Improved Swimming Pool Achieves Higher Reproducibility and Sensitivity to Effect of Food Components as Ergogenic Aids

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Summary A previously developed current swimming pool for mice has been used to evaluate many food components that enhance endurance exercise performance. In this article, to improve reproducibility, reliability and sensitivity of this assay system, we improved the spout part to generate a uniform current and divided the pool into six lanes to avoid physical interference between swimming mice. The stability of the current flow was assessed by measuring the surface current speed and water volume from the spout part. Maximum swimming times of ddY and BALB/c mice were measured to assess the reproducibility of the maximum swimming time. The improvement in sensitivity compared to the original equipment was estimated under three physiological conditions: low carbohydrate diet feeding, low blood hemoglobin level, and carbohydrate supplementation during exercise. The new spout part improved uniformity and quick adjustment of surface current, yielding an increase of workload in a stepwise manner during swimming. Exercise workload was increased in proportion to surface current speed, as evidenced by cadence of kicks and serum lactic acid levels. The improved swimming pool showed higher reproducibility of swimming time until fatigue (p<0.0001). Correspondence between blood hemoglobin concentration and swimming time was improved in the swimming pool. The improved swimming pool yielded higher sensitivity for low carbohydrate diet feeding (p<0.0001) and carbohydrate supplementation during exercise (p<0.01) compared to the original swimming pool. The improvement of the swimming pool achieved higher sensitivity and reproducibility in assessing various diet and food components compared to the original swimming pool.

Key Words graded exercise, swimming pool, mice, endurance exercise

Exercise performance is affected by appropriate diet. Controlled studies using laboratory animals should be conducted to elucidate the biochemical mechanisms underlying the improvement of performance afforded by food components such as ergogenic aids.

We have previously established an adjustable-current swimming pool for evaluating endurance performance in mice (1). This equipment has been used to evaluate many food components and materials that enhance endurance swimming capacity in mice, such as cyclic cluster dextrin (2), (−)-hydroxycitrate (3), capsaisin (4) and its non-pungent analogs (5), green tea extract (6), the Chinese medicine Nanpao (7), royal jelly extract (8) and conjugated linoleic acid (9). In recent years, the equipment has also been used for the evaluation of: 1) a genetically altered mouse model that exhibits decreased mitochondrial respiration (10); 2) a mouse model of asthma that shows alterations in ventilatory function (11); 3) hepatic gene expression in an obese mouse model with exercise training using cDNA microarrays (12); and 4) the effects of the novel endothelial NO synthase enhancer AVE9488 on recovery from hind limb ischemia (13).

There exist some problems in reliability and reproducibility in this swimming pool when measuring maximum endurance swimming time in mice. The problems arise from physical interference between swimming mice. As six mice simultaneously swim in the pool based on previously recommended experimental protocols, swimming mice sometimes interfere with each other at the corner of the pool. The surface area of the pool should thus be separated into six lanes for six mice using five boards to avoid physical interference between swimming mice.

The other problem is instability of the surface current. It required about 2 h to adjust surface current before the start of the experiment. Despite careful pre-
liminary adjustment of surface current, the instability of the surface current often caused daily variation of the swimming time of mice (7) and markedly decreased detection sensitivity of the effect of food components as ergogenic aids.

In relation to the instability of the surface current, it required some technical improvement to change the surface current during swimming of mice. Changing workload is in principle impossible in weight attached swimming exercise but it is a standard exercise protocol for the cycle ergometer (14) in humans and treadmill running in humans (15), rodents (16) and thoroughbred horses (17). The difficulty of performing graded exercise in swimming remained one of the shortcomings of swimming exercise in rodents (18). The difficulty was caused by the instability in surface flow and difficulty in rapid changes of flow speed in the original swimming pool. Therefore technical improvement concerning surface current flow would increase stability and enable graded increase of workload during swimming.

We describe herein the development of an improved swimming pool allowing establishment of graded exercise. Furthermore, we confirmed the superiority of the improved swimming pool and graded exercise protocol over the original swimming pool, and compared the effects of altering whole body carbohydrate storage by dietary manipulation (administration of cyclic cluster dextrin (CCD) or feeding of low carbohydrate (L-CHO) diet) and altering oxygen transport capacity by exsanguination.

**Methods**

**Animals.** Male BALB/c and Std ddY mice (5 wk old) were purchased from Japan SLC, Inc. (Shizuoka, Japan). All experiments were performed using BALB/c mice except for the reproducibility of swimming time until fatigue. Mice were maintained at 24.5˚C with a 12-h light cycle (lights on from 0800 to 2000) and provided ad libitum access to water and food. The mice were fed a commercial diet (MF; CLEA Japan, Inc., Tokyo, Japan) for 7 d before the start of each experiment and accustomed to swimming exercise for 30 min during the preliminary period. All procedures were performed in accordance with the Animal Experimentation Guidelines of Nagoya University.

