CII test Wako).

Animal studies were performed according to the regulations of our laboratory in line with the 1980 guideline Notification No. 6 of the Prime Minister's Office of Japan.

Statistical Analysis Data are expressed as mean \pm S.E. Comparisons of swimming capacity between control and treated groups were assessed using one-way analysis of variance (ANOVA) and the Tukey-Kramer Multiple Comparison Test. The data on metabolic parameters were analyzed by the unpaired *t* test. The data on glycogen concentration were assessed using two-way analysis of variance (ANOVA) followed by Fisher PLSD *post-hoc* analysis. A level of $p < 0.05$ was used as the criterion for statistical significance.

RESULTS

Effects of Astaxanthin on Swimming Exercise In Experiment 1, which involved a 10% body weight load, the 6 mg/kg and 30 mg/kg astaxanthin groups showed a significant increase in swimming time to exhaustion as compared to the control group from the first week. In the 1.2 mg/kg astaxanthin group, a significant increase in swimming time to exhaustion as compared to the control group was evident after 5 weeks (Fig. 1). In order to investigate in detail, the mice had a weight attached 5% body weight for the duration of the swim-to-exhaustion. With a 5% body weight load, the mice in the astaxanthin groups again swam for significantly longer times compared to the control group (Fig. 2).

Effects of Astaxanthin on Blood Lactate, Glucose, and NEFA Concentration during Swimming (Experiment 3) In the astaxanthin groups, blood lactate concentration was significantly lower than in the control group (Fig. 3).

In the control group, plasma glucose was decreased by 15 min of swimming exercise. After the exercise ended, the plasma glucose recovered. However, in the astaxanthin 6 mg/kg, 30 mg/kg groups, plasma glucose was significantly higher than in the control group. In the control group, plasma NEFA concentration was decreased by 15 min of swimming exercise. In the astaxanthin 30 mg/kg group, plasma NEFA was significantly increased by swimming exercise.

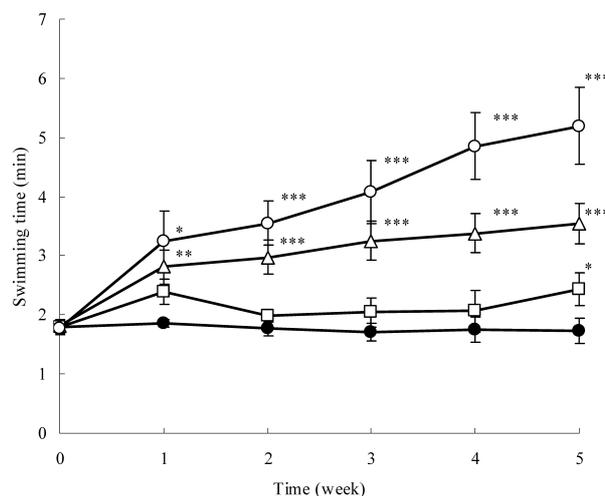


Fig. 1. Effect of Astaxanthin on Swimming Exercise in Mice (Experiment 1)

The mice were given either vehicle (●), or an astaxanthin dose of 1.2 (□), 6 (△), or 30 (○) mg/kg body weight ($n=10$ per group). The mice were made to perform swimming exercise with weights attached to their tails corresponding to 10% of their body weight. Each value represents mean \pm S.E. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ vs. control.

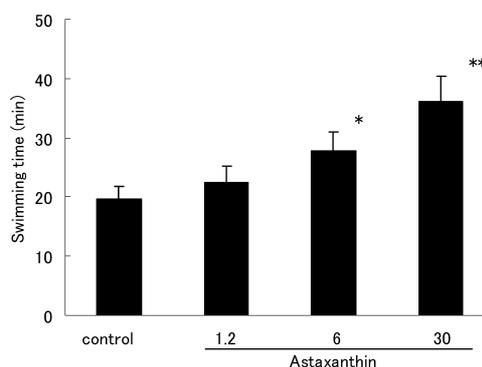


Fig. 2. Effect of Astaxanthin on Swimming Exercise in Mice (Experiment 2)

The mice were given either vehicle or an astaxanthin dose of 1.2, 6, or 30 mg/kg body weight for 3 weeks ($n=10$ per group). The mice swam with weights attached to their tails corresponding to 5% of their body weight. Each value represents mean \pm S.E. Significant difference from corresponding control group (* $p < 0.05$, *** $p < 0.005$).

