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Author(s)	Ishihara, Kengo
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Development of a swimming system for the
evaluation of food components acting on
endurance exercise capacity

Kengo Ishihara

2000

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ABBREVIATIONS

ANOVA	analysis of variance
Hb	hemoglobin
HCA	(-)-hydroxycitrate
MCT	medium chain triglycerides
OBLA	onset of blood lactate accumulation
SEM	standard error of mean
SD	standard deviation
VO ₂	oxygen consumption
VO ₂ max	maximum oxygen consumption
TCA cycle	tricarboxylic acid cycle

GENERAL INTRODUCTION

Endurance exercise capacity, which is one of the various factors constituting physical fitness, is highly associated with health. Endurance exercise capacity is affected by diets and nutrition, and controlled studies using laboratory animals should hence be conducted to elucidate the effect of diets on endurance exercise capacity.

With respect to evaluate the effect of food components on endurance exercise performance, it is advantageous to use mice in a long-term experiment using some food specimens with limited availability. However, there few reports on the use of mice in such studies, since satisfactory equipment for evaluating the work capacity of mice has not been available.

The treadmill, the most widely used apparatus for the evaluation of the work capacity of small laboratory animals, is unsatisfactory when applied to quantitating the effect of diets on exercise performance of mice. Hatta (1994a, 1994b) reported that commercial treadmills are too large for mice. Thus, they either do not support the mice, allow the mice to avoid electric shock, or cause injury to the tail. The frequency of such accidents increases with running speed. Moreover, 4 to 8 wks of preliminary training are required in treadmill because mice do not adapt to the treadmill. Mice that learn slowly must be discarded or the data will be widely distributed. These disadvantages have been pointed out by many investigators (Hatta et al. 1994a, 1994b).

Forced swimming in small laboratory animals has been widely used for studying the physiology and capacity of the organism in response to stress. For the study of exercise physiology, swimming has a number of advantages over other types of exercise such as treadmill running. Swimming requires only simple and inexpensive equipment. No training is required since the typical laboratory rodents such as rat, mouse and guinea pig have natural swimming ability.

A variety of swimming tests have been used as criteria of the physical work capacity of animals under diverse experimental conditions (Armstrong et al. 1974, Beaton and Feleki 1966, Greenen et al. 1988, McArdle et al. 1968, Ryan et al. 1993,

1994, Yang et al. 1960). In order to standardize the work load and reduce the swimming time, weights at specific body weight percentages were added to the chest or tail of the animal (Dawson and Horvath 1970, Montoye et al. 1960, Pierce et al. 1984, Wilber 1959). However, although the attached weight increased the work load by countering buoyancy, it could not be completely confirmed to not hamper the animal's ability to swim by constraining free tail movement and by creating an irregular distribution of body weight.

To obtain a standard work load quantitating maximum swimming capacity without adding weight, we developed a swimming pool with a pump that generates water flow and creates currents. The objective of our present study was to quantitate the maximum swimming capacity of mice in this apparatus, itself designed for the purpose of testing the exercise capacity of mice following various treatments with diets and drugs.

In the present study, the author developed a swimming pool with a pump that generates water flow and creates currents for the evaluation of endurance capacity of mice and quantitated the maximum swimming capacity of mice in this apparatus. This thesis consists of two chapters: In chapter 1, the features of the developed pool was described detailly and the pool was compared to another two exercise methods, treadmill running and swimming with weight. The standard deviation of the maximum swimming time in our developed pool was almost same as that in treadmill and 20% smaller than that in the swimming with weight, and the developed pool were hence highly sensitive to the effect of food on endurance exercise performance compared with swimming with weight. The developed pool also did not require 4 to 8 wks of preliminary training required in treadmill and the exercise time until fatigue was 70% shorter than treadmill and the price of our developed swimming pool was about one-fifth of the treadmill. An adjustable-current swimming pool could consequently reduce the experimental period and cost required for the evaluation of the dietary components on endurance exercise performance.

In chapter 2, the author investigated the effect of some dietary components on endurance swimming capacity with the pool. In particular, Nanpao and (-)-hydroxycitrate (HCA) significantly increased the swimming capacity of mice. The author cleared that the enhanced lipid oxidation caused the increase in endurance exercise capacity of mice treated Nanpao or HCA.

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CHAPTER 1 Development of an adjustable-current swimming pool

Section 1 Development of the system and comparison to another exercise apparatus

In this section, the author described an adjustable-current swimming pool for the evaluation of endurance capacity of mice. To clarify how mice swam in the pool, the author measured the muscle glycogen and serum L-lactic acid concentration. Further, to compare the developed pool with another exercise apparatus, the author administered overdose caffeine, which should reduce endurance exercise capacity (Jacob and Michaud 1961, Kaplan et al. 1994), to mice and made them exercise until fatigue in three kinds of exercise apparatus containing the developed pool, treadmill running, and swimming with weight.

MATERIALS AND METHODS

Animals. Five-week-old male Std ddY mice (a closed colony) and CDF1 mice (an inbred strain) (Japan SLC, Hamamatsu, Japan) were used. They were housed in standard cages (33 x 23 x 12 cm, 6 mice/cage) under controlled conditions of temperature ($22 \pm 0.5^\circ\text{C}$), humidity (50%), and lighting (lights on from 0700 to 1900 h). They were provided a stock diet (type MF; Oriental Yeast Co., Ltd., Tokyo, Japan) and water ad libitum. The care and treatment of the experimental animals conformed to the Kyoto University guidelines for the ethical treatment of laboratory animals.

Design of adjustable current swimming pool. Figure 1 shows the design of the swimming system used. We used an acrylic plastic pool (90 x 45 x 45 cm) filled to a depth of 38 cm with water. The surface of the tank is clear and smooth, which prevents the animal from supporting itself while swimming. The current in the pool is generated by circulating water with a pump (type C-P60H, HITACHI, Tokyo, Japan). We devised a spout and suction parts generating a uniform current. Vertical holes of the appropriate diameter were bored on the nozzle in a straight

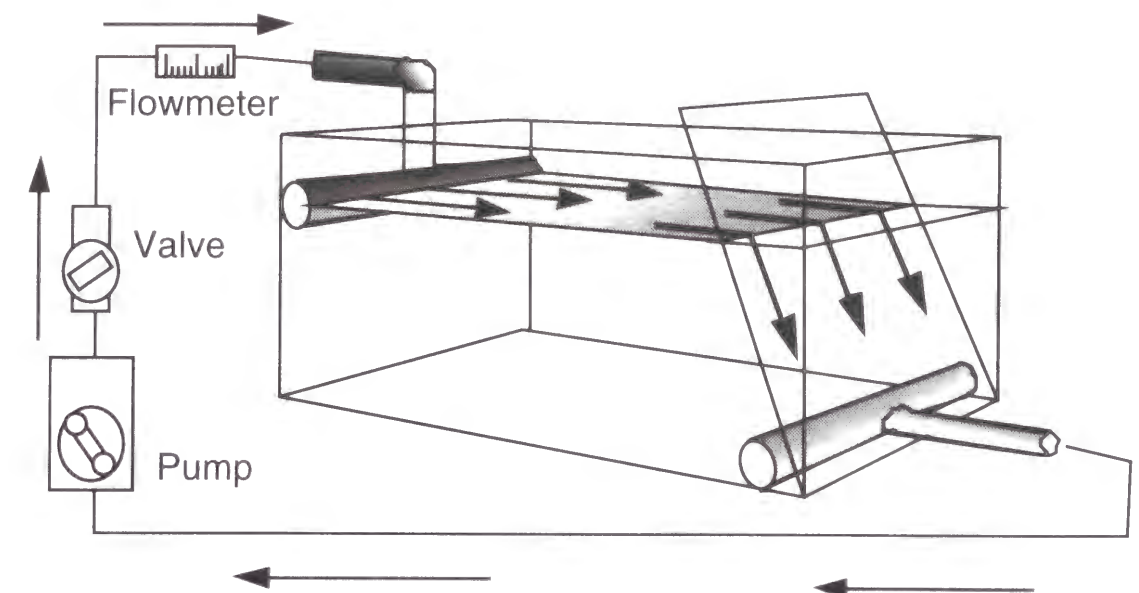


FIGURE 1 Illustration (side view) of the adjustable current pool. The dimensions of each part are described in the Materials and Methods section.

line with precision to the nearest 0.1~0.2mm. Water is returned to the pump through a narrow slit in the plastic pipe set on the bottom of the pool. The strength of the current is adjusted by changing the water flow, which is regulated by opening and closing a valve and is monitored by a water flowmeter (FC-A20, Tokyo Flowmeter Laboratory Co., Tokyo, Japan). The distribution of the surface current speed is measured with a digital current meter (type SPC-5 Sanko Industry Co., Tokyo, Japan) for 24 surface points spaced at regular intervals. The temperature of the water is maintained at 34°C with a water heater and thermostat.

Measurement of maximum swimming time in the adjustable current pool. To accustom them to swimming, all mice were given a 1-wk preliminary period, in which they swam for 30 min at 6L/min flow rate twice a week. They were then made to swim in groups of 6 at a time until fatigue, defined as failure to rise to the surface of the water to breathe within a 7-sec period. We noted characteristic changes of swimming behavior prior to fatigue; their posture became more upright and they were finally, as illustrated in Figure 2, unable to maintain the workload required of them to maintain themselves on the surface of the water, then sinking vertically with frothing. This was so characteristic that we could easily identify imminent fatigue prior to sinking with frothing and followed by greater than 7-sec spent below the surface. In previous studies, various criteria of exhaustion have been used, generally defined as performance inability ranging from 10 sec to 60 sec of submersion (Denadai 1994). The 7-sec interval employed in the present study is

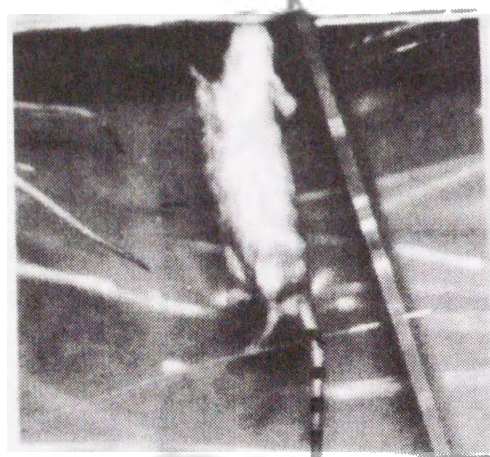
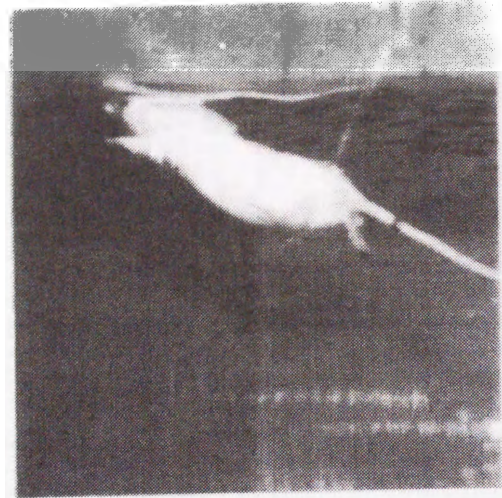
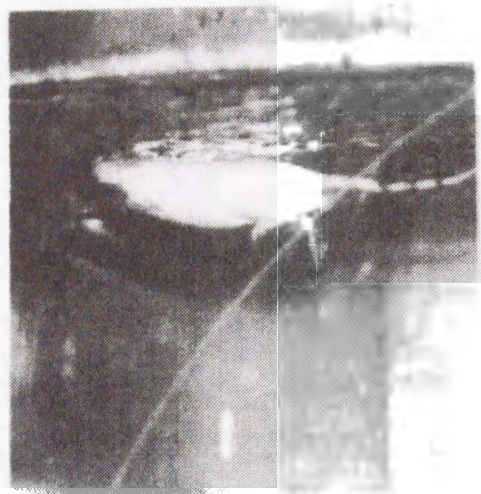


FIGURE 2 The postures of swimming mice at the first (upper left), midterm (upper) and final (left) stages of swimming. Imminent fatigue is signaled by righting of the posture, and the mouse is finally unable to maintain the work load required of it to remain on the surface of the water. This pattern of behavior was so characteristic that we could anticipate the fatigue of the mouse and make preparations to rescue it when it assumed the posture illustrated on the far right.

rather shorter than the previous studies using rats, but we found that drowning occurred frequently at longer intervals. Intervals shorter than 5 sec reduced the reproducibility of the test results. The suction of water at the end of the tank is not strong enough to suck in the mice.

We measured the total swimming period until fatigue as the index of the swimming capacity.

Analysis of kicking and paddling intervals. The swimming of mice in the pool in the current at various flow rates was recorded on videotape. The average numbers of hind limb kicks and forelimb paddlings were counted by inspection of the videoimages played back at slow speed.

Administration of caffeine. Mice administered a large dose of caffeine, which is reported to reduce endurance capacity (Jacob and Michaud 1961, Kitagawa 1968), were used as an inhibited group. The caffeine and placebo groups were subcutaneously injected 30 min before swimming, with 250 uL of caffeine in saline (100 mg/kg) and with 250 uL of saline, respectively, and the performance time until fatigue was measured.

Forced swimming with attachment of weights to the tail. Five-week-old Std ddY mice were accustomed to swimming in the adjustable current pool as described above. After the preliminary treatment, 32 mice were randomly divided into 4 equal groups. Measurement of the swimming time until fatigue with an attachment of weight to the tail was carried out in a static pool in 34°C water. The weights were made of lead thread, cut to the equivalent of 2% or 4% of the body weight of each mouse, and coiled and fixed around the base of the tail. The swimming was started at 1300 h. Mice were subcutaneously administered overdose caffeine as and maximum swimming time was measured as described above.

Comparison of current swimming with treadmill running. Treadmill running was performed after 5 days preliminary training period with progressively increasing in running speed. ddY mice of 5-wk-old ran up a 7% gradient as follows: 1st day; running for 15 min at 5m/min, 2nd day; 15min at 5m/min and then 3min at 8m/min, 3rd day; 5min at 5m/min, 10 min at 7m/min, 1 min at 8m/min, 1min at 9m/min and then 3min at 10m/min, 4th day; gradually increased from 1m/min to 10 m/min for 10 min and from 10 to 15 m/min for 5 min then 5min at 15m/min, 5th day; increased 1 to 15m/min for 10 min and 15 to 20 m/min for 5 min and then 5 min at 20m/min. At the end of the 5 days training session all mice were tested for determination of the maximum running time to fatigue as following protocols, increased in the speed from 1 to 13 m/min for first 10 min and the speed was increased 2m/min in every 20 min. Fatigue was defined as by both the mouse's inability to return to the treadmill belt from the shock grid and failure of the righting reflex (McArdle et al. 1968). Mice were subcutaneously administered overdose caffeine as and maximum running time was measured as described above.

The effects of swimming conditions on the swimming capacity. To examine the effect of the water flow rate on the swimming capacity, we changed the water flow rate (6, 7, 8, 9 and 10 L/min) with other conditions held constant (34°C water, swimming started at 1300 h). The mice were forced to swim at each water

flow rate and the swimming capacity was measured in the same way. To investigate the effect of water temperature, we then changed the water temperature (25, 30, 34 and 37°C), with other conditions again kept constant (water flow rate of 8 L/min, swimming started at 1300 h), and measured the swimming capacity in the same way.