**Design of the improved adjustable-current swimming pool.** Figures 1 and 2 show the design of the improved swimming system used. We used an acrylic plastic pool (90×45×45 cm) filled to a depth of 38 cm with water. The surface of the tank is clear and smooth, preventing the animal from gaining any support while swimming. Each lane has two nozzles (30 mm long, 4 mm inner diameter) with a center-to-center distance of 48 mm.

An acrylic slope at the end of the surface area (67˚ to horizontal) submerges the surface flow to the bottom of the pool. Water is returned to the pump through a narrow slit in the plastic pipe set in at the bottom of the pool (Fig. 2). Current strength is adjusted by changing the waterflow, which is regulated by opening and closing a valve and is monitored using a water flowmeter (FC-A20; Tokyo Flowmeter Laboratory, Tokyo, Japan). Temperature of the water is maintained at 34˚C using a water heater and thermostat.

**Measurement of water volume.** Water volume from the nozzles in each lane was measured as the weight of water collected into a glass beaker within a specified period of time. Water was filled to a depth of 10 cm in the pool during the measurement of water volume from nozzles. Flow rates of the pump were 5 and 10 L/min in the original swimming pool and 10, 12 and 15 L/min in the improved swimming pool.

**Measurement of surface-current speed.** The distribu-
tion of surface-current speed was measured using a digital current meter (type SPC-5; Sanko Industry, Tokyo, Japan) at 10 and 5 cm from the terminal part of the surface area. Water was filled to a depth of 38 cm in the pool during the measurement of surface current speed. Surface flow speed was measured at a flow rate of 10 L/min in both the original and improved swimming pools.

Graded exercise protocol in the improved swimming pool. Mice were fasted for about 3 h before the start of swimming, then made to swim. Mice swam until fatigued, defined as a failure to rise to the surface of the water to breathe within a 7-s period. The characteristic changes in swimming posture accompanying fatigue have been described previously (1). Surface current speed of each lane was measured before and after swimming. In the graded exercise protocol, flow rate of the pump was increased by 1 L/min every 5 min from an initial flow rate of 10 L/min until fatigue.

Analysis of kicking cadence. Swimming mice in the improved swimming pool with current at varying flow rates and swimming mice with attachment of 5 or 10% of body weight in static water were recorded on videotape. Average numbers of hindlimb kicks were counted by inspection of the video images played back at slow speed.

Serum lactic acid concentration during swimming. Mice were exercised in the improved swimming pool for 3 or 6 min at a flow rate of 10 or 15 L/min. Blood was collected by retro-orbital bleed and was treated with 5% perchloric acid. The concentration of L-lactate acid was then measured using a commercial kit (Determiner LA; Kyowa Medix, Tokyo, Japan).

Reproducibility of swimming time until fatigue. Male 7- to 10-wk-old Std ddY (n=104) and BALB/c (n=97) mice were used. Std ddY mice swam until fatigue in the original swimming pool at a constant flow rate of 8 L/min. BALB/c mice swam in the improved swimming pool using the graded exercise protocol.

Effect of L-CHO diet on swimming time until fatigue. Based on the preliminary swimming time until fatigue, 24 of 42 mice were selected for this part of the study using a selection algorithm described elsewhere (19). Mice were divided into two groups, with one group fed an AIN-93-based L-CHO high-fat diet (mixture of 550 g of lard and 1 kg of AIN-93G diet) and the other group fed AIN-93G diet for 3 d before measurement of swimming time until fatigue. Swimming times were measured repeatedly under three different experimental conditions: 1) in the original swimming pool at a flow rate of 7 L/min; 2) in the improved swimming pool at a constant flow rate of 10 L/min; and 3) in the improved swimming pool using the graded exercise protocol. Each swimming exercise was separated by ≥6 d for recovery.

Effect of exsanguination on swimming time until fatigue. Based on the preliminary swimming time until fatigue, 32 of 63 mice were selected for this part of the study using the same selection algorithm described previously (19). Mice were divided into three groups and 300 or 500 µL of blood was collected by a retro-orbital bleed under ether anesthesia. Blood was not collected from the sham operation group. Swimming time until fatigue was determined on the day after exsanguination in the improved swimming pool at a constant flow rate of 13 L/min or using the graded exercise protocol. Blood hemoglobin concentrations were measured.
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after conversion to the cyanometemoglobin derivative (20).

Administration of CCD. Based on the preliminary swimming time until fatigue, 24 of 42 mice were selected for this part of the study according to the selection algorithm (19). Mice were divided into two groups, both of which were fed the AIN-93G diet for the first 3 d of the experiment. Mice were then administered either 0.25 mL of 5% CCD solution or distilled water at 10 min after the start of swimming in the improved swimming pool during the graded exercise protocol. After measurement of swimming time until fatigue, the diet for both groups of mice was changed to an AIN-93-based L-CHO high-fat diet (mixture of 550 g of lard and 1 kg of AIN-93G diet) for 3 d. Mice were then administered either 0.25 mL of 5% CCD solution or distilled water at 10 min after the start of swimming in the improved swimming pool during the graded exercise protocol. Water temperature of the pool was 37˚C in this experiment.