Effects of Astaxanthin on Epididymal Adipose Tissue Weight (Experiment 3)

There was no significant difference in body weight between the control group and astaxanthin groups for 5 weeks (control: 42.1 ± 1.0 g, astaxanthin 1.2 mg/kg: 42.9 ± 1.1 g, 6 mg/kg: 42.3 ± 1.2 g, 30 mg/kg: 42.3 ± 1.2 g). But in the 30 mg/kg astaxanthin group, epididymal adipose tissue weight was significantly ($p < 0.05$) decreased compared to that of the control group (Fig. 4). In the astaxanthin 1.2 mg/kg and 6 mg/kg groups, the epididymal adipose tissue weight tended to be lower than in the control group, but not significantly.

Effects of Astaxanthin on Liver and Muscle Glycogen

Non exercise, astaxanthin had no effect on glycogen concentration in the liver and gastrocnemius muscle. Liver glycogen contents were decreased by swimming exercise. However, liver glycogen contents were significantly higher in the astaxanthin 30 mg/kg groups than in the control group after swimming for 15 min (Fig. 5). In the astaxanthin 6 mg/kg and

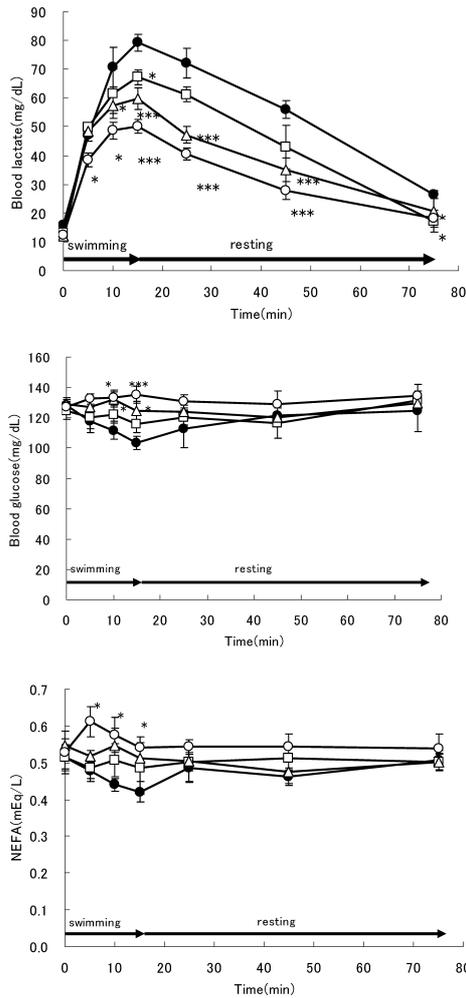


Fig. 3. Effect of Astaxanthin on Blood Lactate, Glucose, and NEFA Concentration during Swimming for 15 min (Experiment 3)

The mice were given either vehicle (●) or astaxanthin 1.2 mg/kg (□), 6 mg/kg (△), or 30 mg/kg (○) (n=10 per group). The mice were made to perform swimming exercise with weights attached to their tails corresponding to 5% of their body weight. Each value represents mean ± S.E. *p<0.05, ***p<0.005 vs. control.

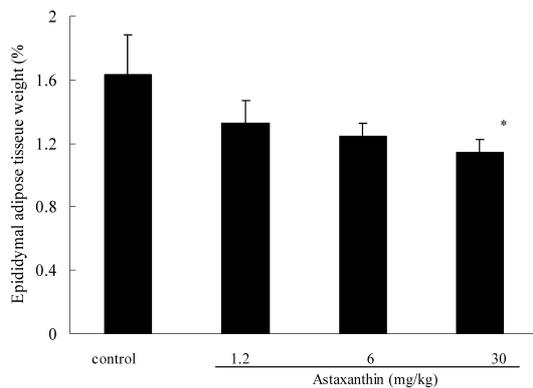


Fig. 4. Effect of Astaxanthin on Epididymal Adipose Tissue Weight after Swimming Exercise for 15 min (Experiment 3)

Each value represents mean ± S.E. *p<0.05 vs. control.