Serum L-lactic acid analysis. Mice were made to swim in the current pool at the water flow rate of 6, 8, or 10L/min. Blood samples were taken from the tail during the 5th, 10th 20th, and 30th min of exercise and during the last min of exercise prior to exhaustion. Blood samples for lactate determination were immediately deproteinized in perchloric acid (0.8N) and centrifuged, and the serum L-lactic acid concentration was determined using a Kyowa Medex Co., Ltd. commercial kit [Determiner³ LA, Tokyo, Japan].

Muscle glycogen analysis. Mice were forced to swim for 10 min at the water flow rate of 6, 8, or 10 L/min, and after the swimming load, they were killed by dislocation of neck. The gastrocnemius and pectoralis muscles were removed immediately, frozen in liquid nitrogen and kept at -80°C until analysis for glycogen concentration. The glycogen content was measured spectrophotometrically by a method employing enzymatic techniques as described elsewhere (Fushiki et al. 1994). Briefly, after hydrolysis of the muscle sample in 0.6N HCl at 100°C for 2 hr, the glucose residues determined using a commercial kit (Glucose CII test Wako, Wako Pure Chemical Industries, Osaka, Japan).

Statistics. Statistical analysis of differences between pairs of groups was performed using Student's t test. Comparisons of the means among more than two groups were performed by one-way analysis of variance (ANOVA) followed by Tukey's test. Statistics were calculated with the InStat software package (Macintosh Version 2.00, GraphPad Software Inc., San Diego, CA). Probability levels of < 0.05 were considered to indicate significance.

RESULTS

Performance parameters of the adjustable current swimming pool.

The mice showed the maximum performance at the temperature of 34°C (data not shown). It was also noted that the reproducibility of the data for the maximum

swimming time was highly dependent on the uniformity of the current. Imprecise boring of the vertical holes on the nozzle disturbed the uniformity of the current, which caused scatter of the data distribution. Therefore, care was taken to bore the holes of the target diameter on the nozzle and in a straight line with precision to the nearest 0.1~0.2mm. The uniform current near the surface of the water extended to at least 3 cm depth below the surface as estimated by using the flow speed meter. The water return via the plastic pipe at the bottom of the pool aided in maintaining uniform current with minimum variation of the swimming data (data not shown).

The uniformity of the current was confirmed by the results of measurement with the digital surface current speed meter (**Fig. 3**). Uniform current was observed at each flow rate, except at the part of the pool immediately in front of the spout, where the mice for the most part did not stay during the swimming tests. At flow rate of less than 4 L/min, constant current speed could not be maintained in the remote part of the pool, suggesting that 4-5L/min might be the lower limit yielding reliable data. As alternative methods, generation of a surface current with air bubbling or with water stirring caused many disturbances, and fine adjustment of flow speed was impossible (data not shown). In these methods, we observed an

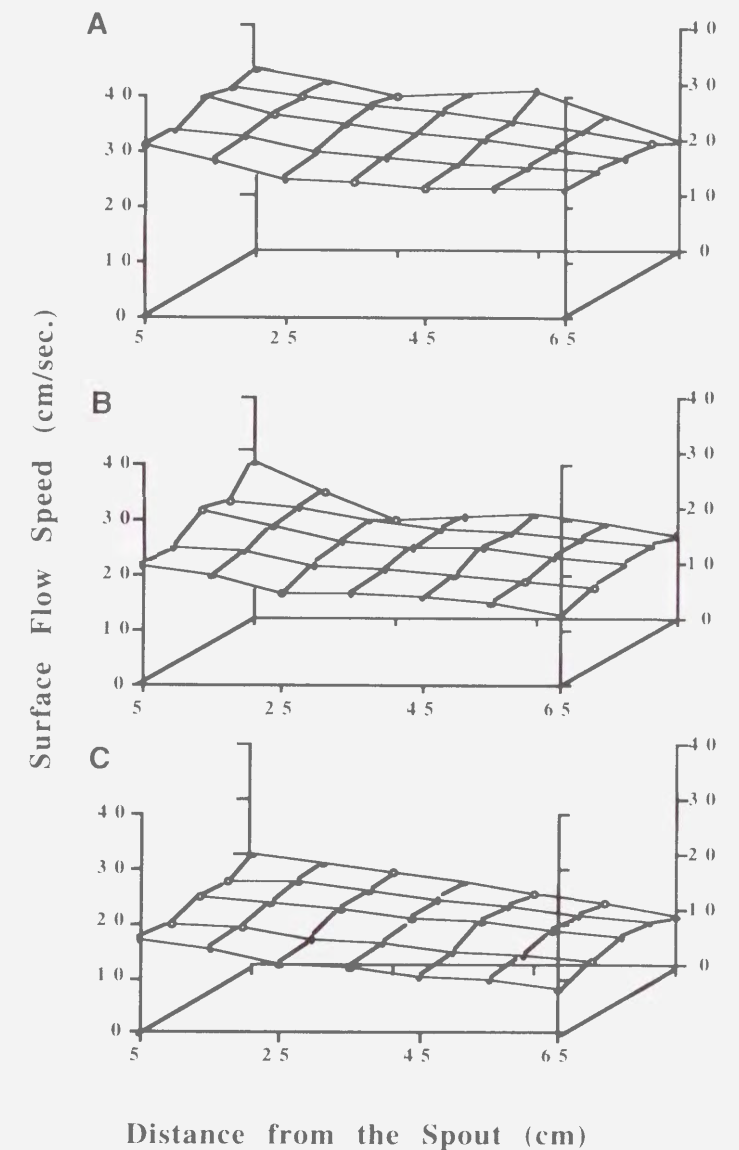


FIGURE 3 Surface currents of each part of the pool. Surface of the pool was divided into 24 grids, and the average current of each was measured with a digital surface meter at the flow rates of 10 (A), 7 (B), and 5 L/min (C). Measurement was repeated at least 3 times, and the average speed is shown for each grid plotted and linked to the neighboring grids.

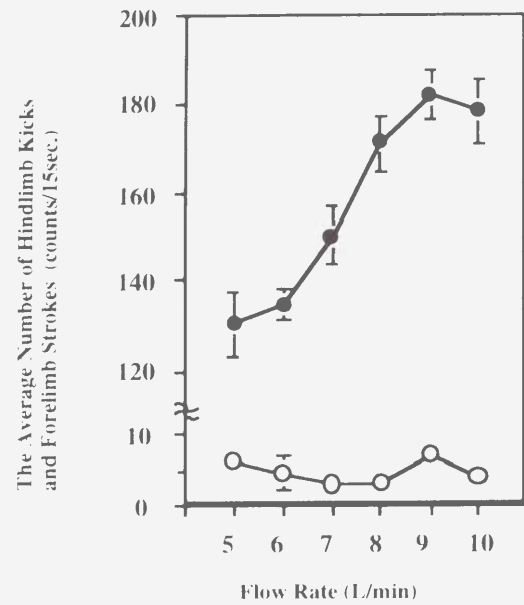


FIGURE 4 The average number of hind limb kicks and forelimb strokes at each flow rate. The movement of the hind limb was observed on videoimages played back at slow speed. Values are means \pm SEM (n=4).

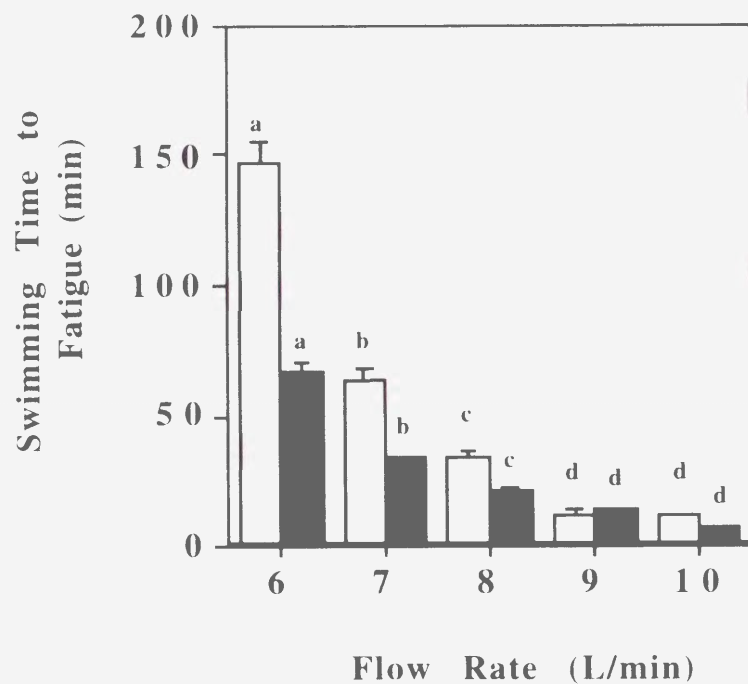


FIGURE 5 The swimming time until exhaustion at various flow rates in ddY (open bars) and CDF1 (solid bars) mice. Swimming time, which is defined in the text, decreased with increase in the flow rate. Values are means \pm SEM for 18-25 mice. Values not sharing common superscript letters within the same strain are significantly different, $P < 0.05$.

irregular current, stagnation, and a surge, with current decay especially prominent in the remote part at the low speed.

As the flow rate was increased, the work load of the mice became greater, which was evidenced by the data for the average number of kicks increased by speeding up current (Fig. 4). The hind limbs were more actively used by the mice than the forelegs in this apparatus; the average number of forelimb strokes was very low and did not increase upon speeding up of the current. The hind limb kicking intervals were not invariably constant, but rather they formed clusters interspersed with rest, which is a common behavior of

rodents in treadmill running. In the swimming apparatus the mice did not become submerged under the water while attempting to rest during exercise.

The analysis of the swimming time until exhaustion at various current speeds in each mouse strain revealed a strong correlation of work load and flow rate, as shown in Figure 5. The maximum swimming time to fatigue in both strains clearly decreased with increase in the flow rate,

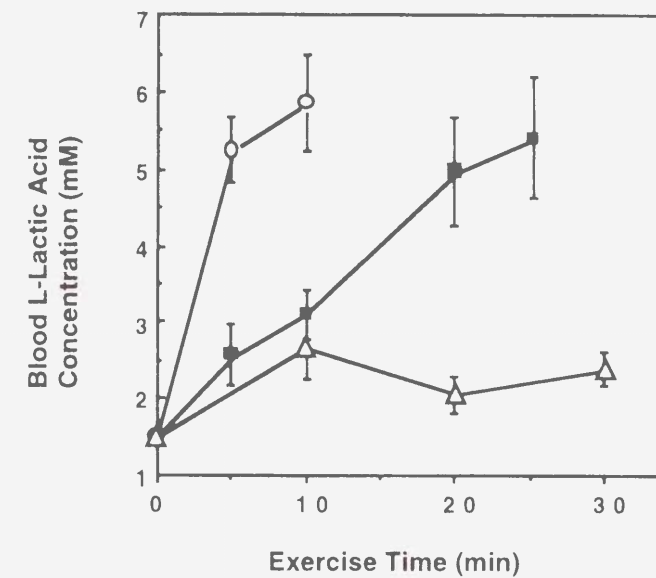


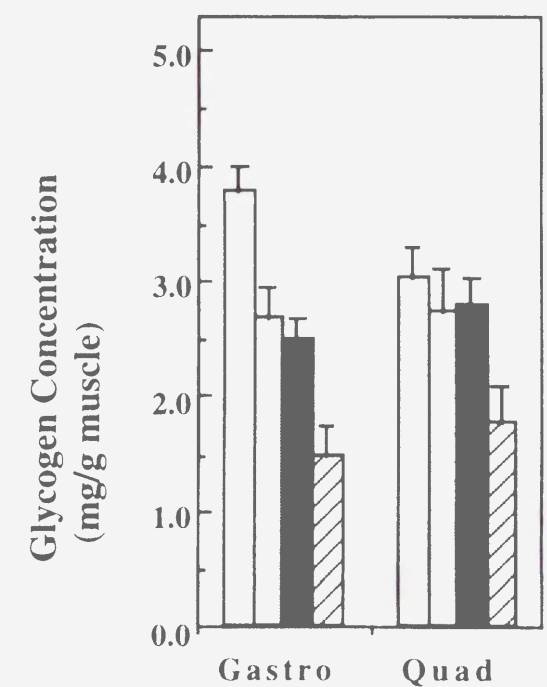
FIGURE 6 Blood L-lactic acid concentration during swimming at 10 (○), 8 (■), and 6 L/min (△). Measurement of L-lactic acid was as described in the text. Values are means \pm SEM for 4-6 mice.

rates of 6, 8, and 10L/min flow are presented in Figure 6. The blood L-lactic acid concentration remained at slightly higher than the resting level when the flow rate was 6L/min, increased abruptly in proportion to exercise time above the rate of 8L/min,

indicating that the work load could be finely regulated by manipulation of the flow rate. Across the flow rates the data for the CDF1 mice showed less variation than those for the ddY mice, perhaps due to the lesser genetic variation.

Biochemical indexes also indicated the correlation between work load and the current speed. The data demonstrating an increase of serum L-lactic acid concentration across the flow

FIGURE 7 Muscle glycogen concentration 10 min after the start of swimming at the various current flow rates. Open bars, sedentary mice; crosshatched bars, 6 L/min; solid bars, 8 L/min; hatched bars, 10 L/min. Gastrocnemius and pectoralis muscles were immediately quickly resected in mice killed by 10 min after the start of swimming, and the glycogen concentration was analyzed. Values are means \pm SEM for 6-9 mice. Values not sharing common superscript letters were significantly different ($P < 0.05$).



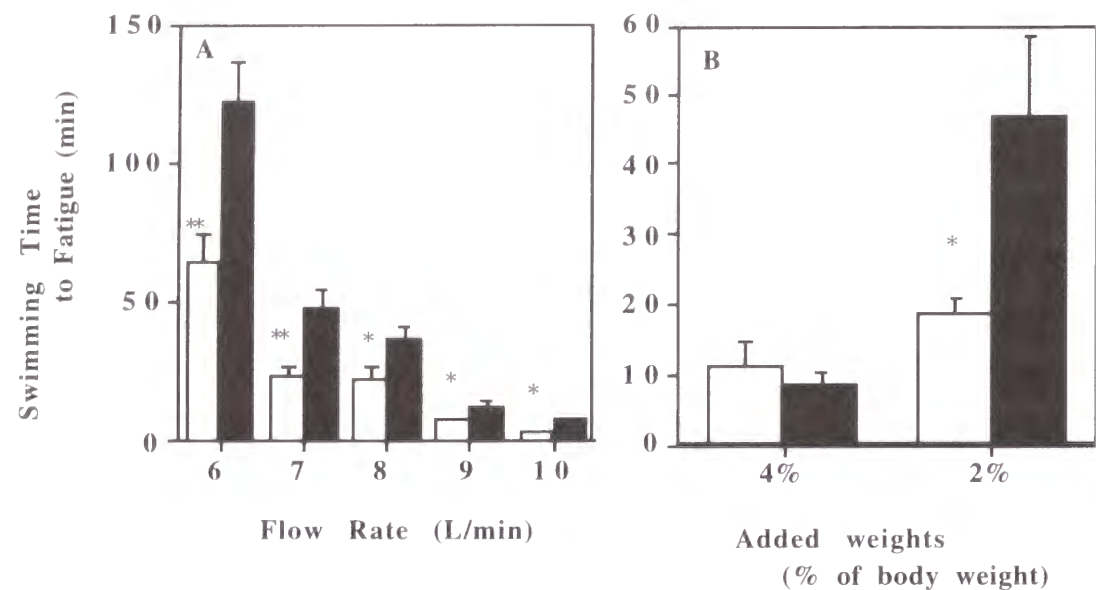


FIGURE 8 Effects of caffeine (100 mg/kg body weight) on swimming time to fatigue. Caffeine solution was injected subcutaneously 30 min before start of swimming. A: swimming time of caffeine (open bars) and placebo (solid bars) groups. B: Swimming time of caffeine and placebo groups with loads equal to 2% and 4% of body weight attached to tail. Values are means \pm SEM for 8-17 (A) and for 8 (B) mice. Significantly different from corresponding control value: * $P < 0.05$; ** $P < 0.01$.

indicating that the blood lactate accumulation (OBLA) point lies about 8 L/min. The glycogen concentration of the gastrocnemius muscle after 10 min of swimming declined with flow rate increase, suggesting that the higher flow rate demanded greater gastrocnemius muscle (hind limb) glycogen consumption. On the other hand, the pectoralis muscle (forelimb) glycogen consumption was rather slow, which is consistent with the data indicating low average number of forelimb strokes during swimming (Fig. 7).

