Effects of *Anoectochilus formosanus* on Endurance Capacity in Mice

Mayumi IKeuchi1,*, Kohji Yamaguchi1, Tomio Nishimura2 and Kazunaga Yazawa1

1 Laboratory of Nutraceuticals and Functional Foods Science, Graduate school of Marine Science and Technology, Tokyo University of Marine Science and Technology, 4—5—7, Konan, Minato-ku, Tokyo 108—8477, Japan
2 Akatsuka Botanical Garden Co., 1868—3, Takanoo-cho, Tsu, Mie 514—2293, Japan
(Received June 17, 2004)

**Summary** The present study was designed to determine the effects of *Anoectochilus formosanus* extract (AFE) on endurance capacity in mice. Four wk-old male mice were given either a vehicle (distilled water) or AFE (500, 1,000 mg/kg) through stomach intubations for 4 wk. Mice were made to perform swimming exercises with weights attached to their tails corresponding to 10% of their body weight. Endurance capacity was evaluated by swimming time to exhaustion. The group treated with 1,000 mg/kg AFE showed a significant improvement (p<0.05) in endurance performance time. The mice were made to swim for 15 min with loads corresponding to 5% of their body weight. In the 1,000 mg/kg body weight of AFE administration group, blood lactate concentration was significantly lower than in the control group. In the AFE administration group, the plasma non-esterified fatty acid (NEFA) was significantly increased by swimming exercise. AFE treatment also significantly decreased fat accumulation. Liver and gastrocnemius muscle glycogen after 15 min of swimming remained at significantly higher levels in the mice fed 1,000 mg/kg of AFE as compared to the control group. These results suggest that AFE activated utilization of lipid more than glucose as the energy source for performance.

**Key Words** endurance capacity, *Anoectochilus formosanus*, lactate

Recently, much attention has been paid to the phenomenon known as fatigue. However, the molecular mechanisms of fatigue still remain unclear. Fatigue is divided into two kinds according to the manner in which it is manifested, physical fatigue and psychological fatigue. Physical fatigue is divided into muscle fatigue and central nervous system fatigue. Muscle fatigue is caused by exercise that activates the physical energy metabolism. Muscle fatigue is induced by a decrease of energy sources in skeletal muscle and an accumulation of contain products that are increased by exercise. On the other hand, central nervous system fatigue is caused by a decrease of blood sugar and the production of fatigue-products in the brain (1—3). These types of fatigue can be prevented and one can recover from them by taking a certain nutrient. Fatigue-related research generally includes an examination of treatments that delay the occurrence of fatigue and enhance physical performance. This often involves nutritional strategies that supply extra fuel to the working muscle, or buffer the buildup of toxic metabolic by-products.

For example, carbohydrate or branched-chain amino acid (BCAA) feedings may delay increases of 5-HT (5-hydroxytryptamine) and improve performance (4). Vitamin E is a major free-radical-trapping antioxidant in plasma low density lipoprotein, and dietary supple-

*To whom correspondence should be addressed.
E-mail: fm03544@edu.s.kaiyodai.ac.jp

**MATERIALS AND METHODS**

**Extract preparation**

Whole plants of *Anoectochilus formosanus* were propagated by tissue culture. The fresh whole plants of *Anoectochilus formosanus* (600 g) were cut and extracted with 99.5% ethanol at room temperature for 24 h. After filtration, the extract was concentrated to give a red powder (5.0 g).
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. They were allowed to do a free feeding until the start of the experiment.

Swimming exercise test protocol

Experiment 1: The mice were allowed to adapt to the laboratory housing for at least 1 week. Thirty mice were divided into three groups (n=10). The mice were given either a vehicle (distilled water), or AFE in doses of 500 or 1,000 mg/kg/d, by stomach intubations 5 d a week for 4 wk. The mice were administered the dosage at 10:00. The mice were submitted to 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. The mice carried out swimming exercise tests every other week. The swimming exercise was carried out in a tank (28×46×29 cm) filled with water to 26 cm in depth and maintained at a temperature of 30±1°C. Swimming exercise was done from 11:00 to 17:00, a period in which minimal variation of endurance capacity has been confirmed in rats, in order to avoid circadian variations in physical activity (10). Blood samples were taken from the tail before swimming exercise. Red blood cells, hemoglobin, triglyceride, and cholesterol were assayed.

Experiment 2: The mice were allowed to adapt to the laboratory housing for at least 1 wk. Twenty mice were divided into two groups (n=10). The mice were given either a vehicle or 1,000 mg/kg/d AFE by stomach intubations 5 d a week for 4 wk. Mice were made to swim for 15 min supporting loads corresponding to 5% of their body weight. 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, and 10, 30, and 60 min after exercise. 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 (NEA 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).

