

## Enhancement of Swimming Endurance in Mice by Highly Branched Cyclic Dextrin

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We investigated the ergogenic effect in mice of administering highly branched cyclic dextrin (HBCD), a new type of glucose polymer, on the swimming endurance in an adjustable-current swimming pool. Male Std ddY mice were administered a HBCD, a glucose solution or water *via* a stomach sonde 10 min before, 10 min after or 30 min after beginning swimming exercise, and were then obliged to swim in the pool. The total swimming period until exhaustion, an index of the swimming endurance, was measured. An ergogenic effect of HBCD was observed at a dose of 500 mg/kg of body weight, whereas it had no effect at a dose of 166 mg/kg of body wt ( $p < 0.05$ ). The mice administered with the HBCD solution 10 min after starting the exercise were able to swim significantly longer ( $p < 0.05$ ) than the mice who had ingested water or the glucose solution. The rise in mean blood glucose level in the mice administered with HBCD, which was measured 20 min after starting swimming, was significantly lower ( $p < 0.05$ ) than that in the mice administered with glucose, although it was significantly higher ( $p < 0.05$ ) than that in the mice administered with water. The mean blood insulin rise in the mice given HBCD was significantly lower ( $p < 0.05$ ) than that in the mice given glucose. The mice administered with HBCD 30 min after starting the exercise swam significantly longer ( $p < 0.05$ ) than the mice who had ingested water, although the enhancement of swimming time was similar to that of the glucose-ingesting mice. The gastric emptying rate of the HBCD solution was significantly faster ( $p < 0.05$ ) than that of the glucose solution. However, this glucose polymer must have spent more time being absorbed because it has to be hydrolyzed before absorption, reflecting a lower and possibly longer-lasting blood glucose level. We conclude that the prolongation of swimming endurance in mice administered with HBCD depended on its rapid and longer-lasting ability for supplying glucose with a lower postprandial blood insulin response, leading to a delayed onset of fatigue.

**Key words:** highly branched cyclic dextrin (HBCD); glucose; swimming endurance; current swimming pool; gastric emptying rate

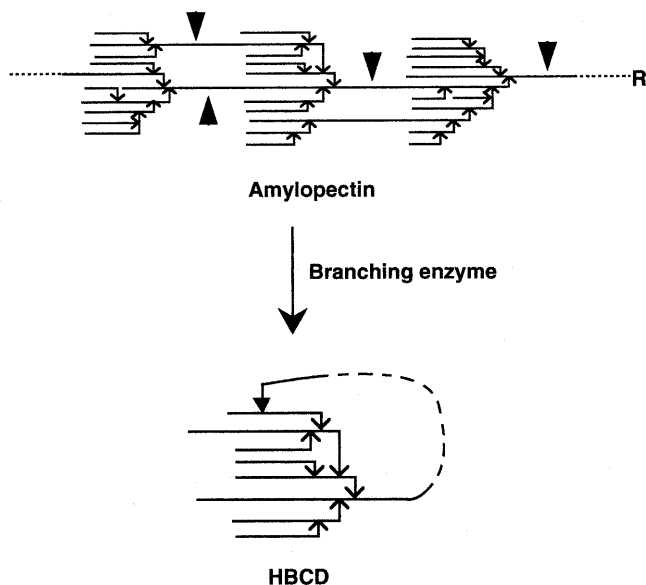
The ability to sustain prolonged exercise is determined to some extent by fuel availability. It has been shown that maintenance of euglycemia and the carbohydrate (CHO) oxidation rate during exercise can delay fatigue, which implies that CHO intake before and/or during exercise may help to prolong the duration of aerobic exercise activities.<sup>1–3</sup> This improvement in performance could be at least partly due to the oxidation of exogenous CHO which could prevent the depletion of the tricarboxylic acid cycle intermediates<sup>4</sup> that has been observed during prolonged exercise.<sup>5</sup> Several studies have, indeed, shown that a large percentage of the CHO ingested during exercise is actually oxidized, providing between 16% and 20% of the total energy expended.<sup>6</sup>