Comparison of the current swimming system with forced swimming with weight load attached to the tails of mice with and without caffeine pretreatment. The subcutaneous administration of 100 mg/kg of caffeine 30 min before

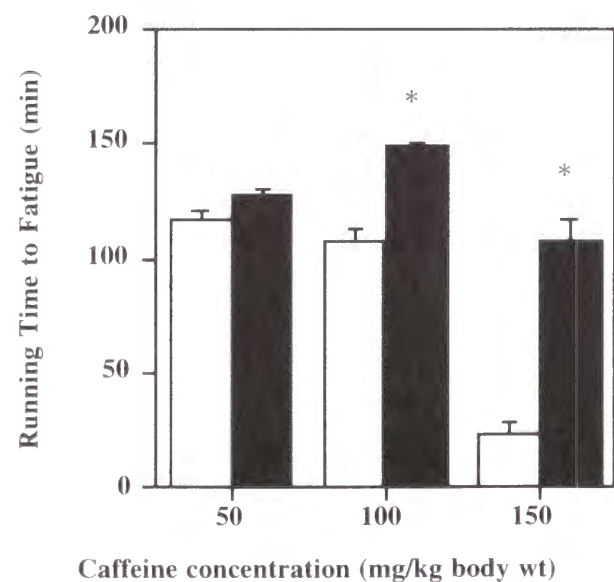


FIGURE 9 Effect of caffeine on running time to fatigue. Caffeine was injected subcutaneously 30 min before start of running. * $P < 0.05$.

swimming markedly decreased the swimming time to fatigue at each current speed, as shown in Figure 8A. A significant difference was observed at every flow rate. Attaching a weight to the tail had similar effect (Fig. 8B). The performance of the mice, however, showed a significant difference in the placebo and caffeine groups only at the 2%, and not at the 4%, added weight.

Comparison of current swimming pool and treadmill exercise protocols. The treadmill measurement of maximum performance required a long time to train the mice to keep running in the narrow lane and to adapt to gradual increase of the belt speed. The subcutaneous administration of 100 mg/kg of caffeine 30 min before swimming markedly decreased the running time to fatigue. Maximum running time was three times longer than maximum swimming time at 8 L/min of flow rate shown in Figure 8A, though the author detect the effect of overdose caffeine administration (Fig. 9). The result indicated that using many animals were difficult in treadmill running.

DISCUSSION

Forced swimming of animals has been employed as a criterion of their physical work capacity. Dawson and Horvath (Denadai 1994) pointed out that swimming has advantages over other forms of exercise, including the treadmill. Training is not required, as rodents have a natural swimming ability, and they are assumed to be highly motivated to avoid drowning when fatigue is imminent, assuring a high level of performance. However, as McArdle and Montoye (1966) noted in their review, certain problems arise with these tests in rats. Many of the rats immediately submerge themselves to the bottom in an apparent attempt to escape. If the tank is relatively shallow they learn to sink to the bottom to rest and push off to return to the surface. Weights have been attached to the tail to standardize the work load and reduce the swimming time in the static water pool. However, the artificial addition to the body weight by such attachment of weights may not always eliminate the effect of weight differences as a factor in swimming time (Denadai 1994, McArdle 1966). Furthermore, as a result of these problems, investigators are increasingly disinclined to use the swimming work load. In contrast

to rats, mice, we have noted, never learn to sink to the bottom to rest, as was also observed by Kaplan et al. (1994), who used a static water pool. Therefore, in mice, the swimming system potentially offers greater advantages.

At higher water flow speed (more than 9 L/min), the mice showed fatigue accompanied by high blood lactate concentration, suggesting that at some point they began to rely heavily on anaerobic metabolism to maintain themselves on the surface against the current. As we noted above, at such extreme work load, the mice never spontaneously submerged to the bottom to rest, perhaps due to the strong motivation to avoid drowning; this is another aspect of this system contributing to its reproducible work load quantification, especially at high work load intensity. The accumulation of blood lactate during swimming also supports the notion that swimming at fairly high flow rate (8-10 L/min) causes the mice to depend highly on anaerobic metabolism. On the other hand, at flow rates below 8 L/min, there was more modest accumulation of blood lactic acid during swimming, suggesting that the work load was still below the anaerobic threshold for mice.

The swimming system we have designed is not well suited for studies in the rat, because rats do rest on the bottom of the pool, as has been noted in previously reported swimming systems (Sturek et al. 1984). A similar disadvantage was noted by Flaim et al. (Flaim et al. 1979) when they applied swimming exercise in rats in their detailed studies of the cardiovascular response to acute aquatic and treadmill exercise. They noted a data discrepancy between treadmill and aquatic exercise and suggested that there appears to be a significant "learning" component involved in the aquatic form of exercise in addition to physical variables including the swimming tank structure and animal buoyancy affected by air trapped in the fur and the percent contribution to the body weight of adipose tissue.

Wilbur (1959) reported that there was a logarithmic decrease in swimming time with increased weight loading in guinea pigs. However, as reported by Scheer (1947), the variability in the specific gravity of rats contributes to the variability in swimming times in such methods. Given the other drawbacks of weight attachment described in the introduction to the present article, the degree of quantitative regulation of work load achieved by adding a weight to a rodent's tail or chest seems open to debate. Flaim et al. (1979) also pointed out that the rate of exercise and the magnitude of the workload can be more precisely controlled in treadmill exercise than in aquatic exercise with attached weights and continuous agitation of water. Adjustment

according to the specific gravity and production of quantitatively uniform work load in growing animals in a long-term experiment is time-consuming and painstaking if not outright impossible. Our present system offers clear advantages in this regard.

The increase of workload by weight attachment differed in its effects on the relative swimming capacities for the drug and placebo groups in comparison with the swimming in the current pool. As previously reported (Jacob and Michaud 1961, Kitagawa 1968), under the addition to the root of the tail of a weight made of a thread of lead equivalent to 2% of the body weight, administration of 100 mg of caffeine markedly reduced the maximum swimming time. A tail-weight equivalent to 4% of the body weight seems to have an effect corresponding to flow rate of about 9-10 L/min in our current pool, and that of 2% an effect comparable to about 7 L/min flow rate as derived from the data for the swimming time until fatigue.

Flaim et al. (1979) reported that the increase in cardiac output and the distribution of blood flow in swimming are very different from those in running, so that, for example, heart rate is not elevated at all by swimming compared to resting values, in contrast to treadmill running where typically a cardiovascular response is observed. In the present study using mice, the average number of hind limb kicks was well correlated with the flow speed. In the present investigation, we did not measure the cardiac responses, but the clear increase in the frequency of hind limb kicks with the current speed may likely have some effect on cardiovascular response like that seen in treadmill running. The recent study reported by Kaplan et al. supports these deductions; they cited a marked difference between the cardiac adaptations to chronic exercise with free swimming for 4 weeks in a rat model and those in a mouse model. They observed that diving was not a prominent behavioral component in the mice, and the induction of mitochondrial glycolytic enzyme was a readily documented feature of the exercise-associated response.

The swimming exercise in our pool system evoked a significant increase in endurance capacity. The swimming training in the pool 3 times per week for 2 weeks gradually increased the maximum swimming time, suggesting that swimming in a current pool is not only a stress but also enhances the endurance capacity as occurs in treadmill running (Dudley et al. 1982, Hickson 1981, Holloszy 1967, Holloszy et al. 1984, Pattengale et al. 1967). We observed a similar increase in maximum swimming time in our previous studies using the prototype of the current pool

described here in (Fushiki et al. 1995, 1994).

In conclusion, our current pool system offers many advantages in the evaluation of the endurance capacity of mice. In mice, the data obtained show higher reproducibility than those obtained for treadmill running. The apparatus we employed is also useful for detecting the effects of dietary differences and drug pretreatment on the endurance capacity.

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Section 2 Changes of maximum swimming time in various conditions which are considered to effect on physical fitness of mice

In section 1, the author developed an adjustable-current swimming pool and the way to measure the maximum swimming time until fatigue. The author also elucidated that the work intensity could be controlled by regulating the flow rate of the pump. The author described some advantages of the pool compared to the treadmill and swimming with weight.

In this section, the author clarified that the maximum swimming time correlated with endurance exercise capacity by confirming that the swimming time was changed as might have been expected from the condition in which the endurance exercise capacity clearly would change. For example, a chronic endurance training increases the endurance exercise performance and lipid oxidation capacity expressed as the enzyme capacity of TCA cycle, β -oxidation, and so on (Holloszy and Booth 1976). Iron deficiency (Perkkiö et al. 1985, Ohira et al. 1981) decrease oxygen carrying capacity and decrease the endurance exercise capacity.

MATERIALS AND METHODS

Animals. All the animals were housed in standard cages (33 x 23 x 12 cm; 6 mice/cage) under controlled conditions of temperature (22 ± 0.5 °C), humidity (50%), and lighting (lights on from 1800 h to 0600 h). They received humane care as outlined in the Guide for the Care and Use of Laboratory Animals (Kyoto University Animal Care Committee according to NIH #86-23; revised 1985).

Apparatus. The gas analyzer used for the assessment of metabolic rate in the mice consists of six acrylic metabolic chambers, CO₂ and O₂ analyzers (model RL-600, AlcoSystem), and a switching system (model ANI6-A-S, AlcoSystem) to sample gas from each metabolic chamber. Each metabolic chamber has a 125.4-cm² floor and is 6.5 cm in height. Room air was pumped through the chambers at a rate of 1.0 l/min. Expired air was dried in a cotton thin column and then directed to an O₂/CO₂ analyzer. Air from each chamber was sampled for 60s. During the last

1s the O₂ and CO₂ concentrations were measured 100 times and the average was used for the calculation of oxygen consumption (VO₂) and respiratory quotient (RQ). Therefore, the data of each chamber could be obtained every 7 minutes and the data were stored in a spreadsheet.

The running system used in the experiment was the treadmill (model Simplex II, Columbus) for running of mice. Three mice could run at the same time in the running lane (28.8 x 4.7 x 4 cm; LWH) and the system was placed in the acrylic metabolic chamber with an inside volume of about 1000 cm³ and an electric air fan in it for mixing the air.

In the experiment to measure the maximum oxygen consumption (VO₂max), the animals were forced to run up a 10% incline at 6 m/min and the running speed was progressively increased to 9 m/min for 4 min. After 4 min, the running speed was increased by 3 m/min every 1 minute. Exhaustion was defined as the point at which the mice were unable to maintain the pace and to avoid the shock grid set at the rear of the treadmill. Respiratory gas was monitored during running, and oxygen consumption, RQ, carbohydrate oxidation, lipid oxidation and VO₂max was calculated.

Experimental design

Experiment 1: the effect of a chronic training on maximum swimming time until fatigue . Sixteen male Std ddY mice (5 wk old Japan SLC, Hamamatsu, Japan) were used in the present study. They were given free access to water and a commercial diet (type MF; Oriental Yeast, Tokyo, Japan) containing the following (g/kg diet): water, 80; protein, 246; fat, 56; fiber, 31; carbohydrates, 523. All the mice were measured maximum oxygen consumption, and the maximum swimming time to fatigue and divided into two groups. Ten mice were made to swim for 1 hr/d at 5 L/min of flow rate 6 days per wk for 6 wk. The other four mice were given no exercise training. On 4th and 7th wk, the swimming time until fatigue and maximum oxygen consumption were measured in both groups of mice. On the last day of experiment, mice were killed by decapitation and the gastrocnemius and quadriceps muscles, epididymal and perirenal adipose tissue, liver, spleen, heart, and kidney were removed and weighed. Muscles and liver were immediately frozen in liquid N₂ and kept at -80 °C until analysis. The glycogen content was measured spectrophotometrically by a method using enzymatic techniques as described

elsewhere (Passonneau and Lauderdale 1974). Measurement of enzyme activity of citrate lyase, the gastrocnemius muscle tissues were described in elsewhere (Stitt 19xx, Murakami et al. 1994). Protein concentrations are measured using a commercial kit (Protein Assay, Bio-Rad, CA).

Experiment 2: The effect of iron deficiency on maximum swimming time until fatigue. Forty male Std ddY mice (3 wk old Japan SLC, Hamamatsu, Japan) were used in the present study. They were divided into four groups with equal body weights. Three groups were given an iron deficient diet containing 5 mg of iron per kg diet and the other group were given a normal diet containing 40 mg of iron per kg diet. All the mice were given free access to water and diets for 3 wk. Three wk later, all the mice swam until exhaustion and the maximum swimming time until fatigue were measured. Three groups which had fed 5 mg/kg diet were successively given 5, 25, or 40 mg/kg diet, respectively, and the diet of only group fed 40 mg of iron diet per kg were not changed. Then they were made to swim until exhaustion every 5 days and maximum swimming time was measured. The concentration of hemoglobin were measured 10 times during the experimental period. Body weights and food intakes were measured everyday during experimental period. On the last wk of experiment, their maximum oxygen consumption during running were measured. Mice were killed by decapitation.

Statistical analysis. Data are presented as means \pm SEM; however, in some figures SEMs are not plotted. All the statistical analyze were performed by StatView version 4.5. Body weight, organ weights, serum parameters, and glycogen concentrations were analyzed by ANOVA, and post-hoc comparisons were made using Fisher's test. Maximum swimming time until fatigue, carbohydrate and lipid oxidation, and RER are analyzed by two-way repeated measures of ANOVA, and post-hoc comparisons were made using Fisher's test.

RESULTS

Experiment 1: the effect of a chronic training on maximum swimming time until fatigue. The chronic endurance training significantly reduced increasing of body weight. There are no difference among groups before starting the training; however, after 6 wk, the sedentary mice weighed 4.8 g more than the trained mice

($P < 0.05$). The weight of perirenal and epididymal adipose tissues tended to be larger in the sedentary mice, but the differences were not significant. The gastrocnemius muscle weight were significantly larger in the trained mice (**Table 1**).

The chronic endurance training significantly increased maximum swimming time until fatigue. There are no difference among groups before starting the training; however, the maximum swimming time of trained mice was significantly longer in trained mice after 3 wk ($P < 0.05$) and was 105 ± 16.5 in trained mice and 49.8 ± 17.3 in sedentary mice after 6 wk. Maximum oxygen consumption were significantly increased after 3 wk of endurance training ($P < 0.05$) and the citrate synthase activity was significantly higher in the trained mice ($P < 0.05$, Table 1).

