Effects of Fenugreek Seeds (Trigonella foenum graecum) Extract on Endurance Capacity in Mice

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(Received March 1, 2006)

Summary The present study was designed to determine the effect of fenugreek seed extract (FG) on endurance capacity in male mice aged 4 wk. Mice were given orally either vehicle or FG (150, 300 mg/kg body weight) by stomach intubation for 4 wk. The 300 mg/kg FG group showed a significant increase in swimming time to exhaustion as compared to the control group. In the FG groups, blood lactate concentration 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 FG group, NEFA and plasma glucose were significantly increased by swimming. FG treatment also significantly decreased fat accumulation. These results suggest that improvement in swimming endurance by the administration of FG is caused by the increase in utilization of fatty acids as an energy source.

Key Words fenugreek, endurance capacity, fatigue

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. Thirdly, through these processes, exercise causes the production and accumulation of metabolism-related substances, 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 internal reduction substances.

During in vivo screening for endurance capacity and anti-fatigue foods, an extract of fenugreek seeds was found to have a potent enhancing effect on endurance capacity in mice.

Fenugreek (Trigonella foenum graecum) is an annual plant from the family Leguminosae, cultivated in Mediterranean countries and India. The seeds of fenugreek have long been used as a traditional medicine and a traditional food consumed during pregnancy and lactation. Moreover, fenugreek seeds have pungent aromatic properties, and they are used as one of the spices in curry. The nutrient composition of fenugreek seeds is (in %): moisture 9.0; protein 26.0; fat 7.0; saponins 8–10; total dietary fibre 48.0 (4, 5). Recently, it has been reported that fenugreek improves diabetes (6–8), hypercholesterolemia (9), gastric ulcer (10), hyperthyroidism (11), neoplasia (12), and inflammation (13). However, the chronic effects of fenugreek seeds on endurance capacity have not been demonstrated. In the present study, we investigated endurance capacity by administering fenugreek seed extract to mice and then subjecting the animals to exercise in the form of swimming.

MATERIALS AND METHODS

Extract of fenugreek seeds. The extract of fenugreek seeds used in this study was supplied by TSI (Technical Sourcing International, Inc., USA). Fenugreek seed was extracted with ethanol, and the solution was evaporated. This extract included more than 20% 4-hydroxyisoleucine.

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 Nouran, 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 wk. Thirty mice were divided into three groups (n=10), which were given either a vehicle (distilled water), or extract of fenugreek seeds (FG) in doses of 150 or 300 mg/kg body weight, by stomach intubation at 10:00 5 d a week for 4 wk. The mice were subjected 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 (14). 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. To avoid circadian variations in physical activity, swimming exercise was performed from 11:00 to 17:00, a period in which minimal variation of endurance capacity has been confirmed in rats (15). Blood samples were taken from the tail before the final swimming exercise. Food was withdrawn at 6:00 and exercise was performed at 12:00, so the blood sample was collected after at least 5 h of fasting. Red blood cells, hemoglobin, triglyceride, and cholesterol were assayed.

Experiment 2: The protocol was the same as above except that the loads corresponded to 5% of 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.

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 (14). 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. 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. 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, Ltd., Osaka, Japan). Glucose was assayed by 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. 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. (16). 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).

Animals studies were done according to the regulations of our laboratory in line with the 1980 guideline entitled Notification No. 6 of the Prime Minister’s Office of Japan.

Statistical analysis. Data are expressed as mean ± SE. Comparisons of swimming capacity between control and treated groups (150, 300 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 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 fenugreek seeds extract on swimming exercise

In Experiments 1 and 2, the mice were assessed to be fatigued when they failed to rise to the surface of the water to breathe within 5 s. In Experiment 1, which involved a 10% body weight load, the 300 mg/kg FG

![Fig. 1. Effects of fenugreek seeds extract on swimming exercise in mice. The mice were given either vehicle (control, □) or an FG dose of 150 (▲) or 300 mg/kg body weight (●) (n=10 per group). The mice swim 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, ***p<0.005).](image1)

![Fig. 2. Effects of fenugreek seeds extract on swimming exercise in mice. The mice were given either vehicle (control) or an FG dose of 150 or 300 mg/kg body weight (n=10 per group). The mice swim with weights attached to their tails corresponding to 5% of their body weight. Each value represents mean±SE. Significant difference from corresponding control group (*p<0.05, ***p<0.005).](image2)
Increase in Swimming Endurance Capacity by Fenugreek

The mice in the fenugreek groups again swam for significantly longer times compared to the control group (Fig. 2). Hemoglobin (Hb) concentrations did not differ at 4 wk after administration (Table 1). The concentration of plasma triglyceride (TG) in the 300 mg/kg FG group was significantly lower than in the control group. The concentration of total cholesterol (TC) tended to be lower than in the control group, but not significantly.

Effects of fenugreek seeds extract on blood lactate, glucose, and NEFA concentration during swimming (Experiment 3)

In the FG 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 FG 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, but in the FG groups, plasma NEFA was significantly increased by swimming exercise.