Muscle and liver glycogen analysis

Mice were made to swim for 15 min and immediately after swimming were killed by dislocation of the neck. Liver and muscle samples were removed and stored at −20°C. The liver and muscle glycogen content was determined using the method of Lo et al (11). Briefly, portions of the muscle and liver were put into a tube containing 1.5 mL of 30% KOH saturated with Na2SO4 and immersed in a boiling water-bath for 30 min, before glycogen was assayed using a commercial kit (Glucose CII test Wako).

Statistical analysis

Data are expressed as mean±SE. Comparisons of swimming capacity between control and treated groups (500, 1,000 mg/kg) 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 muscle and 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

Effect of Anoectochilus formosanus on the swimming exercise

The mice were given either a vehicle (distilled water), or AFE in doses of 500 or 1,000 mg/kg/d, by stomach intubations 5 d a week for 4 wk. The mice were submitted to swimming exercise supporting constant loads 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. There was no significant difference in body weight between the control group and AFE groups for 4 wk (control: 44.1±1.3 g. AFE 500 mg/kg: 44.6±1.4 g. AFE 1,000 mg/kg: 44.6±1.3 g). The 1,000 mg/kg body weight AFE group showed a significant increase (61%) in their swimming time to exhaustion as compared to the control group (p<0.05). In the 500 mg/kg body weight AFE group, no clear effect of AFE was observed (Fig. 1).

Fig. 1. Effect of Anoectochilus formosanus on the swimming exercise in mice. The mice were given either a vehicle (□), or AFE dose of 500 (▲), or 1,000 mg/kg body weight (●) (n=10 per group). Mice were made to perform swimming exercise with weights attached to their tails corresponding to 10% of their body weight. Each value represents mean±SE. Significant difference from corresponding control group (*p<0.05, **p<0.01).
Table 1. Effect of Anoectochilus formosanus on RBC, hemoglobin, plasma triglyceride, plasma total cholesterol, and plasma glucose.

<table>
<thead>
<tr>
<th></th>
<th>RBC (×10⁴/mm³)</th>
<th>Hb (g/dL)</th>
<th>TG (mg/dL)</th>
<th>TC (mg/dL)</th>
<th>GLU (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>554±27.5</td>
<td>14.4±0.23</td>
<td>145.2±10.8</td>
<td>149.5±5.3</td>
<td>124.8±7.2</td>
</tr>
<tr>
<td>A. formosanus</td>
<td>557±15.6</td>
<td>14.7±0.22</td>
<td>129.9±9.5</td>
<td>131.2±2.2*</td>
<td>117.3±5.2</td>
</tr>
</tbody>
</table>

Each value represents mean±SE; n=10 for each group. *p<0.05 vs. vehicle group.

The hemoglobin concentration showed no difference at 4 wk after administration (Table 1). The concentration of total cholesterol in the AFE group was significantly lower than in the control group. The concentration of triglyceride tended to be lower than in the control group, but not significantly.

Effect of Anoectochilus formosanus on blood lactate concentration during swimming

In the 1,000 mg/kg body weight AFE administration group, blood lactate concentration was significantly lower (25–40%) than in the control group (Fig. 2).

Effect of Anoectochilus formosanus on plasma glucose and NEFA concentration during swimming

In the control group, plasma glucose was decreased by 15 min of swimming exercise. After the exercise finished, the plasma glucose recovered. However, in the 1,000 mg/kg body weight AFE group, 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. However, in the 1,000 mg/kg body weight AFE group, plasma NEFA was significantly increased by swimming exercise. After 10 min of exercise, the concentration of NEFA in the AFE group was suddenly and became the same as in the control (Fig. 3).

Fig. 2. Effect of Anoectochilus formosanus on blood lactate concentration during swimming for 15 min. Twenty mice were divided into two groups (n=10). The mice were given either a vehicle (□), or 1,000 mg/kg body weight AFE (●) for 4 wk (n=10 per group). Mice were made to perform swimming exercise with weights attached to their tails corresponding to 5% of their body weight. Each value represents mean±SE. *p<0.05, ***p<0.005 vs. control group.

Fig. 3. Effect of Anoectochilus formosanus on plasma glucose (A), non-esterified fatty acid (B) concentration during swimming for 15 min. The mice were given either a vehicle (□), or 1,000 mg/kg body weight AFE (●) for 4 wk (n=10 per group). Mice were made to swim for 15 min supporting loads corresponding to 5% of their body weight. Each value represents mean±SE. *p<0.05, ***p<0.005 vs. control group. #p<0.05 vs. 1,000 mg/kg. ###p<0.005 vs. 1,000 mg/kg body weight AFE at 0 min.