Experiment 2: the effect of iron deficiency on maximum swimming time until fatigue. The concentration of hemoglobin were gradually decreased during 5 mg of iron per kg diet feeding during the experimental period. Mice fed 5 mg/kg diet showed lower hemoglobin concentration on -12 d of the experiment because the mice were fed the diet from -21 d. The difference of hemoglobin concentration were significant on d 0 ($P < 0.0001$). After changing the diet, hemoglobin concentrations showed rapid increase in mice fed 40 mg/kg diet and smaller increase in mice fed 25 mg/kg diet (**Fig. 1**).

TABLE 1

Effect of chronic training on endurance exercise capacity		
	Trained	Untrained
	Swimming time [min]	
Pre-training	70.8 \pm 8.4	69.6 \pm 15.3
3wk-training	126.0 \pm 16.0 *	41.4 \pm 10.3
	VO ₂ max [ml/kg/min]	
Pre-training	129 \pm 2.2	131 \pm 3.2
3wk-training	135 \pm 3.3 *	124 \pm 2.4
Gastrocnemius muscle	[% of body weight]	
	1.049 \pm 0.02 *	0.885 \pm 0.07
Citrate synthase	[U/mg protein]	
	0.513 \pm 0.04 *	0.320 \pm 0.02

Values are means \pm SEM, compared with untrained group, * $P < 0.05$

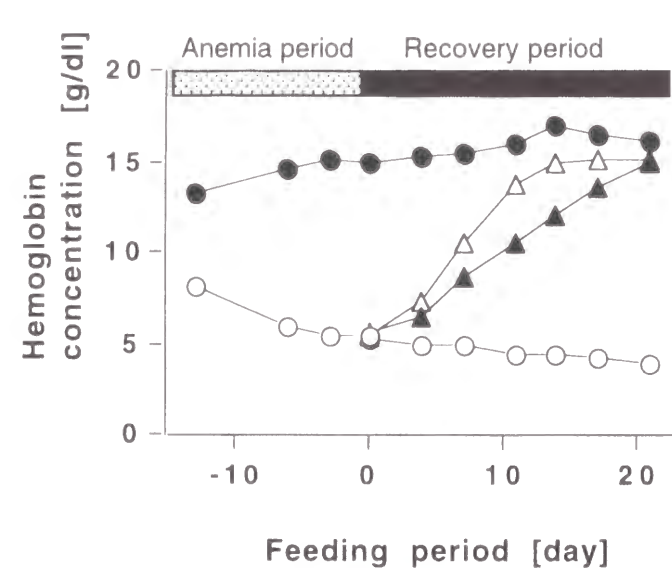


FIGURE 1 Hemoglobin concentration in iron-deficient mice. They were fed diets with iron content of 5 (○) or 40 mg/kg diet (●) from 3 to 6 wk of age. At 42 days of age (day 0), iron-deficient mice were divided into three groups and fed diets with iron content of 5 (○), 25 (▲), or 40 mg/kg diet (△). Values are means ± SEM for 10 mice. Values are significantly different each other among the same experimental day except the first and last day.

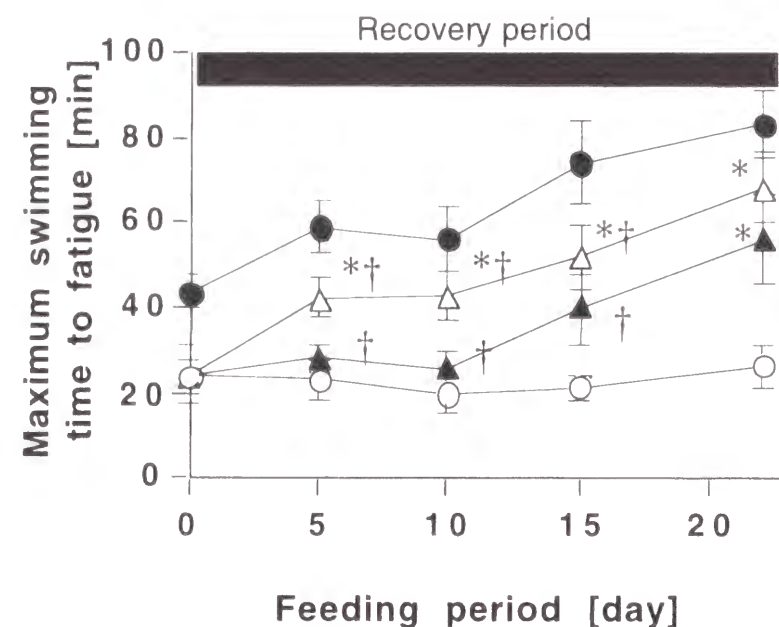


FIGURE 2 Swimming time in iron-deficient mice. Diets and symbols are described in legend of Fig.1. They were made to swim until fatigue during recovery from anemia, from 42 to 63 days of age. Values are means ± SEM for 10 mice. Anemia group were significantly different from nonanemia group at each day, and recovery groups were follows: *P < 0.05 from anemia group and †P < 0.05 from nonanemia group.

The iron deficiency significantly decreased maximum swimming time until fatigue. The swimming time on d 0 were significantly higher in mice fed 40 mg/kg diet than in mice fed 5 mg/kg diet ($P < 0.0001$). After changing the diet, the swimming time showed rapid increase in mice fed 40 mg/kg diet and smaller increase in mice fed 25 mg/kg diet (Fig. 2). The maximum oxygen consumption, the index of oxygen career capacity, measured on the last wk were significantly

lower in anemia group than the other three groups (Fig. 3).

The iron deficiency significantly reduced the growth weight in all the groups fed iron deficient diets. There are no difference among groups for the first 2 wk of feeding of iron deficient diet, however, after 3 wk, the 40mg/kg fed mice showed greater growth rate compared with the other groups. Similarly, after changing the diets, the growth rate depended on the content of iron in the diets, that is, body weights on the last day of experimental period was higher in the rank (data not shown).

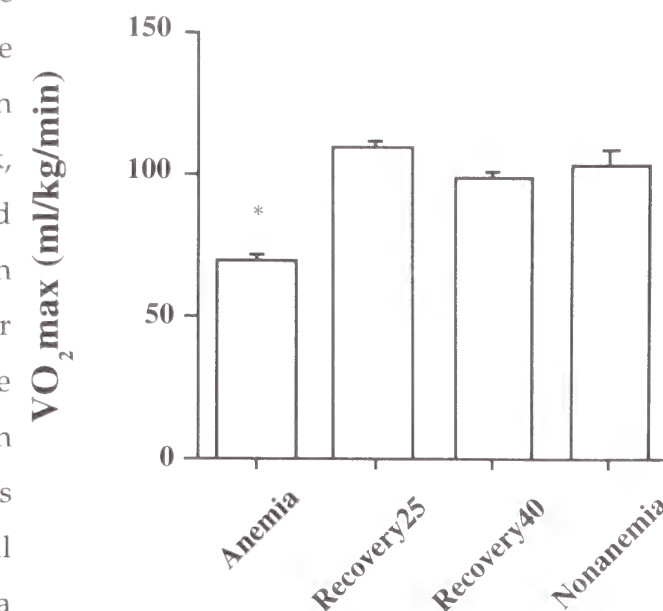


FIGURE 3 Maximum oxygen consumption on last day of experiment. Recovery 25 and 40 means groups whose diet were changed to diet with iron content of 25 and 40 mg iron contained diet. Values are means ± SEM, *P < 0.05.

DISCUSSIONS

The present study was designed to investigate the effects of chronic training and the iron deficiency on the swimming time in mice. It was revealed that the maximum swimming time were increased in chronically trained mice and decreased in iron deficient mice.

Chronic endurance training is one of the most effective way to increase endurance exercise performance. Hollozy et al. (1976) established the training protocol that increase the endurance exercise performance of rats. Briefly, young rats are trained to run for progressively longer periods on a motor-driven treadmill up an 8° incline until at the end of 12 week they are running at 31 meters per minute for two hours per day, five days per week. This program produces a high level of training, with a large increase in endurance but no muscle hypertrophy (Gollnick 1972, Holloszy 1967, Pattengale 1967). The earlier study of swimming training in which rats were trained in static water concluded that the swimming training was less effective than running for inducing adaptive changes in the skeletal muscles, as these animals do

only the minimum work necessary to keep afloat. Six hours of daily swimming for 14 wk produced only 35 % as great an increase in the respiratory capacity of leg muscles in rats as does the running program just described (Baldwin et al. 1975). As in the previous study, swimming in the current system gave mice higher work intensity than that in the static water.

Endurance exercise performance is dependent on the ability of the gas transport system to deliver oxygen to exercising muscles and the oxidative capacity of the active muscles. A primary index of an individual's ability to sustain the exercise is maximal oxygen uptake (VO_2max) which depends on both central (O_2 delivery) and peripheral (O_2 extraction) factors. The mechanisms limiting VO_2max are multiplicity and interrelated but the transport of oxygen by blood is regarded as a major factor. Hemoglobin (Hb) concentration is one of the most important determinant of VO_2max . It is well established that reduction in Hb concentration impairs maximal aerobic power. At sea level, anemia causes both VO_2max and physical work capacity to decrease (Celsing 1986, Clement 1984) even when there is only as little as one to two gram percent decrease in Hb concentration (Woodson 1984). The greater severity of anemia, the greater decrease in work capacity. The effect of Hb concentration on VO_2max has been shown to occur not only by convective (oxygen delivery) factor, but also by diffusive (oxygen extraction) factor (Hogan 1990).

In severe iron deficiency, two types of exercise capacity are impaired. In a brief hard exercise, maximal O_2 consumption (VO_2max) is decreased (Dallman 1982). Additionally, the ability to perform prolonged submaximal endurance exercise is also diminished (Dalman 1982, Finch et al. 1976, Koziol et al. 1982). There is little doubt about the physiological and economic handicaps of severe iron deficiency. Some studies indicate that the decrease in work capacity in iron deficiency is roughly proportional to the degree of iron deficiency (Basta 1979, Edgerton 1979, 8).

The lowering of endurance exercise performance with anemia is frequently observed in female athletes. Several etiological factors may explain storage iron depletion in athletes; these include: (Balaban 1992, Weight 1992, Newhouse 1988, Haymes 1989, Smith 1995) gastrointestinal blood loss, increased loss of iron in sweat, increased iron loss in urine, intestinal iron malabsorption, and iron malnutrition. Though there have not been established the animal model for sports anemia, the effect of dietary iron deficiency on endurance exercise performance were investigated

(Perkkiö 1985, Tobin 1989). Rats had received diets with iron contents ranging between 9 and 50 mg/kg diet from 3 to 6 wk of age. Both VO_2max and duration until exhaustion in a treadmill exercise showed significant depression between a Hb of 10 and 8 g/dl. In conclusion, the maximum swimming time until exhaustion measured in the current swimming system showed significant increase or decrease along with physical condition of mice which are chronically trained, given diet containing iron deficient diet, or aged.

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Section 3 Improvement of current swimming system

In the former part of section 3, the author investigated the experimental condition in which the SD of swimming time became smaller. Decreasing in SD of swimming time would contribute to increase the reliability and sensitivity of the current swimming system. The deviation in swimming time could be derived from genetic nonuniformity in Std ddY strain. Thus the author tested another strains of mice and find the standard deviation of the swimming time was quite smaller in BALB/c mice.

In the latter part of section 3, the author investigated the exercise intensity during swimming. O₂ consumption reflects energy expenditure (Perez and Eatwell 1980, Hoover-plow and Nelson 1985), and the exercise intensity can be represented as the percentages of oxygen consumption during submaximal exercise to the VO₂max (%VO₂max) (Ohira 1981). It is important to clarify the exercise intensity as well as frequency and duration in research about exercise. However, there are no examples measuring the oxygen consumption during swimming in mice because of difficulties to collect slight respiratory gas of mice. The author measured oxygen consumption during swimming in various flow rates in the current swimming system using BALB/c mice.

MATERIALS AND METHODS

Animals. All the animals were housed in standard cages (33 x 23 x 12 cm; 6 mice/cage) under controlled conditions of temperature (22 ± 0.5 °C), humidity (50%), and lighting (lights on from 1800 h to 0600 h). They received humane care as outlined in the Guide for the Care and Use of Laboratory Animals (Kyoto University Animal Care Committee according to NIH #86-23; revised 1985).

Experimental design

Experiment 1: The effect of strain of mice on maximum swimming time. Four-week-old male BALB/c and C57BL/6 mice (weighing 15~20g, an inbred

strain) and Std ddY mice (a closed colony) (Japan SLC, Hamamatsu, Japan) were used. They were housed in standard cages (33 x 23 x 12 cm, 6 mice/cage) under controlled conditions of temperature ($22 \pm 0.5^\circ\text{C}$), humidity (50%), and lighting (lights on from 1800 to 0600 h). They were provided a stock diet (type MF; Oriental Yeast Co., Ltd., Tokyo, Japan) and water ad libitum. The care and treatment of the experimental animals conformed to the Kyoto University guidelines for the ethical treatment of laboratory animals.

All the mice were given a swimming training at 7 L/min of flow rate for 20 min on the evening at which they were purchased. Three days later, they swam until fatigue at 7 L/min of flow rate and the maximum swimming time was measured. Four mice swam at the same time in the current swimming system. The swimming test was repeated 5 times for 2 wk.

Experiment 2: The oxygen consumption during swimming in the current swimming system. Nine BALB/c mice (4 wk old male) were used in the present study. They were submerged in the static water for 30 min at 34°C everyday for 7 d. O_2 consumption at rest were measured for 12 hr using acrylic chamber as described in section 2. O_2 consumption during swimming in the current swimming system was measured for 10 minutes at the flow rate of 0, 5, and 7 L/min on another day. Mice were swum in the metabolic chamber specially designed for the present purpose. Each measurement at the different flow rate were performed on the different day. After measurement of O_2 consumption in the current swimming system, $\text{VO}_{2\text{max}}$ of all the mice were measured with treadmill as described in section 2.

RESULTS

Experiment 1: the effect of strain of mice on maximum swimming time. The representative swimming time of BALB/c, C57BL/6, and Std ddY mice were shown in **Table 1**. It is apparent from the percentage of standard deviation for average swimming time that the personal errors of swimming time of mice were quite smaller in BALB/c mice than Std ddY mice. Sample sizes necessary for statistical significance were calculated using measured standard deviations of each strains and smallest differences that the author wish to detect as statistically significant. The author set 15 min, which is the difference of swimming time between control

and Nanpao treated group, as the smallest differences (Table 1). The measurements of swimming time were repeated for 4-5 times and the reproducibility of the tendency were confirmed. The sample sizes necessary for statistical significance are 37 in BALB/c strain, 71 in C57BL/6 strain, 131 in Std ddY strain at 7L/min, and 99 in Std ddY strain at 8L/min. Using BALB/c strain, the effect of food components on swimming times could be detected with smaller number of mice.

Experiment 2: The oxygen consumption during swimming in the current swimming system. The oxygen consumption at rest varied from 40 to 80 ml/kg body wt/min. The oxygen consumption during swimming are 52, 96, and 124 ml/kg body wt/min at the flow rate of 0, 5, 7 L/min, respectively (**Fig. 1**). Percentage of oxygen consumption for maximum oxygen consumption were 36.2, 66.7, and 85.8 at the flow rate of 0, 5, 7 L/min, respectively.