30 mg/kg groups gastrocnemius muscle glycogen contents tended to decrease, but not significantly. And, gastrocnemius muscle glycogen contents significantly higher in the astaxanthin 6 mg/kg and 30 mg/kg groups than in the control group after swimming for 15 min.

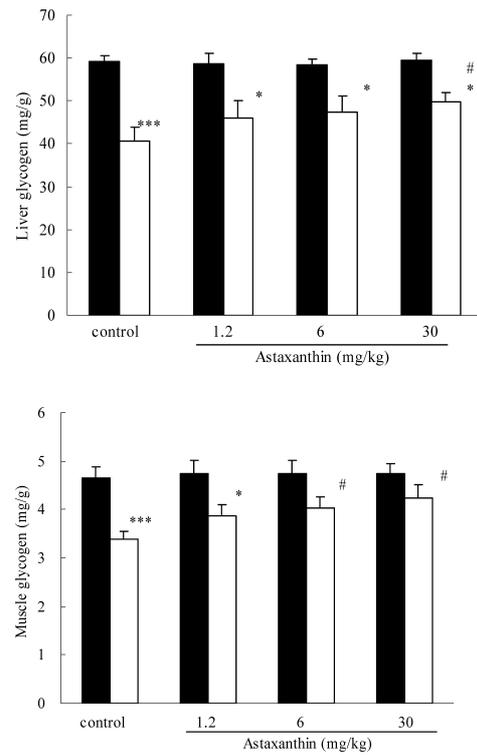


Fig. 5. Effect of Astaxanthin on Liver and Muscle Glycogen after 15 min of Swimming Exercise

The mice were given either vehicle or astaxanthin 1.2 mg/kg, 6 mg/kg, or 30 mg/kg for 5 week. ■: no-exercise, □: exercise. The mice were made to perform swimming exercise with weights attached to their tails corresponding to 5% of their body weight. Each value represents mean ± S.E. *p<0.05, ***p<0.005 vs. no-exercise. #p<0.05 vs. exercise control.

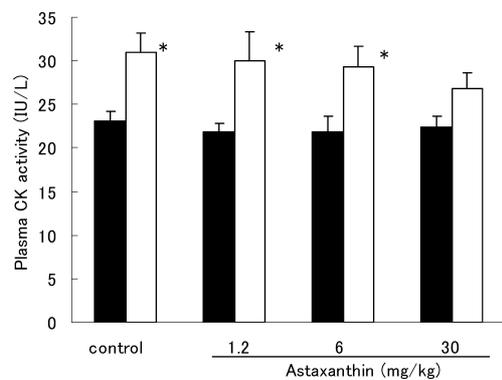


Fig. 6. Effect of Astaxanthin on CK Activity in Plasma after 15 min of Swimming Exercise

The mice were given either vehicle or astaxanthin 1.2 mg/kg, 6 mg/kg, or 30 mg/kg for 5 week. ■: non-exercise, □: exercise. The mice were made to perform swimming exercise with weights attached to their tails corresponding to 5% of their body weight. Each value represents mean ± S.E. *p<0.05 vs. non-exercise.

Plasma CK Activity Plasma CK activity was increased by exercise. But increased was reduced in the astaxanthin 30 mg/kg group (Fig. 6).

DISCUSSION

Many aspects of fatigue have been studied over the years, but adequate methods for objective evaluation of fatigue have not yet been established. In the present study, male mice exercised to fatigue, and the effect of astaxanthin supplementa-

tion on endurance capacity and fatigue was evaluated. Swimming time was significantly prolonged by administering astaxanthin. The present study aimed to clarify the manner of this effect.