Substitution of the glucose polymer for glucose would allow an increased content without any increased osmolality, and may also have taste advantages for sweetness; therefore, the available evidence suggests that use of the glucose polymer rather than free glucose would enhance the exercise performance.<sup>1,7</sup> A study has suggested that solutions of long-chain glucose polymers are more readily oxidized by the muscles during exercise than a glucose or fructose solution,<sup>8</sup> but another study has found no difference in the oxidation rate between ingested glucose and the glucose polymer.<sup>9</sup> There is a discrepancy concerning the glucose polymers between these studies, because most of the commercial glucose polymers (dextrins) used in these studies were mixtures with a broad size distribution including glucose, maltose and oligosaccharides. The composition of dextrins may have affected the postprandial blood glucose and insulin response. The postprandial blood insulin response may in turn have influenced the endurance in these studies. There are no studies that have used dextrins with a narrow molecular weight distribution.

HBCD is a new type of dextrin that is produced from waxy corn starch by the cyclization reaction of a branching enzyme (BE, 1,4- $\alpha$ -D-glucan: 1,4- $\alpha$ -D-glucan 6- $\alpha$ -D-(1,4- $\alpha$ -D-glucano)-transferase, EC 2.4.1.18; Fig. 1).<sup>10</sup> This dextrin is highly soluble in water and has a relatively low propensity for retrogradation. The average molecular weight of HBCD is 160,000 with a narrow size distribution, whereas most of the commercial dex-

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Abbreviation: HBCD, highly branched cyclic dextrin



**Fig. 1.** Production of HBCD by the Cyclization Reaction of BE from Amylopectin.

Solid lines indicate a 1,4- $\alpha$ -D-glucan chain. Arrows are  $\alpha$ -1,6 glucosidic linkages. The reducing end of a glucan chain is shown by "R." Closed triangles indicate the  $\alpha$ -1,4 linkages cleaved by BE. Both cyclic molecules and reducing (non-cyclic) molecules are produced by the action on glucan with a reducing end.

trins are mixtures with a broad size distribution, including glucose, maltose and oligosaccharides, and have a higher propensity for retrogradation.<sup>11)</sup> Furthermore, HBCD is as digestible as commercial dextrins to glucose by  $\alpha$ -amylase and  $\alpha$ -glucosidase that are present in the small intestine.<sup>12)</sup> These results could imply that HBCD would be slowly absorbed after digestion to glucose and give a lower postprandial glycemic response.

We hypothesize that HBCD would particularly produce a lower glycemic and insulin response than glucose from its structure which would provide longer-lasting glucose availability during submaximal exercise. In addition, a reduced insulin response might prevent rebound hypoglycemia. A more sustained glucose availability could supply CHO to maintain the energy substrates' oxidation when the glycogen stores have been depleted, thus leading to enhanced exercise capacity. The purpose of this study is to elucidate whether HBCD supplementation before or during exercise could enhance the endurance of mice.

## Materials and Methods

**Animals.** Five-week-old Std ddY mice (a closed colony from Japan Shizuoka Laboratory Center, Hamamatsu, Japan) were used. They were housed in cages (33  $\times$  23  $\times$  12 cm; 6 mice per cage) under controlled conditions of temperature (22  $\pm$  0.5°C), humidity (50%), and lighting (lights on from 18:00 to 6:00). They were provided with a stock diet (type MF; Oriental Yeast, Tokyo, Japan) and water *ad libitum*. The care and treatment of the experimental animals conformed to the Kyoto University guidelines for ethical treatment of

laboratory animals.

**Blood glucose and insulin response after CHO ingestion during resting.** Groups of 7 to 9 mice were used per experiment. After being fasted for 17 h (20:00–13:00), each mouse was orally administered with 300  $\mu$ l of a 5% HBCD or glucose solution *via* a stomach sonde. The mice were killed by decapitation 0 to 30 min after the administration of the solution. Blood samples were collected from the severed neck veins, serum being obtained by centrifugation and then maintained at –20°C until assayed.