TABLE 1

Errors of swimming time depends on strain

Strain	Flow rate [L/min]	Swimming time [min]	[%] ¹	n ²
BALB/c	7L/min	36.35±11.39	31.3	37
C57BL/6	7L/min	29.19±12.32	42.2	71
Std ddY	7L/min	46.47±26.86	57.8	131
	8L/min	26.79±13.39	50.0	99

Values are means \pm SD (n=21-50)

1 percentage of SD to means

2 sample sizes necessary for statistical significance

DISCUSSIONS

In this section, the author improved the swimming system in following two points; 1) BALB/c strain promised the smaller standard deviation of maximum swimming time compared to Std ddY strain, 2) the oxygen consumption during swimming correlated to the flow rate of the pump in mice accustomed to swimming for 1 wk.

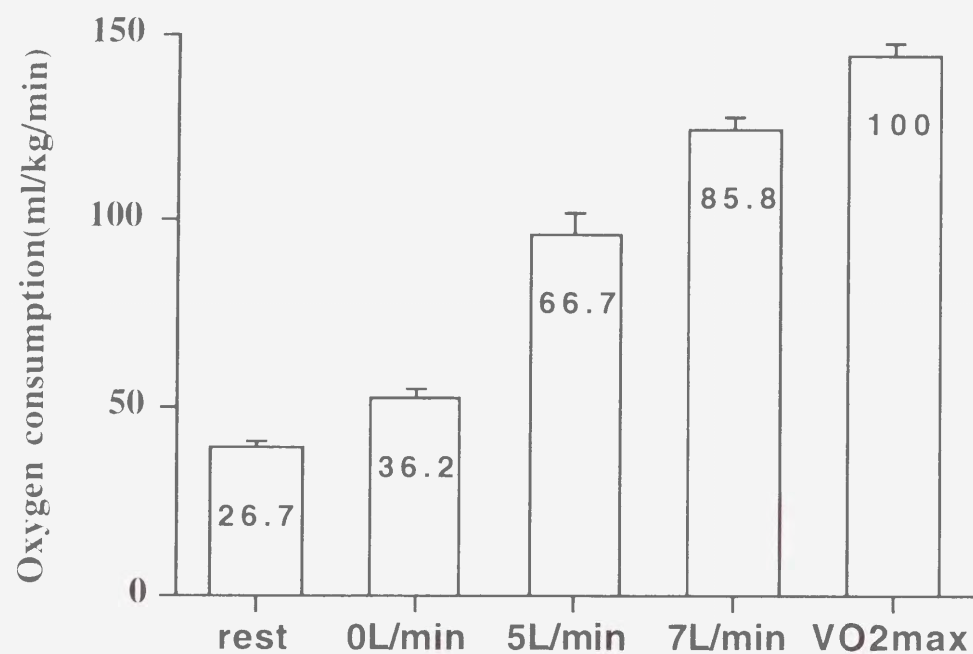


FIGURE 1 Oxygen consumption and exercise intensity in various flow rates on an adjustable-current swimming pool. Exercise intensity was expressed in each column as %VO₂max, percentages of VO₂ to VO₂max.

In the previous sections, the author have elucidated that the current swimming system is useful for the evaluation of the effect of food components on endurance exercise performance. However, the standard deviation of swimming time sometimes reached to 50% of the average swimming time. The large SD could be persuaded by three reasons; 1) some mice could swim at the posture of upright at the terminal point of the pool, 2) those mice not only kicked each other but also hindered a mice that were not fatigued yet, 3) because of above reasons, it was quite difficult to identify the exhaustion of mice, thus the identification of exhaustion was a little different in observers (between the author and colleagues). These problems were manifest when mice grew up and their body sizes are larger than that they used to be.

To improve above problems, a slower growth rate of mice were desired. That is, smaller mice easily sinked and did not swim at the posture of upright at the terminal point, thus the author investigated the BALB/c and C57BL/6, whose body weight gains are smaller and they are generally used in many researches. Both strains of mice did not swim at the posture of upright at the terminal point of the pool and their swimming time were shorter than Std ddY. In this section, four mice

swam at the same time in the pool compared with the previous sections in which six mice swam at the same time. These changes decreased the frequency that mice kick each other, thus the identification of fatigue became easier, which reduction resulted in the smaller standard deviation in BALB/c and C57BL/6 strain compared with Std ddY strain. The SDs of body weights were also clearly smaller in BALB/c and C57BL/6 strain than Std ddY (data not shown), which might indicate the genetic uniformity of the former two strains. The author confirmed that there were no correlation or relation between their body weights and maximum swimming time in BALB/c, C57BL/6, and Std ddY (data not shown).

The maximum swimming time clearly correlated to the frequency of fights (or battle) between mice. Mice who bullied by an another mouse or mice who bullied another mouse always swam for a extremely short time compared to the other mice who were independent of fights. In mice who were involved in fights, the weak, sometimes injured, swam for a slight shorter time compared to the strong. The wasting of energy accompanied with fight might shorten maximum swimming time. BALB/c strain is the most quiet strain of the three strains.

The oxygen consumption is the most frequently used index in the study of sports exercise. In many studies, work intensities during endurance exercise are expressed as percentages of maximum oxygen consumption or heart rate. However, it has been difficult to collect the respiratory gas of mice during swimming exercise. In this section, the airtight chamber which does not disturbs the surface current is designed and thereby the respiratory gas during swimming could be collected. When mice were put into the pool, their oxygen consumption was about 130 ml/kg body wt/min even in the static water, whose value were quite large compared to their previously reported maximum oxygen consumption of about 130 ml/kg body wt/min (Fernand et al. 1993, Schefer and Talan 1996). After accustomed to swimming for 1 wk, their oxygen consumption decreased to 100 ml/kg body wt/min in the static water. In conclusion, BALB/c strain improved the evaluation in the pool and the exercise intensities at various flow rates were clarified.

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CHAPTER 2 Application to the evaluation of food components acting on endurance exercise capacity

Section 1 Chronic (-)-hydroxycitrate administration spared carbohydrate utilization and promoted lipid oxidation during exercise in mice

Recently the effect of (-)-hydroxycitrate (HCA) on lipid metabolism (Kriketos et al. 1999, McCarty 1994, McCarty 1995) has been reported. HCA is an active ingredient that is extracted from the rind of the Indian fruit *Garcinia cambogia* (Lewis and Neelakantan 1965), which is available as a herbal supplement, and decrease adipose tissue weight after a few wk's feeding period (Chee et al. 1977, Greenwood et al. 1981, Rao and Sakariah 1988). HCA is a competitive inhibitor of ATP: citrate lyase (EC4.5.3.8), inhibits fatty acid synthesis, and reduces appetite in rodents (Watson et al. 1969). Whether HCA administration effects on lipid oxidation and endurance exercise performance is unobvious. We expect that HCA may aid aerobic performance, but the research from such viewpoint is not carried out. The purpose of the present study was to investigate the possibilities of HCA as dietary supplement to increase endurance exercise performance. We reported here that lipid oxidation were increased during slight intensities of exercise in male Std ddY mice chronically administered HCA.

MATERIALS AND METHODS

Animals and diets Seven wk old male Std ddY mice (Japan SLC, Hamamatsu, Japan) were used in the present study. They were housed in standard cages (33 x 23 x 12 cm; 6 mice/cage) under controlled conditions of temperature (22 ± 0.5 °C), humidity (50%), and lighting (lights on from 1800 h to 0600 h). They were given free access to water and a commercial diet (type MF; Oriental Yeast, Tokyo, Japan) containing the following (g/kg diet): water, 80; protein, 246; fat, 56; fiber, 31; carbohydrates, 523. All animals received humane care as outlined in the Guide for the Care and Use of Laboratory Animals (Kyoto University Animal Care Committee

according to NIH #86-23; revised 1985). HCA used in the present study was a free acid and provided from Nihon Shinyaku Co. (Kyoto, Japan). In all the experiment, HCA was soluted in distilled water and orally administered as 0.48 mol/L solution.

Apparatuses The gas analyzer used for the assessment of metabolic rate in the mice consists of six acrylic metabolic chambers, CO₂ and O₂ analyzers (model RL-600, AlcoSystem), and a switching system (model ANI6-A-S, AlcoSystem) to sample gas from each metabolic chamber. Each metabolic chamber has a 125.4 cm² floor and is 6.5 cm in height. Room air was pumped through the chambers at a rate of 1.0 L./min. Expired air was dried in a cotton thin column and then directed to an O₂/CO₂ analyzer. Air from each chamber was sampled for 60 s. During the last 1 s the O₂ and CO₂ concentrations were measured 100 times and the average was used for the calculation of oxygen consumption (VO₂) and respiratory exchange ratio (RER). Therefore, the data of each chamber could be obtained every 7 min and the data were stored in a spreadsheet.

The swimming system used in the experiment for the measurement of the swimming time of mice to exhaustion was an adjustable-current swimming apparatus for mice. The details were as described previously (Ishihara et al. 1997, Matsumoto, et al. 1996). Briefly, an acrylic plastic pool (90 x 45 x 45 cm) was filled with water to a depth of 38 cm; and the current was generated with a pump (type C-P60H; Hitachi, Tokyo, Japan). This swimming apparatus has previously been used to evaluate the effect of food and nutrients such as capsaicin (Kim, et al. 1997, Kim, et al. 1998a, Kim, et al. 1998b), overdose caffeine (Ishihara, et al. 1997, Matsumoto, et al. 1996), Nanpao (Saito, et al. 1998) and medium-chain triglycerides (Fushiki et al. 1995).

The running system used in the experiment was the treadmill (model Simplex II, Columbus) for running of mice. Three mice could run at the same time in the running lane (28.8 x 4.7 x 4 cm; LWH) and the system was placed in the acrylic metabolic chamber with an inside volume of about 1000 cm³ and an electric air fan in it for mixing the air.

Experimental design

Experiment 1: Effect of a single oral HCA administration on respiratory gas, serum parameters, and glycogen accumulation. After 1 wk's preliminary period, 60, 54, and 12 mice were used to measure single oral HCA administration on respiratory gas, serum parameters, and glycogen accumulation, respectively. Sixty

mice were separated into three groups with equal body weights and were placed into a metabolic chamber designed to measure respiratory gas and prohibited to access to a diet and water from 0930 h. At 1000 h they were orally administered 100 or 300 µl of 0.48 mol/L HCA solution or water and the respiratory gas was analyzed for 2 h after the administration. To measure serum parameters, Another 54 mice were separated into three groups and prohibited a diet and orally administered as described in detail in above paragraph. They were killed by decapitation 30 min and 100 min after the administration, respectively. Blood was rapidly corrected immediately from the neck and the concentration of serum glucose, FFA, and triglycerides are measured. To investigate the effect of HCA on glycogen accumulation, another 12 mice were separated into three groups as described in detail in above paragraph and given free access to a commercial diet after administration and killed by decapitation 16 h after administration. The gastrocnemius muscle and liver were rapidly removed, frozen in liquid N₂, weighed and kept at -80 °C to analyze the concentration of tissue glycogen.

Experiment 2: Effect of HCA administration on the maximum swimming time until fatigue. Eighteen mice were forced to swim to exhaustion at a flow rate of 8 L/min twice at 3 d intervals during the preliminary period of 1 wk and were divided into three groups. They were orally administered 100, or 300 µl of 10 % HCA solution or 100 µl of water at about 1700 h and given free access to a commercial diet and water. From the next day, mice swam everyday until fatigue at the flow rate of 8 L/min and the maximum swimming time was measured. They were orally administered HCA solutions everyday after swimming (about 1700 h).

Experimental design 3: Effect of chronic HCA administration on lipid oxidation in mice. Eighteen mice were divided into two groups for that the mean body weights were equal in both groups and they were orally administered 100 µl of 0.48 mol/L HCA or water twice a day (first administration; from 1000 h to 1100 h, second administration; from 1700 h to 1800 h) for 25 d. They were given free access to diet and water and body weight and food intake was measured everyday. After first administration of 26 d, they were placed into a treadmill chambers, and allowed to rest for 1 h, followed by 1 h run at the speed of 15 m/min and the respiratory gas was monitored. The respiratory gas was obtained through the experiment. On the 27 d, they were killed by decapitation and the gastrocnemius and quaqdriceps muscles, epididymal and perirenal adipose tissue, liver, spleen, heart, and kidney

were removed and tissue weights measured.

Muscle glycogen, serum glucose, free fatty acid, triglyceride analysis

The glycogen content was measured spectrophotometrically by a method using enzymatic techniques as described elsewhere (Passonneau and Lauderdale 1974). Briefly, after hydrolysis of the muscle sample in 0.6 mol/L HCl at 100 °C for 2 h, the glucose residues were determined with a commercial kit (glucose CII test Wako, Wako Pure Chemical Industries, Osaka, Japan).

Blood was collected from the severed neck veins, and serum was obtained by centrifugation and stored at -80 °C until analysis. Serum glucose, FFA, triglycerides are measured with a commercial kit (glucose CII, NEFA C, triglyceride G test Wako, Wako Pure Chemical Industries, Osaka, Japan).

Statistical analysis Data are presented as means \pm SEM; however, in some figures SEMs are not plotted. All the statistical analyze were performed by StatView version 4.5. Body weight, organ weights, serum parameters, and glycogen concentrations were analyzed by ANOVA, and post-hoc comparisons were made using Fisher's test. Maximum swimming time until fatigue, carbohydrate and lipid oxidation, and RER are analyzed by two-way repeated measures of ANOVA, and post-hoc comparisons were made using Fisher's test.

RESULTS

Effect of a single HCA administration on respiratory gas, serum parameters, and glycogen accumulation (Experiment 1). The HCA administration significantly increased RER in 300 μ l administered group, but not in 100 μ l administered group (Fig. 1). Oxygen consumption was not different among all the experimental groups (data not shown). Carbohydrate oxidation increased and lipid oxidation decreased in 300 μ l administered group for 3 h after administration, but not in 100 μ l administered group (data not shown).

Serum FFA concentration in 100 μ l administered group was significantly higher 100 min after administration but not 30 min after the administration. However serum FFA significantly did not change in 300 μ l administered group in 30 and 100 min after administration. Serum glucose and triglycerides are not changed in all the

experimental groups (Table 1).