In the control group, plasma glucose was decreased by swimming exercise. In the astaxanthin groups, plasma glucose was significantly higher than in the control group. In addition, liver and muscle glycogen contents were significantly higher in the astaxanthin groups than in the control group after swimming for 15 min. These results indicate that the supply of glucose can be used more smoothly and/or that glucose utilization may be decreased during exercise in the astaxanthin. The glycogen-sparing effect of astaxanthin could provide an important survival advantage in situations requiring extended periods of prolonged endurance exercise because glycogen depletion is associated with physical exhaustion, and slower utilization of glycogen results in improved endurance exercise performance. As one of the sources of blood glucose, liver glycogen plays an important role in controlling the availability of cellular energy. It is possible that astaxanthin may have promoted glycogenolysis restraint and/or gluconeogenesis. In addition, in the astaxanthin groups, the blood lactate concentration was significantly lower than in the control group. Lactic acid is produced as a result of carbohydrate metabolism. These results indicate that astaxanthin caused a decrease in glucose utilization during exercise.

Increased fatty acid utilization during exercise reduces the glycogen depletion rate and improves endurance exercise performance.¹⁵⁾ Therefore, increased fatty acid utilization is thought to be important for endurance performance. These increases are associated with enhanced lipolysis and sparing of stored glycogen, resulting in a delay of complete glycogen depletion by increasing circulating catecholamines. The enhanced availability of NEFA is thought to cause greater fat metabolism in the active muscles, which in turn decreases carbohydrate utilization and leads to increased exercise capacity.¹⁶⁾ These concepts agree with our research results. In the control group, plasma NEFA concentration was decreased by 15 min of swimming. However, in the astaxanthin 30 mg/kg group, plasma NEFA was significantly increased by swimming. Astaxanthin activated utilization of lipid to a greater extent than glucose as an energy source for performance. There was no significant difference in body weight between the control group and astaxanthin groups for 4 weeks. However, in the 30 mg/kg astaxanthin group, adipose tissue weight was significantly ($p < 0.05$) decreased compared to that of the control group. The metabolic effects of astaxanthin on endurance performance appear to be caused by the increase in fatty acid utilization as an energy source, with sparing of glycogen. The glycogen thus saved could become an available energy source for the later stages of exercise, thus delaying the onset of fatigue.

Improvement of cardiopulmonary function and increase of oxygen supply to tissues as a result of an increase of hemoglobin are commonly stated to be major factors that increase endurance capacity. In the present study, the hemoglobin concentration after 4 weeks of administration did not differ from that at the start of the study (data not shown). These results indicate that astaxanthin did not influence the supply of oxygen to tissues provided by hemoglobin.

It is well documented that a bout of aerobic physical exercise markedly increases O_2 uptake and consumption due to the increased skeletal muscle energy requirement. This increased O_2 consumption further augments the generation of reactive oxygen species (ROSs) when the scavenging capacity of both nonenzymatic and enzymatic defense mechanisms is overwhelmed. This is especially the case during an acute bout of exhaustive exercise. ROSs have been reported to cause modifications in cellular biochemical components such as protein, lipid, and DNA.^{17–19)} Furthermore, ROSs, like lactate anion and protons, have been suggested to be implicated in oxidative skeletal muscle fatigue. It is reported that ROS alter such transport systems as potassium transport and thus contribute to the onset of fatigue.²⁰⁾ Polyunsaturated fatty acids are another ROS target, and their peroxidation may lead to fluidity and permeability alterations. Moreover, lactate anion, independently from proton and thus pH modifications, may decrease muscle force production by inhibiting Ca^{2+} release from the sarcoplasmic reticulum and/or by changing ionic strength.²¹⁾ Astaxanthin has been reported to be more effective than other antioxidants, such as vitamin E and β -carotene, in preventing lipid peroxidation in solutions and in various biologic membrane systems.^{4–6)} Astaxanthin can attenuate exercise-induced damage in mouse skeletal muscle and heart, including an associated neutrophil infiltration that induces further damage.²²⁾ In the present study, plasma CK concentration after exercise was increased. But the increase in plasma CK activity was inhibited by treatment with astaxanthin. It is possible to say that astaxanthin does to free radicals, but stabilizes membranes, delays muscle fatigue, and thereby enhances endurance.

In conclusion, our data suggest that astaxanthin may have beneficial effects on endurance capacity. The administration of astaxanthin causes an increase in utilization of fatty acids as an energy source, which spares glycogen.

However, comprehensive chemical and pharmacological research is required to determine the mechanism by which astaxanthin affects endurance capacity.

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