**Current swimming pool.** An adjustable-current water pool was used for measuring the swimming endurance. The details have been described elsewhere.<sup>13)</sup> We used an acrylic plastic pool (90  $\times$  45  $\times$  45 cm; Anitec Co., Otsu, Japan) filled to a depth of 38 cm with water. The inner surface of the pool is flat and smooth to prevent an 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). Water is returned to the pump through a narrow slit in a plastic pipe set at the bottom of the pool. The strength of the current can be adjusted by changing the water flow, which is regulated by opening or closing a valve, and is monitored by a water flow meter (FC-A20, Tokyo, Flow Meter Laboratory, Tokyo, Japan). The distribution of the surface-current speed is measured with a digital current meter (type SPC-5, Sanko Industry, Tokyo, Japan) at 12 surface points spaced at regular intervals. The temperature of the water is maintained at 34°C with a water heater and thermostat. The high reproducibility and sensitivity of this apparatus for evaluating the maximum endurance of mice have been reported.<sup>13–15)</sup>

**Measurement of the maximum swimming time in the adjustable-current pool.** To avoid circadian variations in physical activity, the experiments were done from 13:00 to 17:00, a period during which minimal variation of endurance has been confirmed in mice. After a 1 preliminary-wk period during which the mice became accustomed to swimming, the maximum swimming time (endurance) was measured at a flow rate of 7 liter/min. The mice were assessed to be fatigued when they failed to rise to the surface of the water for breath within a 7-second period. A period of longer than 7 seconds frequently resulted in drowning. The swimming endurance of two treatment groups, such as water versus HBCD or HBCD vs. glucose (or glucose vs. water in the case of administration 10 min after starting swimming) was measured by a cross-experiment, and the paired data were analyzed.

**Analysis of metabolic parameters during exercise.** The mice were made to swim in the current pool with a water flow rate of 7 liter/min. Blood samples were taken from the neck vein by decapitation 20 min after beginning swimming (10 min after HBCD ingestion). Immediately after taking 5  $\mu$ l of blood from a micro tube containing whole blood, it was deproteinized in perchloric

ic acid (0.8 N) to determine lactate. After each blood sample had been centrifuged, the L-lactic acid concentration in the blood was determined with a Kyowa Medex commercial kit (Determiner LA, Tokyo, Japan). Residual blood samples were kept in an ice bath until the blood glucose and insulin were measured. After centrifugation, the blood glucose concentration was measured by the glucose oxidase method with a commercial kit (glucose CII test Wako, Wako Pure Chemical Industries, Osaka, Japan). The blood insulin concentration was determined by an immunoassay with a commercial insulin measurement kit (Morinaga, Yokohama, Japan).

**Muscle glycogen analysis.** Immediately after the blood had been collected, the gastrocnemius muscles were removed, frozen in liquid nitrogen, and kept at  $-80^{\circ}\text{C}$  until being analyzed for glycogen concentration. The glycogen content was measured spectrophotometrically by the glucose oxidase method as described elsewhere.<sup>15)</sup> Briefly, after hydrolyzing the muscle sample in 0.6 N HCl at  $100^{\circ}\text{C}$  for 2 h, the glucose residues were determined with a commercial kit (see the method for blood glucose determination).

**Gastric emptying rate of the CHO solution.** After being fasted for 17 h (20:00–13:00), each mouse was orally administered with 300  $\mu\text{l}$  of water, a 5% HBCD solution or a 5% glucose solution. Each animal was killed by dislocating the neck 5 min after the administration, the stomach was immediately extirpated, and the gastric juice was weighed. The gastric emptying rate is represented by the solution volume transferred from the stomach to the small intestine.