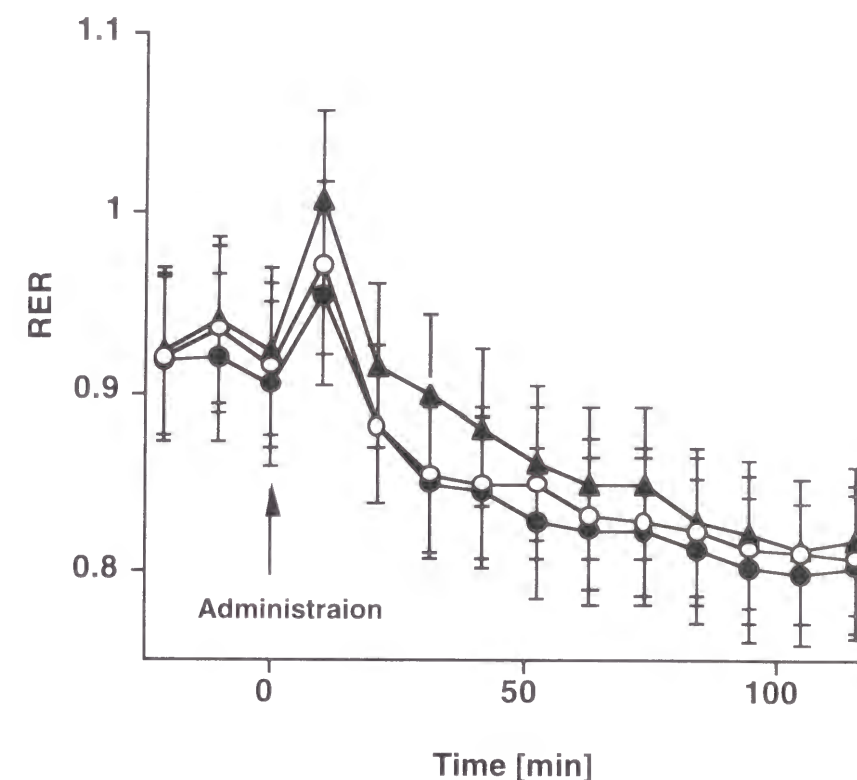


FIGURE 1 Effect of a single HCA administration on respiratory exchange ratio in mice (Experiment 1). Each mouse was housed in a metabolic chamber and orally administered 100 (●, n=20), 300 (▲, n=20) μ l of 10% HCA solution or water (O, n=20) at time 0 in a resting condition and respiratory gas was collected. Values are means \pm SEM. Administration of 300 μ l of HCA significantly increased respiratory exchange ratio (RER) compared with the control group ($P = 0.014$, two-way repeated measures of ANOVA), but that of 100 μ l did not effect on respiratory exchange ratio.

TABLE 1

Serum parameters after a single administration of HCA (Experiment 1)			
	Control	HCA	
		(mg administration/mouse)	
		10mg	30mg
FFA, mEq/l			
30min	0.393 \pm 0.039	0.391 \pm 0.060	0.376 \pm 0.039
100min	0.473 \pm 0.026	0.560 \pm 0.022*	0.483 \pm 0.025
Glucose, mg/dl			
30min	260.4 \pm 11.8	242.9 \pm 12.3	210.2 \pm 11.3
100min	228.9 \pm 7.1	239.3 \pm 6.7	232.0 \pm 5.5
Triglycerides, mg/dl			
30min	178.6 \pm 33.3	114.0 \pm 41.7	182.4 \pm 23.8
100min	166.1 \pm 10.3	185.9 \pm 6.5	196.4 \pm 14.2

1 Values are means \pm SEM, n=4 for 30min; n=18 for 100min.

2 *Significantly different from control (ANOVA, $P < 0.05$).

The concentration of glycogen in the gastrocnemius muscle and liver (per muscle

weight) in mice were measured. The glycogen concentration in mice administered 10 mg of HCA was significantly higher in gastrocnemius muscle, and slightly (but not significantly) higher in liver. The administration of HCA at a higher dose (30 mg) tended to decrease liver glycogen concentration (Table 2).

Effect of HCA administration on the maximum swimming time (Experiment 2). The effect of HCA administration on the swimming time to fatigue (maximum swimming time) was shown (Fig. 2). In the mice administered 10 mg of HCA, the maximum swimming time on the day after the first administration was slightly longer than that in the control mice, and the time on the 3 d of the administration was significantly longer compared with control group ($P < 0.05$). No significant increase was observed in mice administered 30 mg of HCA.

TABLE 2

Glycogen concentration 16 h after HCA administration (Experiment 1)			
	Control	HCA (mg administration/mouse)	
		10mg	30mg
Gastrocnemius muscle	4.31 ± 0.22	5.65 ± 0.45*	4.36 ± 0.30
Liver	34.13 ± 4.50	38.26 ± 3.67	29.46 ± 3.28

1 Values are means ± SEM, n=4.

2 *Significantly different from control (ANOVA, $P < 0.05$).

FIGURE 2 Effect of HCA on swimming time to fatigue in mice (Experiment 2). Mice were orally administered 100 (●, n=6), 300 (▲, n=6) μl of 0.48 mol/L HCA solution or water (○, n=6) at 1700 h and swimming time to fatigue were measured from 1300 h on d 0-3. Datas are expressed as increased time in swimming time from that on d 0. Values are means ± SEM. Administration of 100 μl of HCA significantly increased swimming time until fatigue compared with the other two groups on d 3 of experiment ($P < 0.05$, two-way repeated measures of ANOVA).

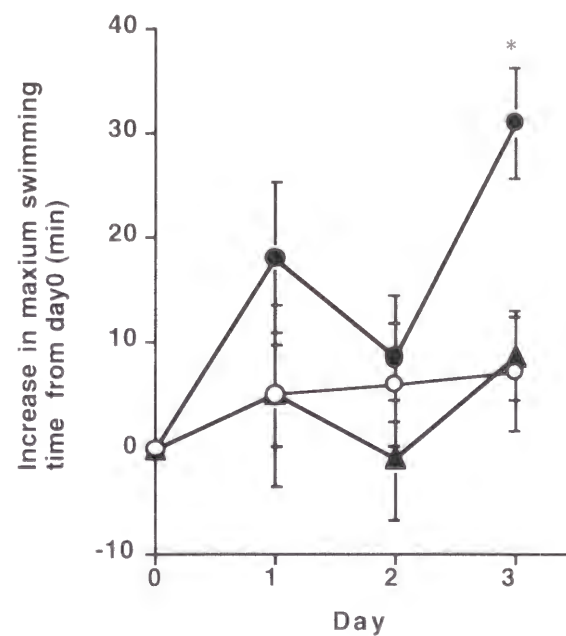


Table 3

Organ weight in mice administered 10mg HCA for 3 weeks (% of body weight)

	HCA	Control
Gastrocnemius muscle	0.508 ± 0.040	0.541 ± 0.056
Quadriceps muscle	0.892 ± 0.360	0.917 ± 0.350
Liver	4.252 ± 0.181*	3.837 ± 0.471
Heart	0.404 ± 0.034	0.398 ± 0.025
Spleen	0.309 ± 0.032*	0.235 ± 0.057
Kidney	1.605 ± 0.115	1.579 ± 0.168
Perirenal fat	0.520 ± 0.295	0.740 ± 0.219
Epididymal fat	1.526 ± 0.499	2.142 ± 0.759

1 Values are means ± SEM, n=10 for control; n=9 for HCA.

2 *Significantly different from control (t-test, $P < 0.05$).

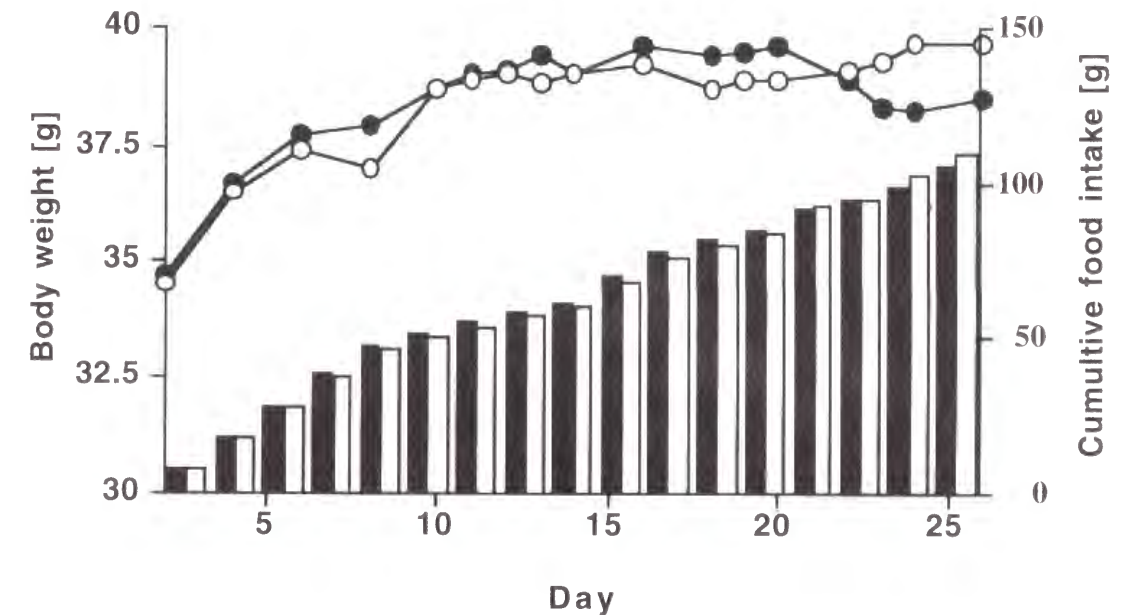


FIGURE 3 Effect of chronic administration of HCA on body weight and food intake in mice (Experiment 3). Mice were orally administered 100 μl of 0.48 mol/L HCA (n=9) solution or water (n=9). Body weight changes are expressed as line for HCA (closed circle) and control (open circle) and food intake are expressed as closed bars (HCA) and open bars (control). Values are means of the group. HCA significantly reduced body weight on d 26 of experiment ($P < 0.05$, Student t-test).

Effect of chronically administered HCA (Experiment 3).

HCA administration for 25 d did not decrease the food intake (Fig. 3). There were no differences in body weight among the groups before starting the diets; however, after 3 wk, the HCA administered group significantly reduced body weight 3.5 g

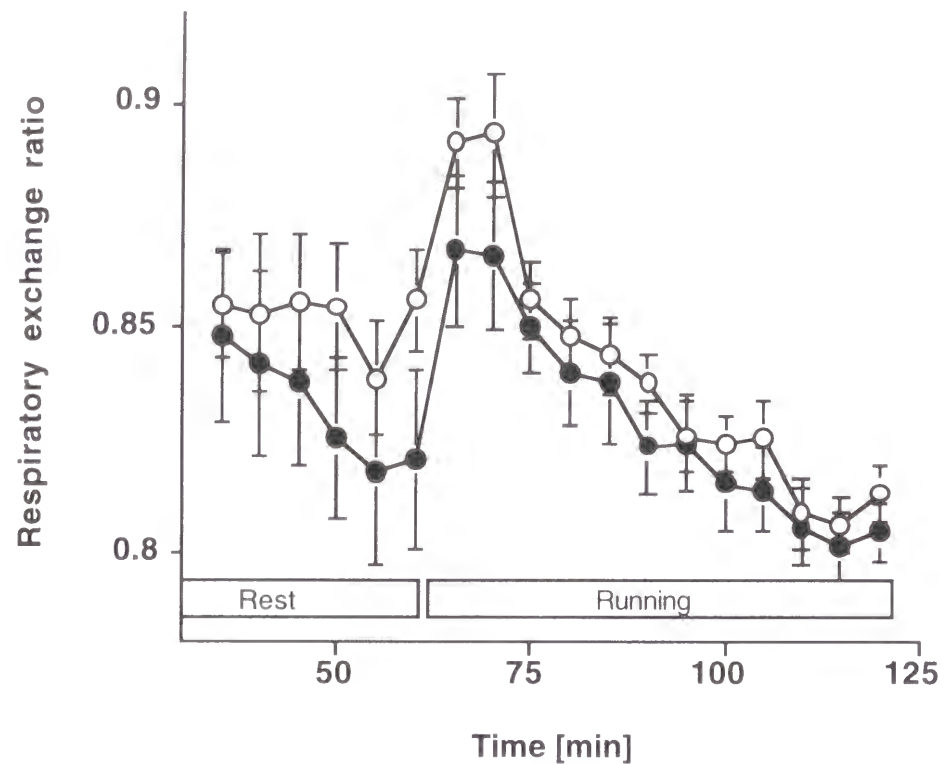


FIGURE 4 Effect of chronic HCA administration on respiratory exchange ratio (RER) (Experiment 3). Mice were orally administered 100 μ l of 0.48 mol/L HCA solution (closed circle) or water (open circle) twice a day for 25 d. After administration on 26 d, they were kept in a metabolic chamber in a resting condition for 1 h, then they were forced to run on the treadmill at the speed indicated as a dotted line. Statistical analyses were separately performed about rest (0-60 min) and exercise (65-120 min) by using two-way repeated ANOVA. RER was significantly smaller in HCA administered mice during running ($P < 0.01$).

lighter than the control group ($P < 0.05$). Chronic HCA administration had significant effect on organ weights (**Table 3**). HCA administration tended to reduce adipose tissue weight by about 33 % compared with the control group ($P = 0.24$ and 0.18 for epididymal and perirenal fat tissue, respectively). HCA administration was associated with significantly larger livers and spleens ($P < 0.05$).

On 26 d administration, metabolic rate was monitored after the administration. HCA solution was given 60 min before the start of the running exercise (0 min in **Fig. 4**). Thirty min's after the administration, RER began to decrease in mice administered HCA. RER in control group and HCA group was 0.86 ± 0.01 and 0.82 ± 0.02 at 1 h after administration, respectively (60 min). RER abruptly increased by 0.05 in both groups after the start of the running exercise (65 min), but gradually decreased after the belt speed reached the set value of 15 m/min

(75 min). RER reached about 0.8 at the end of the running exercise in both groups and was significantly smaller in the HCA-administered group throughout the running ($P < 0.01$). Oxygen consumption of both group mice gradually decreased to 80 ml/kg body wt during resting in the metabolic chamber and increased to 100-120 ml/kg body weight during running (data not shown).

The lipid oxidation, calculated from RER and oxygen consumption, decreased in control group but did not decrease in HCA administered mice before the start of exercise ($P = 0.07$). Lipid oxidation at the first 20 min's of running was significantly higher in HCA group than the control group ($P < 0.0001$, **Fig. 5**). Though there was no difference before the start of running on the carbohydrate oxidation, carbohydrate oxidation quantity of the HCA group was always higher during 60 min's running than the control group ($P < 0.0001$, **Fig. 5**).

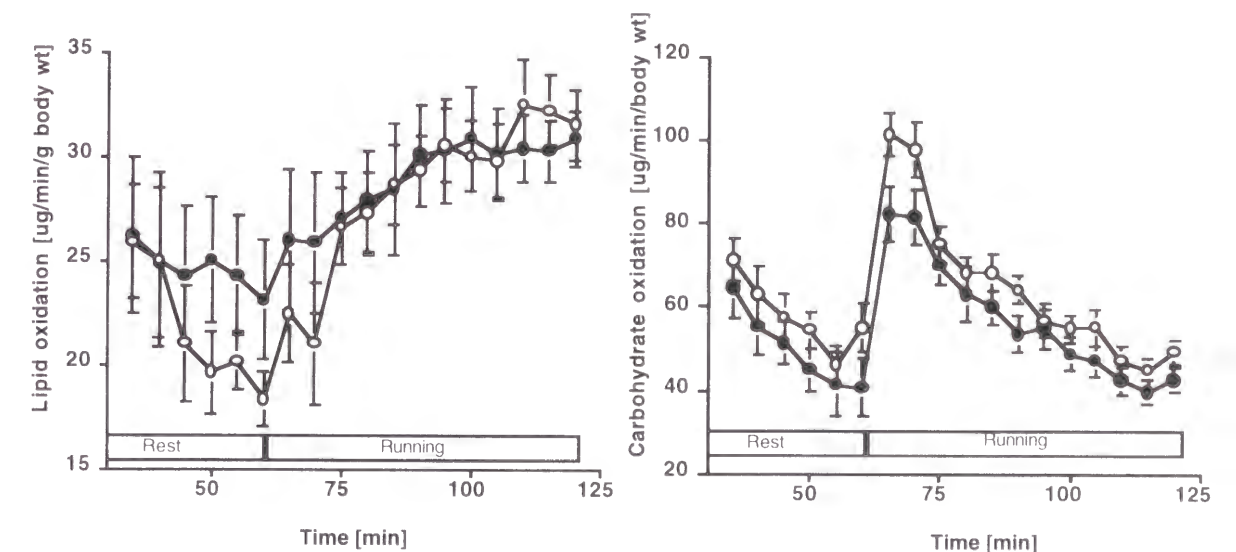


FIGURE 5 Effect of chronic HCA administration on lipid and carbohydrate oxidation (Experiment 3). Mice were treated as detailedly described in the legend of Figure 4 and text. Lipid and carbohydrate oxidation was calculated from RER (**Fig. 4**) and oxygen consumption (not shown). The running speed was 15 m/min. Statistical analyses were separately performed about rest (0-60 min) and exercise (65-120 min) by using two-way repeated ANOVA. Lipid oxidation was significantly larger and carbohydrate oxidation was significantly smaller in HCA administered mice during running ($P < 0.01$).