Effects of fenugreek seeds extract on epididymal adipose tissue weight

There was no significant difference in body weight

| RBC ($\times 10^6$/mm$^3$) | Hb (g/dL) | TG (mg/dL) | TC (mg/dL) | Glu (mg/dL) |
|---------------------------|-----------|------------|------------|-------------|
| Control                   | 564±36.5  | 14.7±0.54  | 149.6±17.2 | 158.5±18.9  | 123.0±5.9   |
| FG 150 mg/kg              | 554±32.4  | 14.7±0.66  | 147.5±7.5  | 146.3±30.8  | 136.0±10.3  |
| FG 300 mg/kg              | 569±40.4  | 14.4±0.99  | 136.3±7.1  | 144.3±22.6  | 133.3±8.9   |

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

Fig. 4. Effects of fenugreek seeds extract on epididymal adipose tissue weight. Mice were divided into three groups (n=10). The mice were given either vehicle (control) or an FG dose of 150 or 300 mg/kg body weight. Each value represents mean±SE, n=10 for each group. * p<0.05 vs. control group.
between the control group and FG groups for 4 wk (control: 42.1±1.0 g, FG 150 mg/kg: 42.9±1.1 g, FG 300 mg/kg: 42.3±1.2 g). But in the 300 mg/kg FG group, epididymal adipose tissue weight was significantly (p<0.05) decreased compared to that of the control group (Fig. 4).

**Effect of fenugreek seed extract on liver and muscle glycogen**

Liver and gastrocnemius muscle glycogen contents were significantly higher in the FG groups than in the control group after swimming for 15 min (Fig. 5).

**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 fenugreek seed extract supplementation on endurance capacity and fatigue was evaluated. Swimming time was significantly prolonged by administering FG. The present study aimed to clarify the manner of this effect.

In the control group, plasma glucose was decreased by swimming exercise. In the FG groups, plasma glucose was significantly higher than in the control group. In addition, liver and muscle glycogen contents were significantly higher in the FG groups than in the control group after swimming for 15 min. These results indicate that the supply of glucose can be used smoothly and/or that glucose utilization may be decreased by the use of fenugreek as an energy source during exercise. The glycogen-sparing effect provides 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. The need of skeletal muscle for concentrated carbohydrate energy sources can be met either by intracellular glycogen stores or by blood glucose. Blood glucose levels are maintained by dietary sources, gluconeogenesis, and glucogenolysis. As one of the sources of blood glucose, liver glycogen plays an important role in controlling the needs of cellular energy. After carbohydrate ingestion, absorbed glucose can be used as the substrate for liver glycogen synthesis or exit the liver and travel to peripheral tissues such as muscle. Metabolic byproducts of glucose metabolism, such as lactate, in these peripheral tissues can return to the liver for use as a gluconeogenic substrate (17, 18). The glucose 6-phosphatase formed in liver gluconeogenesis is either hydrolyzed to glucose and returned to the circulatory system or used for the synthesis of glycogen (19, 20). It is possible that fenugreek may have promoted glycogenolysis restraint and/or gluconeogenesis. In addition, in the FG 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 fenugreek caused a decrease in glucose utilization during exercise.

Increased fatty acid utilization during exercise reduces the glycogen depletion rate and improves endurance exercise performance (21). Therefore, increased fatty acid utilization is thought to be important for endurance performance. Caffeine is a meaningful source because increased plasma NEFA concentration reduces glycogen depletion (22). The oral administration of capsaicin successively improved endurance capacity during prolonged exercise (23, 24). These increases were associated with enhanced lipolysis and sparing of stored glycogen, which results in delaying complete glycogen depletion by increasing circulating catecholamine. 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 (25). In the control group, plasma NEFA concentration was decreased by 15 min of swimming. But in the FG group, plasma NEFA was significantly increased by swimming. These results accord with our research results, and it is likely that FG activated utilization of lipid more than glucose as an energy source.
source for performance. There was no significant difference in body weight between the control group and FG groups for 4 wk. But in the 300 mg/kg FG group, adipose tissue weight was significantly \( (p<0.05) \) decreased compared to that of the control group. FG increases hormone-sensitive lipase activity and enhances fat mobilization from adipose tissues, which results in increased plasma NEFA. The metabolic effects of FG on increasing 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.

Commonly, improvement of cardiopulmonary function and increase of oxygen supply to tissues by an increase of hemoglobin are stated to be major factors that increase endurance capacity. In the present study, the hemoglobin concentration was not different after 4 wk of administration. These results suggest that FG did not influence the supply of oxygen to tissues 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 (ROS) 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. ROS have been reported to cause modifications in cellular biochemical components such as protein, lipid, and DNA (26–28). Furthermore, ROS, like lactate anion and protons, are 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 (29). Polyunsaturated fatty acids are another ROS target, and their peroxidation may lead to fluidity and permeability alterations. Moreover, lactate anions, independently from protons and thus pH modifications, may decrease muscle force production by inhibiting \( Ca^{2+} \) release from the sarcoplasmic reticulum and/or by changing ionic strength (30). It has been reported that fenugreek seeds have antioxidant properties (31). It is possible to say that fenugreek seed extract does to free radicals, but stabilizes membranes, delays muscle fatigue, and thereby enhances the endurance.

The fenugreek seed extract used in this study contained 4-hydroxyisoleucine in high concentration (about 20%). However, when we did an experiment with 4-hydroxyisoleucine, we did not obtain the same effects as with fenugreek seed extract, suggesting that other components of the extract must be responsible for its activity.

In conclusion, our data suggest that FG may have beneficial effects on endurance capacity. The administration of FG causes an increase in fatty acid utilization as an energy source, which spares glycogen. However, comprehensive chemical and pharmacological research is required to determine the exact mechanism by which this extract affects endurance capacity and to identify the active constituent responsible for this effect.

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