**Statistics.** Each value is expressed as the mean  $\pm$  SEM. Comparisons of the swimming endurance between the means of two groups were done by the paired Student's *t* test. Data for the metabolic parameters and gastric emptying rate of the CHO solution were analyzed by ANOVA. Statistical data were calculated with the INSTAT software package (Macintosh Version 3.00, GraphPad Software Inc., San Diego, CA, U.S.A.). A level of  $p < 0.05$  was used as the criterion for statistical significance.

## Results

### Blood glucose and insulin responses following HBCD ingestion

It is well known that the ingestion of CHO has a direct influence on the blood glucose concentration, and that blood glucose affects insulin secretion. These phenomena lead to various metabolic changes and would influence the ability to exercise. To reveal the postprandial blood glucose concentration and its change following HBCD ingestion, we administered an HBCD solution or glucose solution to mice. The mean blood glucose and insulin responses in the mice are expressed relative to the fasting levels. Figure 2A shows that the blood glucose level after administering the HBCD solution was lower at all points after 10 min than that of the glucose solution, the difference at 10 min being statisti-

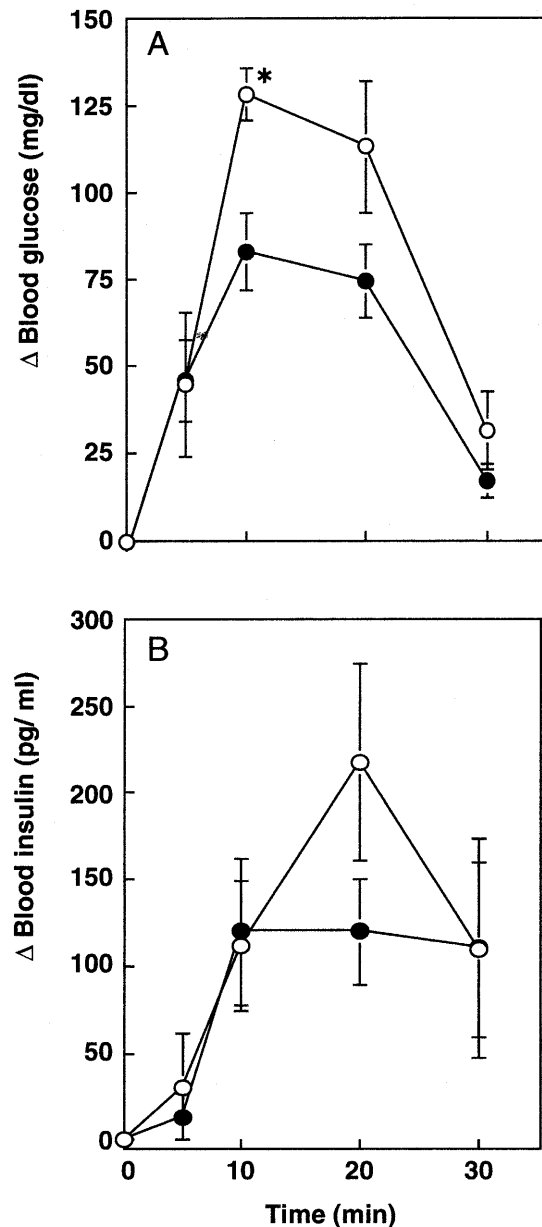


Fig. 2. Changes in the Blood Glucose and Insulin Concentration.

Glycemic response is expressed as blood glucose above the fasting level (A) and insulin response is expressed as blood insulin above the fasting level (B) after the ingestion of a 5% glucose (open circle) or 5% HBCD solution (closed circle). Mice were administered with 500 mg CHO/kg of body weight, and blood samples were collected at 0, 5, 10, 20 and 30 min after the CHO loading. Each value represents the mean  $\pm$  SEM for 9 mice.

\*Significantly different between two groups ( $p < 0.05$ ).

cally significant ( $p < 0.05$ ). The blood insulin level after HBCD loading, which is presented in Fig. 2B, was not significantly different at any point from that of the glucose solution, although the difference at 20 min was marked ( $p = 0.10$ ). These results suggest that the blood glucose response in mice that had ingested HBCD was less than that in mice that had ingested glucose, since HBCD might be digested more slowly and be more gradually absorbed from the gut.