DISCUSSION

The present study was designed to investigate the effects of HCA administration on endurance exercise performance in mice. The major findings are

that, after a single oral administration of 10 mg HCA, 1) serum FFA was increased 100 min after an administration, 2) administration of HCA promoted the accumulation of glycogen in skeletal muscle 16 h after an administration, 3) maximum swimming time until fatigue was significantly increased after 3 d's oral administration, and 4) lipid oxidation was increased and carbohydrate utilization were spared during slight intensities of running.

Mice administered 10 mg of HCA exhibited the increased serum FFA concentration 100 min after administration and the slightly lower RER (not significant) compared to those of control group mice, which might suggest that their lipid metabolisms were promoted and carbohydrate utilization were spared. Therefore, 16 h after administration, the glycogen concentration of gastrocnemius muscle and liver were significantly higher in mice administered 10 mg of HCA. Hellerstein et al. reported that administration of 0.263 mmol/kg/d (1.64 mg/d/mouse) increased liver glycogen accumulation in rats intravenously administered HCA and refed glucose (Hellerstein and Xie 1993).

There were no differences in body weight among the groups before HCA administration. However, HCA administration significantly reduced body weight gain, though energy intake was not different among groups over the course of the study. Chronic HCA might increase energy expenditure during the 3 wk of the experimental period. In studies from the University of South Carolina, Subjects received a slight administration of 750 mg/d/person (0.375 mg/d/mouse) resulted in the weight loss of 4-5 kg over the 8 wk study period (Conte 1993). Another recent study has shown that rats fed a high carbohydrate diet (75% carbohydrate) and given (-)-HCA supplementation (dose was 52 mmol (-)-HCA/kg of 70% dextrose diet, 3.74 kcal/g) over 28 d resulted in a 12.6 % increase in 24 h EE with no change in RER (Vasseli, J.R., Shane, E., Boozer, C. N. and Heymsfield, S. B., *Garcinia cambogia* extracts inhibits body weight gain via increased energy expenditure (EE) in rats. FASEB Experimental Biology, 1998 (San Francisco) Abstract A505).

In mice chronically administered 10 mg of HCA, RER was tended to be decreased in HCA administered mice for 60 min after administration at rest ($P = 0.54$). During 60 min of running, RER was significantly smaller in the HCA-administered group ($P < 0.01$). These data suggested that the chronic HCA administration promoted lipid oxidation and spared carbohydrate utilization during slight intensities of exercise.

Numerous reports have now documented that the enhancement of endurance exercise performance by promoted lipid oxidation. Chronic high fat diet feeding are reported to be one of the dietary manipulation to increase endurance exercise performance (Lambert et al. 1994, Starling et al. 1997). A single or short terms feeding of high fat diet elevated serum FFA concentration but did not increase endurance exercise performance (Okano et al. 1998, Okano et al. 1996, Whitley et al. 1998). It is thought that the continuous higher serum FFA concentration by chronic high fat diet feeding improved lipid oxidation capacity. Correspondingly, in the present study, serum FFA concentration was significantly elevated by the administration of 10 mg of HCA (Table 1) but RER and endurance exercise performance was not effected. However, mice chronically administered 10 mg of HCA enhanced lipid oxidation capacity during exercise (Fig. 4, 5, 6).

Recently Kriketos et al. (1999) did not detect an effect of HCA administration on lipid oxidation either during rest or during moderately intense exercise on a cycle ergometer in male adults. However in their studies subjects received a daily dose of 3.0 g per subject (nearly equal to 1.5 mg/d/mouse) for 3 d seemed to be insufficient to effect on energy metabolism. Chee et al. (1977) observed that the rates of fatty acid synthesis was depressed by an single intraperitoneally injection of 0.4 mmol (83.2 mg/d/mouse) or a single meal feeding containing 52.6 mmol/kg diet (32.8 mg/d/mouse) in rat and chicken livers. Rao and Sakariah (1988) reported that rats fed diet containing 2% HCA for 15 d resulted in significant reduction epididymal fat. The dose of HCA selected for this study was based on above two studies and a previous pilot study conducted by our laboratory.

Acute administration of 30 mg of HCA clearly increased RER and depressed lipid oxidation for the first 3 h after administration. In our pilot study, 30 mg of HCA also reduced food intake thus the dose seemed not to suit the object to investigate the effect of HCA on endurance exercise performance. The mechanism responsible for the observed alteration in RER is not known. An administration of 30 mg of HCA increased carbohydrate oxidation and resulted in decreasing of the glycogen concentration in gastrocnemius muscle and liver at the time 16 h after administration.

In summary, oral administration of 10 mg HCA elevated serum FFA concentration and increased muscle glycogen concentration in mice at rest. Mice administered long term administration (twice daily) of HCA significantly decreased RER after during their running. Thus lipid oxidation increased and carbohydrate

utilization attenuated in the early stages of running. These data indicated that the enhancement of endurance exercise by orally administered HCA in mice was caused by in the attenuation of glycogen consumption caused by the promotion of lipid oxidation during running.

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MATERIALS AND METHODS

Section 2 Effect of Nanpao⁶⁾, a mixture of 31 Chinese crude drugs, on increasing endurance exercise performance of swimming mice.

Nanpao consists of a mixture of 31 Chinese crude drugs (Table 1), each of whose components has been widely used in China as a tonic, especially, for the purpose of enhancing physical fitness of elderly people, such as an improvement of decreasing of physical fitness accompanied with aging. The effects of many tonics used in China on physical fitness have not well been investigated by scientists except herb doctors.

In this section, the author investigate the effect of Nanpao treatment on endurance exercise performance in mice. Mice fed Nanpao showed significant increase in swimming time until fatigue and biological parameters such as the glycogen concentration in gastrocnemius muscle and serum lactic acid showed the enhancement of endurance exercise performance.

TABLE 1

Components and their contents in daily dose on Nanpao (Yamamura et al. 1993)

		(mg)	
Cervi Parvum Cornu	鹿茸 67	Curculiginis Rhizoma	仙茅 33
Canis Penis et Testis	広狗腎 53	Trigonellae Semen	胡蘆巴 33
Donkey Penis et Testis	黑雄腎 235	Ginseng Radix	人參 133
Hippocampus	海馬 67	Astragalli Radix	黃耆 67
Cuscutae Semen	菟絲子 33	Atractylodis Rhizoma	白朮 33
Psoraliae Semen	補骨子 33	Glycyrrhizae Radix	甘草 16.7
Cistanches Herba	肉蓯蓉 33	Cinnamomi Cortex	桂皮 53
Cynomorii Herba	鎮陽 33	Aconiti Tuber	炮附子 33
Eucommiae Cortex	杜仲 67	Corni Fructus	山茱萸 33
Dipsaci Radix	川續斷 16.7	Rubi Fructus	覆盆子 33
Morindae Radix	巴戟天 33		
		Ophiopogonis Tuber	麥門冬 33
		Lycii Fructus	枸杞子 67
		Rhemanniae Radix	熟地黃 67
		Angelicae Radix	當歸 133
		Asini Gelatinum	阿膠 67
		Scrophulariae Radix	玄參 33
		Moutan Cortex	牡丹皮 33
		Achyranthis Radix	牛膝 16.7
		Hoelen	茯苓 67
		Epimedii Herba	淫羊藿 67

Animals. Five-wk old male Std ddY mice, obtained from Japan SLC (Hamamatsu, Japan) were housed in standard cages (33 x 23 x 12 cm; 6 mice/cage) under controlled conditions of temperature (22 ± 0.5°C), humidity (50 ± 5%), and lighting (lights on from 0600 h to 1800 h). They had free access to water and a control diet whose composition was shown in TABLE 2 for 1 wk of preliminary period. All animals received humane care as outlined in the Guide for the Care and Use of Laboratory Animals (Kyoto University Animal Care Committee according to NIH #86-23; revised 1985). HCA used in the present study was a free acid and provided from Nihon Shinyaku Co. (Kyoto, Japan).

Apparatus and procedures

One hundred and forty two mice swum until fatigue for 5 times at the flow rate of 8 L/min and maximum swimming time until fatigue was measured during 2 wk's preliminary period. They were divided into three groups with equal body weights and averaged swimming times. Each group of mice were given free access to water and diets containing

0, 0.1, and 1% of Nanpao powder, respectively. Diet compositions were shown in TABLE 2. They swum 3 times for 1 wk until fatigue and maximum swimming time until fatigue was measured for 3 wk (day 1, 3, 5, 8, 10, 12, 15, 17, 19). Body weights and food intakes are measured everyday. On 21 d, half of each group of mice were killed before swimming and the other mice were killed after 20 min's swimming. They were killed by decapitation and blood were

TABLE 2
Composition of the experimental diet (g/kg diet)

Constituent	0.1% Nanpao	1% Nanpao	Control
Casein ¹	200	200	200
Corn starch ¹	541	541	541
Soybean oil ²	100	100	100
Nanpao powder ³	1	10	0
Mineral mixture ¹	40	40	40
Vitamin mixture ¹	22	22	22
Cellulose powder ¹	94	85	95
L-Methionine ²	2	2	2

¹ Purchased from Oriental Yeast Co. (Tokyo, Japan).

² Purchased from Wako Pure Chemical Industries (Osaka, Japan).

³ Obtained from Tanabe Seiyaku Co. (Osaka, Japan).

collected from the severed neck veins and gastrocnemius muscle, perirenal and epididymal fat tissue, heart, kidney, liver, and spleen are rapidly removed and weighed. Muscle, fat tissues, liver, and serums were immediately frozen in the liquid N₂ and kept at -80 ° until analysis.

Muscle glycogen, serum lactic acid analysis. The glycogen content was measured spectrophotometrically by a method using enzymatic techniques as described elsewhere (Fushiki et al. 1995). Briefly, after hydrolysis of the muscle sample in 0.6 mol/L HCl at 100°C for 2 h, the glucose residues were determined with a commercial kit (glucose CII test Wako, Wako Pure Chemical Industries, Osaka, Japan). Serum lactic acid was measured with a commercial kit (F-kit, Berlinger Mannheim).

Statistical analysis. Data are presented as means ± SEM. All the statistical analyze were performed by Student's t-test between control group and Nanpao treated groups. All analyses were performed using InStat 2.00 (Graphpad Software, CA).

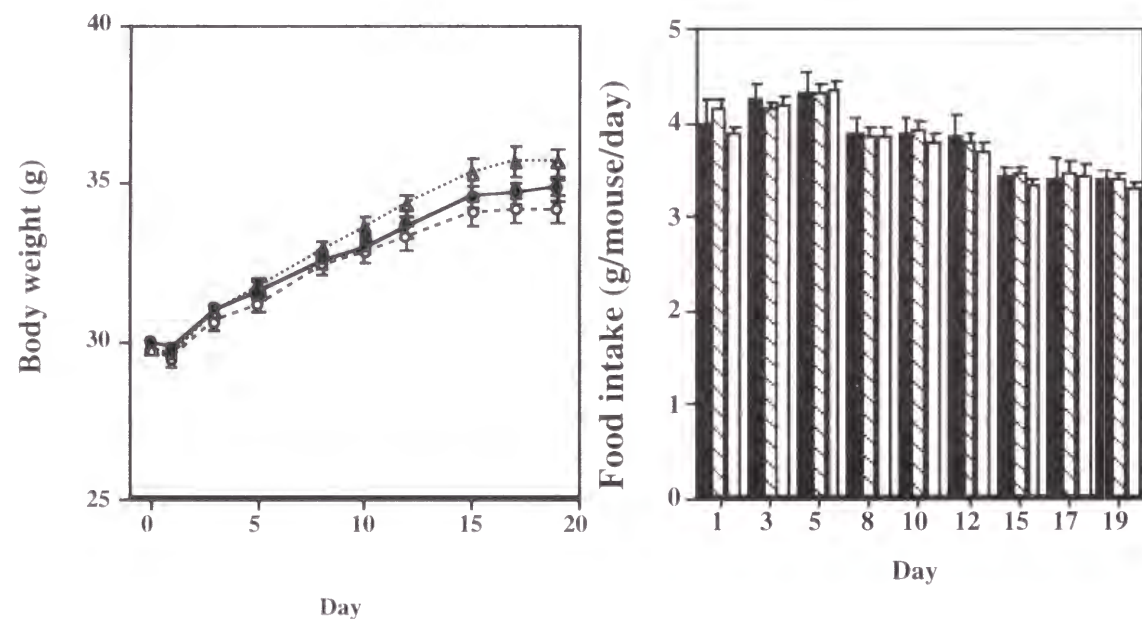


FIGURE 1 Body weight change (left) in mice fed control (open circle) , 0.1% Nanpao (closed circle), and 1% Nanpao (open triangle). Food intake are expressed for control (open bars) , and 0.1% Nanpao (solid bars), and 1% Nanpao (hatched bars). Values are means ± SEM.

RESULTS

The Nanpao treatment did not effect on food intake and body weight. Total food intakes for 3 wk of experimental period were 72.3, 73.0, and 72,3 g/mouse for control, 0.1%, and 1% Nanpao group, respectively. Body weight showed daily increasing and there are no differences among groups during all the experimental period (**Fig. 1**).

The effect of Nanpao administration on the swimming time to fatigue (maximum swimming time) was shown (**Fig. 2**). Maximum swimming times gradually increased in all groups for the first 8 d of the experiment; however, after 10 d, the 0.1% Nanpao administered group showed significantly greater gain in swimming time over the course of study compared with the control group. The average swimming time on d 15, 17, and 19 was 47, 49 and 45 in the 0.1% administered group and 37, 38, and 35 in the control group, respectively. No significant increase was observe in mice administered 1% Nanpao.