Statistics. Data are expressed as mean±standard error of the mean (SE) for the indicated number of individual experiments. Statistical analysis of differences between pairs of groups was performed using Student’s t-test. Comparisons of means among more than two groups were performed using one-way analysis of variance followed by Tukey’s test. Statistical analyses and calculation of the coefficient of variation (CV) were performed using the Prism software package (Windows version 4.03; GraphPad Software, San Diego, CA). Values of p<0.05 were considered statistically significant.

Results

Disappearance of physical interference between swimming mice in the improved swimming pool

Physical interference between swimming mice disappeared when the surface area of the pool was separated into six lanes by the five acrylic boards (Fig. 2). This resolution of physical interference enabled a more objective definition of fatigue. Experimenters needed less training to define fatigue for mice in the improved swimming pool compared to the original swimming pool (data not shown).

Improved uniformity of water volume from the spout part

Uniformity of water volume from all nozzles at the spout part is required to obtain a uniform surface current in each lane of the swimming pool. Water volume from the nozzles in the improved swimming pool (CV, 1.4, 1.6 and 1.5 at flow rates of 10, 12 and 15 L/min, respectively) showed smaller variations than seen in the original swimming pool, which had 11 jet holes in the spout part (CV, 7.0 and 8.7 at flow rates of 5 and 10 L/min; Fig. 3).

Improved uniformity of surface current speed

When the surface area of the pool was divided into six areas by the five acrylic lanes, surface current speed showed large variations among lanes in the original swimming pool. Surface flow speeds measured at 10 cm from the terminal part of the surface current were 1.5 to 2 times higher than those measured at 5 cm from the terminal part in the original swimming pool. Such reductions in surface current speed indicate a large variation in workload across the anteroposterior position for swimming mice in the original swimming pool. Reductions in surface flow speeds from 10 to 5 cm from the terminal part were within 10% in the improved swimming pool, indicating a more uniform workload in the improved swimming pool (Fig. 4).

Analysis of kicking cadence

Workload increases with increasing flow rate, as evidenced by average kicking cadence increasing with increased flow rate within a range of 8–14 L/min in the improved swimming pool. Almost no change in kicking cadence was seen below 8 L/min or over 15 L/min. Maximum kicking cadence in the swimming pool was almost the same as that in weight-attached swimming, but showed reduced individual differences, indicating...
Improvement of Swimming Pool for Mice

uniformity of the workload in the improved swimming pool (Fig. 5).

**Lactic acid concentration during swimming**

Serum lactic acid concentration was increased in proportion to swimming time. The flow rate of 15 L/min showed markedly increased lactic acid concentration compared to 10 L/min (Fig. 6).

**Reproducibility of swimming time until fatigue**

The coefficient of correlation for swimming time until fatigue was higher with graded exercise in the improved swimming pool than with a constant workload in the original swimming pool (Fig. 7). Both coefficients of correlation were higher compared to those for swimming with weights of 5% or 10% of body weight (data not shown).

**Effect of L-CHO diet on swimming time until fatigue**

Swimming time was significantly lower in mice fed the L-CHO diet than in those fed the normal diet in a constant workload in original swimming pool (Fig. 8A, p<0.01). Improvement of the swimming pool appara-
Endurance exercise performance is affected by oxygen transport ability. Many different doping agents have been used to enhance performance. The most common agent in recent use has been erythropoietin, a red blood cell-stimulating hormone. Other medical interventions have been employed to increase oxygen transport ability such as blood transfusions. Meanwhile, endurance exercise performance is substantially decreased in patients with anemia induced by iron deficiency, exercise or drugs.

Maximum exercise duration until fatigue is reproducible when exercise is performed at the velocity achieving maximum oxygen consumption ($VO_{2\text{max}}$) for the individual (23) and does not always correspond to changes in $VO_{2\text{max}}$ (24). Decreased hemoglobin concentrations markedly decrease $VO_{2\text{max}}$ during exercise.
glycogen storage. In the previous study (1), when they were fed the L-CHO diet to reduce muscle fatigue time was observed after the administration of CCD (27). On the other hand, significant increase of swimming time caused by some food habits were detected after L-CHO diet feeding but not after the physiological alterations affecting exercise performance, such as muscle glycogen storage, oxygen transport capacity and exogenous carbohydrate supplementation, can be detected as changes in swimming time until fatigue. These improvements in the swimming pool system and exercise protocol reduce individual variations in swimming time until fatigue and decrease the amount of time and number of animals required for experiments compared to the original swimming pool. The superiority of this improved swimming pool could contribute to the assessment of novel affects of food components as ergogenic aids.

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