Effect of Anoectochilus formosanus on epididymal Adipose tissue weight

There was no significant difference in body weight between the control group and AFE groups for 4 wk (control: 43.3±0.8 g; AFE 1,000 mg/kg: 43.6±0.7 g). But in the AFE group, epididymal adipose tissue weight was significantly (p<0.05) decreased compared to that of the control group (Fig. 4).
Effects of Seasonings on Ascorbic Acid

Effect of Anoectochilus formosanus on liver and muscle glycogen

Liver and gastrocnemius muscle glycogen contents were significantly higher in the 1.000 mg/kg body weight AFE administration group than in the vehicle group after swimming for 15 min (p<0.01) (Fig. 5).

DISCUSSION

This study showed that AFE was found to improve endurance capacity. Swimming time of mice was significantly prolonged by administering AFE. The present study aimed to clarify the manner of this effect.

In the AFE administration group, the blood lactate concentration was significantly lower than in the control group. This result indicates that AFE may attenuate production or enhance clearance of blood lactate. Blood lactate is supposedly generated during anaerobic metabolism, and lactate production is associated with fatigue. These facts indicate that AFE administration may decrease muscle fatique. Further investigation is needed to clarify the effect of AFE on production or removal of blood lactate.

In the control group, plasma glucose was decreased by swimming exercise. In the AFE administration group, plasma glucose was significantly higher than in the control group. These results indicate that blood glucose as an energy source for exercise can be supplied smoothly by AFE. AFE administration might reduce glucose consumption in exercise. In addition, liver and muscle glycogen contents were significantly higher in the AFE administration group than in the control group after swimming for 15 min. It is possible that AFE promoted gluconeogenesis or decreased glycolysis. The saved glycogen could become an available energy source for the following phases of exercise, which would delay the onset of fatigue.

In the control group, plasma non-esterified fatty acid (NEFA) concentration was decreased by 15 min of swimming exercise. However, in the 1.000 mg/kg body weight AFE group, plasma NEFA was significantly increased by swimming exercise. In addition, in the AFE group, epididymal adipose tissue weight was significantly decreased compared to that of the control group. These results suggested that AFE increased blood NEFA by enhancing lipolysis in adipose tissue. It is reported that NEFA mobilized from adipose tissue was utilized in contractile muscle during moderately intense endurance exercise (12). It is possible that blood NEFA increased by AFE administration was utilized in muscle as an energy source, and thus endurance capacity was improved.

Kim et al. demonstrated that the oral administration of capsaicin successively improved endurance capacity during prolonged exercise trials (13). This improvement was associated with enhanced lipolysis and sparing of stored glycogen, which results in delaying complete glycogen depletion by increasing circulating catecholamine. Furthermore, catecholamine activated hormone sensitive lipase. The enhanced availability of NEFA was thought to cause greater fat metabolism in the active

---

Fig. 4. Effect of Anoectochilus formosanus on epididymal adipose tissue weight. Each value represents mean±SE. n=10 for each group. *p<0.05 vs. control group.

Fig. 5. Effect of 1,000 mg/kg dose of Anoectochilus formosanus on liver and muscle glycogen after 15 min of swimming exercise. The mice were given either a control or 1,000 mg/kg body weight AFE for 4 wk (n=10 per group). ■: pre exercise. □: post exercise. Mice were made to swim for 15 min supporting loads corresponding to 5% of their body weight. Each value represents mean±SE. n=10 for each group. *p<0.05 vs. pre exercise group. **p<0.01, ***p<0.005 vs. post exercise control group.
muscles, which in turn decreases carbohydrate utilization and leads to increased exercise capacity (14). Results of this report accord with our research results. It is suggested that AFE also activated utilization of lipid more than glucose as energy source for performance.

It is known that improvement of cardiopulmonary function and increase of oxygen supply to tissues by hemoglobin improve endurance capacity. In the present study, AFE did not affect the hemoglobin concentration. This result suggested that AFE was not concerned with the increase of oxygen which is supplied to tissue by hemoglobin.

The swimming time of the control group showed a tendency to decrease in the first week (Fig. 1). The reason for this decrease may be derived from the stress of the swimming exercise and stomach intubations.

In conclusion, the data of our study suggests that AFE may have beneficial effects on endurance capacity. AFE activated utilization of lipid more than glucose as the energy source for performance. Further pharmacological research and identification of the active constituent will be required.

REFERENCES