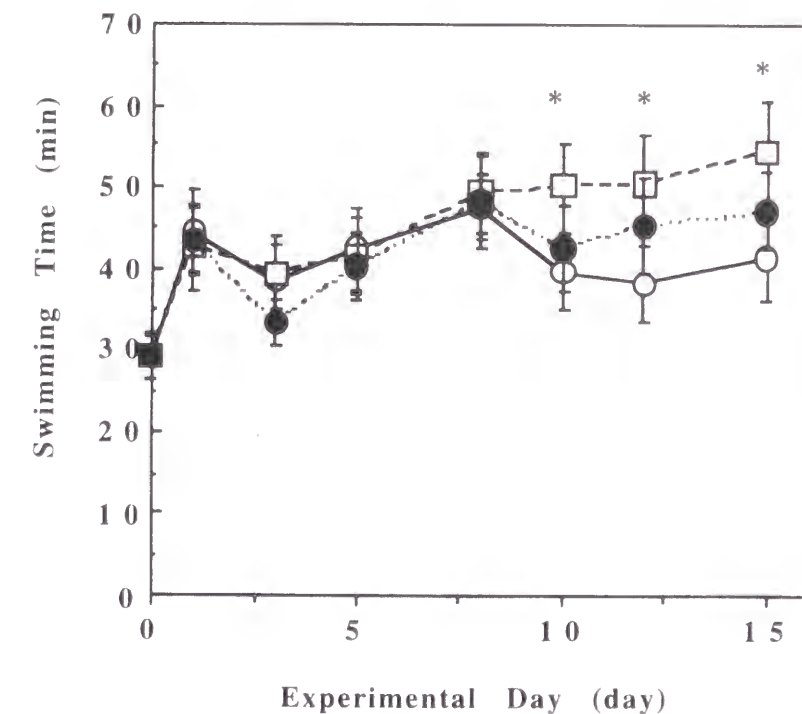


FIGURE 2 Swimming time of mice fed control (open circle), 0.1% Nanpao (open square), or 1% Nanpao (closed circle). Values are means ± SEM, *significantly different from control, (P < 0.05).

Serum lactic acid concentrations after 20 min swimming were 4.25 mM in control group, 4.0 mM in 1% Nanpao group, and 3.8 mM in 0.1% Nanpao group, respectively (Fig. 3). Serum lactic acid tended to be lower in 0.1% Nanpao group compared to control group ($P < 0.10$).

The glycogen concentration in gastrocnemius muscle were significantly affected by Nanpao treatment (Fig. 4). There were no differences in the glycogen concentration among groups before exercise. However, muscle glycogen concentration after 20 min's swimming were significantly higher in 0.1% Nanpao group compared to the other two groups ($P < 0.05$). The glycogen consumption during swimming, which were calculated from the differences of glycogen concentration between before and after, were about twice smaller in 0.1% Nanpao mice compared to the other two groups.

Relative organ weights after 3 wk of Nanpao feeding was shown in TABLE 3. The Nanpao treatment did not affect on organ weights.

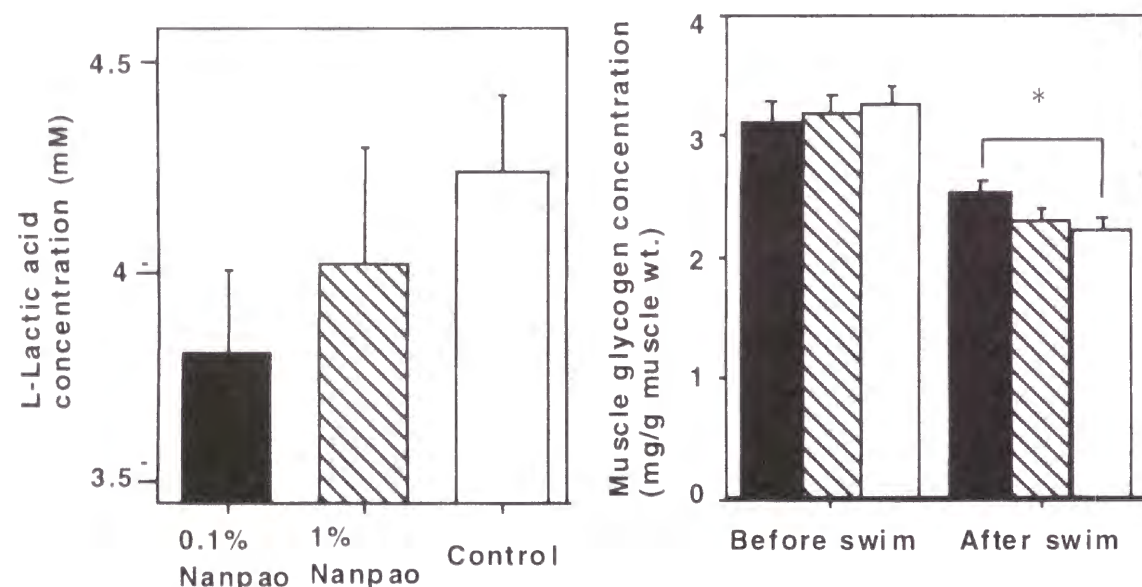


FIGURE 3 Mice were killed by decapitation in 20 min swimming on last day of experiment. Blood was collected. Values are means \pm SEM (n=19-20).

FIGURE 4 Each group of mice were divided into two subgroups on last day of experiment. They were killed by decapitation before and after 20 min swimming, respectively. Values are means \pm SEM, * $P < 0.05$.

TABLE 3
Relative organ weights
(% of body weight)

	0.1% Nanpao	1% Nanpao	Control
M. Gastrocnemius	0.51 \pm 0.01	0.51 \pm 0.01	0.53 \pm 0.01
Liver	4.34 \pm 0.06	4.32 \pm 0.06	4.22 \pm 0.06
Heart	0.38 \pm 0.01	0.38 \pm 0.01	0.38 \pm 0.01
Spleen	0.30 \pm 0.01	0.28 \pm 0.01	0.29 \pm 0.01
Kidney	1.32 \pm 0.03	1.34 \pm 0.03	1.34 \pm 0.02
Epididymal fat	2.58 \pm 0.08	2.64 \pm 0.11	2.42 \pm 0.10
Perirenal fat	0.92 \pm 0.05	0.95 \pm 0.06	0.92 \pm 0.06

DISCUSSION

The present study was designed to investigate the effects of chronic feeding of Nanpao, a Chinese medicine, on endurance exercise performance in mice by using an adjustable current swimming pool. The major findings are that, after a 3 wk feeding of 0.1% Nanpao containing diet, 1) swimming time until fatigue was increased 100 min after an administration, 2) serum lactic acid concentration after 20 min swimming was lower, 3) glycogen utilization during 20 min swimming was smaller.

Panax ginseng is the most famous and mostly investigated Chinese medicine in the Western world (Avakian and Evonuk 1979, Avakian and Sugimoto 1980a, 1980b, Avakian et al. 1984, Benxiang et al. 1983, Forgo and Kirchdorfer 1981, Knapik et al. 1983, Peralisi et al. 1991, Teves et al. 1983, Wang et al. 1983), and the number of study about the other Chinese medicine are has been smaller than that of ginseng. However, some researchers have been interested in Nanpao, which has been widely used as a tonic in China (Kobayashi et al. 1996, Yamamura et al. 1993).

Swimming time in the current pool was an index of endurance exercise capacity and has been used to estimate the effect of food components such as medium chain triglycerides (Fushiki et al. 1995), caffeine (Matsumoto et al. 1996), capsaicin (Kim et al. 1997, 1998a), and its analog (Kim et al. 1998b). There being no

Nanpao were suspended in distilled water and orally administered 30 min before swimming. Swimming time until fatigue was not affected by the single administration of Nanpao (data not shown).

There are cases that chronic administration has no effect but an acute administration effects on endurance exercise capacity in some kind of dietary components. An acute administration of MCT did not effect but a chronic feeding for 2 wk increased maximum swimming time. Chronic MCT administration increased serum ketone body concentration, citrate synthase, malate dehydrogenase, and 3-oxoacid coA transferase activity, which is the key enzyme of ketone body utilization, in the gastrocnemius muscle. Chronic higher concentration of ketone body could induce the key enzyme for a few weeks (Fushiki et al. 1995).

After 3 wk of feeding period, the glycogen concentration was significantly higher in 0.1% Nanpao group compared to the other two groups after 20 min swimming ($P < 0.05$). There are no differences in glycogen concentration among groups before swimming. Thus the glycogen consumption during 20 min of swimming was smaller in 0.1% Nanpao group. The 8 L/min of current speed used in the present study, which corresponds to the intensity of exercise, was almost equivalent to comparably higher intensity. Serum lactic acid concentration was an index of fatigue and was higher in the control group compared to 0.1% Nanpao group after 20 min of swimming. These two biological parameters suggested the higher depending in 0.1% Nanpao group on oxidative energy productive pathway than glycolytic pathway. It is reported that oxidation of muscle triglycerides and serum FFA as energy substrate is important to increase endurance exercise capacity, which results in the lower accumulation of serum lactic acid (Holloszy and Booth 1976, Hurley et al. 1986, Hergreaves et al. 1995, Hickson et al. 1977).

Yamamura et al. (1993) reported that Nanpao administration antagonistically affected on FFA increase 24 hr after swimming. Kobayashi et al. (1996) reported that chronic Nanpao treatment on matured rats suppressed serum FFA increasing accompanied with aging. These two reports suggested that Nanpao had an effect to depress FFA increasing which is brought about by exercise and aging. Enhancement of endurance exercise capacity in the current swimming pool by oral administration of capsaicin was associated with increasing of FFA by way of epinephrine secretion from adrenal gland. In opposition to this, in the present study, Nanpao might enhance the oxidative pathway of FFA, though enzyme activities concerned with

lipid oxidation were not measured.

The dose of Nanpao added to diet was 0.1 and 1% (g/g diet), which corresponds to 100 and 1000 mg/kg body wt/d calculated from the food intakes and body weight. These corresponds 5 and 50 times larger compared with the doses of human daily doses, respectively. In the study by Kobayashi et al. (1996), mice were administered 30, 100, or 300 mg/kg body wt/d, respectively. The dose of 100 mg/kg body wt/d was reported to improve reproduction and mobility, however, that of 300 mg/kg body wt/d aggravated the function of kidney, aging of testis, and the rate of success of conception. The dose of 1% of diet did not effect on body weight, food intake, and organ weight, but the effect on maximum swimming time was lower compared to the dos of 0.1% of diet. We did not investigate an effect by lower dose than 0.1% of diet because small laboratory animals are usually treated higher doses than those treated in human.

In the present section, the effect on Chinese medicine on endurance exercise capacity was investigated using new developed current pool for evaluating endurance capacity of mice. Chronic feeding of Nanpao for 3 wk spared glycogen utilization during swimming and lowered serum lactic acid accumulation, which resulted in the enhancement of endurance exercise performance and maximum swimming of mice are significantly higher than the control group.

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SUMMARY

The author developed a swimming pool with a pump that generates water flow and creates currents for the evaluation of endurance capacity of mice and quantitated the maximum swimming capacity of mice in this apparatus.

The findings in each chapter are summarized as follows:

CHAPTER 1

Section 1 A new forced swimming apparatus for determining maximum swimming time in mice was devised for the evaluation of the endurance capacity following various diet and drug treatments. With the apparatus, a water current is generated by circulating water with a pump in a swimming pool. A spout and suction slit were contrived to generate a constant current while the strength of the current is regulated by a valve. The decrease of the leg kicking intervals of mice accompanying increase in the current speed confirmed that the work load is adjustable by regulation of the current speed. The swimming time until fatigue was observed to decrease with increasing current speed in the two strains of mice. Biochemical index, the blood lactate and muscle glycogen levels, correlated to increase of flow rate. SD of maximum swimming time in our developed pool was almost same as that in treadmill and 20% smaller than that in the swimming with weight, and the developed pool were hence highly sensitive to the effect of food on endurance exercise performance compared with swimming with weight. The developed pool also did not require 4 to 8 wks of preliminary training required in treadmill and the exercise time until fatigue was 70% shorter than treadmill and the price of our developed swimming pool was about one-fifth of the treadmill. An adjustable-current swimming pool could consequently reduce the experimental period and cost required for the evaluation of the dietary components on endurance exercise performance. These results indicate that the apparatus employed in the present study is suitable for the evaluation of the endurance capacity of mice, and that it is useful for detecting the effects of dietary differences and drug pretreatments on this capacity.

Section 2 Maximum swimming time was investigated in two conditions, chronic endurance training and iron deficiency, which would enhance or decrease

endurance exercise performance, respectively. Five-wk-old Std ddY mice swam in the current pool 5 days per week for 6 weeks at the flow rate of 5 L/min. Maximum swimming time significantly increased compared to sedentary mice. The author confirmed their augmentation of endurance exercise capacity by increase of maximum O₂ consumption, muscle hypertrophy, and higher activity of citrate synthase, the TCA cycle enzyme. Three-wk-old Std ddY mice fed diets containing 5 or 40 mg of iron per kg diet for 3 wk as anemia or nonanemia, respectively. Diets of anemia mice were changed to diets containing 5, 25, or 40 mg of iron per kg diet. Recovery of hemoglobin concentration were highly correlated to recovery of swimming time until fatigue. Those two experiments suggested that the endurance exercise performance was highly correlated to maximum swimming time and maximum swimming time was thus the index of endurance exercise performance.

Section 3 The author found the experimental condition in which the SD of swimming time became smaller. The author tested BALB/c and C57BL/6 mice and confirmed that the deviation in swimming time could become smaller in BALB/c mice compared to Std ddY. The author cleared the exercise intensity during swimming by measuring O₂ consumption during swimming. Nine BALB/c mice (4 wk old male) were swum at various flow rate and measured O₂ consumption during swimming followed swimming in the static water for 30 min at 34 °C everyday for 7 d. The exercise intensity could be represented as the percentages of oxygen consumption during swimming.

CHAPTER 2

Section 1 The effects of Nanpao[®], a mixture of 31 Chinese crude drugs widely used in China as a tonic, was tested for its effect on the endurance capacity of mice by using an adjustable-current swimming pool. Seven-week-old male Std-ddY mice were fed with 20% casein diet supplemented 0.1% Nanpao[®] powder for 3 weeks. The mice were subjected to forced swimming 3 times a week for 3 weeks, and the swimming time until fatigue was compared with that of the control. The 0.1% Nanpao[®] treatment significantly prolonged the swimming time and lowered serum L-lactic acid level ($P = 0.1$) and gastrocnemius muscle glycogen depletion rate ($P < 0.05$) after 20 min of swimming exercise. These findings suggested that Nanpao[®] might improve lipid utilization during endurance exercise, and thereby endurance

exercise performance was augmented in Nanpao administered mice.

Section 2 (-)-Hydroxycitrate (HCA) is an active ingredient that is extracted from the rind of the Indian fruit *Garcinia cambogia*, which is available as a herbal supplement, and promoted as a weight loss agent. In the present study, acute and chronic effects of HCA on energy metabolism were examined in male Std ddY mice. Mice were placed into a metabolic chamber and were orally administered 10 mg of HCA or water. Serum FFA levels were significantly higher 100 min after administration in HCA group, but respiratory exchange ratio was not different compared with control group. The concentration of glycogen in the gastrocnemius muscle was higher in HCA group 16 h after the administration and their maximum swimming time until fatigue tended to be longer than the control mice, and the difference was significant after continuous administration for 3 d. Another mice were orally administered 10 mg of HCA or water twice a day for 25 d. On 26 d, they were placed into a treadmill chambers after administration, and allowed to rest for 1 h, followed by 1 h run at the speed of 21 m/min and the respiratory gas was monitored. Respiratory exchange ratio was significantly lower in HCA administered mice for both resting and exercising condition. These results suggested that the chronic HCA administration promoted lipid oxidation and spared carbohydrate utilization at rest and during running in mice.

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