### Effect of HBCD on swimming endurance

After becoming accustomed to swimming, the swimming time to exhaustion was measured for all mice, the average time being  $64 \pm 5$  min (mean  $\pm$  SEM). A dose-dependent effect of HBCD on the swimming endurance was initially observed when it had been orally administered 30 min after starting swimming (Fig. 3). The mean swimming time for the mice that had ingested 500 mg HBCD/kg of body wt. was  $75 \pm 4$  min, whereas that for the mice that had ingested 166 mg HBCD/kg of body wt. was  $65 \pm 3$  min, the difference being statistically significant ( $p=0.02$ ). Therefore, we subsequently administered the HBCD or glucose solution to the mice at a dose of 500 mg/kg of body wt. throughout this study.

To make clear the effect of HBCD on the swimming endurance, 300  $\mu$ l of a 5% HBCD solution, 5% glucose solution or water (as a vehicle control) was orally administered to individual mice 10 min before, or 10 or 30 min after beginning the swimming experiment. The swimming time to fatigue was measured at a flow rate of 7 liter/min (19 cm/s of surface current speed). We investigated the relationship between the swimming time to fatigue (endurance) and the timing and quantity of CHO ingestion. As shown in Fig. 4, the mice that had been given HBCD 10 min before swimming did not show any more ergogenic effect on the endurance than those given water, whereas the mice given glucose showed 40% poorer swimming endurance than those given HBCD (Fig. 4-A1 and -A2). The mice administered with the HBCD solution 10 min after beginning swimming were able to swim significantly longer than the mice administered with water or glucose ( $p<0.05$ ) (Fig. 4-B1 and -B2). The mice administered with

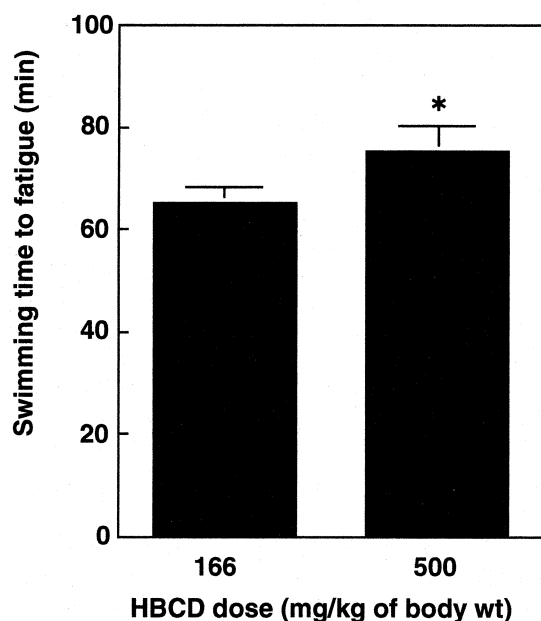


Fig. 3. Effect of HBCD Dose on the Swimming Endurance in Mice.

Mice were administered with a HBCD solution at a dose of 166 or 500 mg/kg of body wt. 30 min after starting swimming. The swimming time to fatigue was measured to evaluate the swimming endurance, and is presented as the mean  $\pm$  SEM for 16 mice.

\*Significantly different ( $p<0.05$ ).

HBCD 30 min after starting swimming swam 20% longer than the mice administered with water, although the endurance was similar to those administered with glucose (Fig. 4-C1 and -C2). These results indicated that 10 min after the onset of swimming was the best time to administer HBCD to the mice. To confirm the reproducibility among cross-experiments, we performed one more cross-experiment concerning the glucose vs. water treatment groups. The mean swimming times to fatigue by the mice administered with water, the glucose solution and the HBCD solution 10 min after beginning swimming from 3 cross-experiments, were  $65 \pm 5$ ,  $68 \pm 5$  and  $86 \pm 6$  min, respectively (Fig. 5).

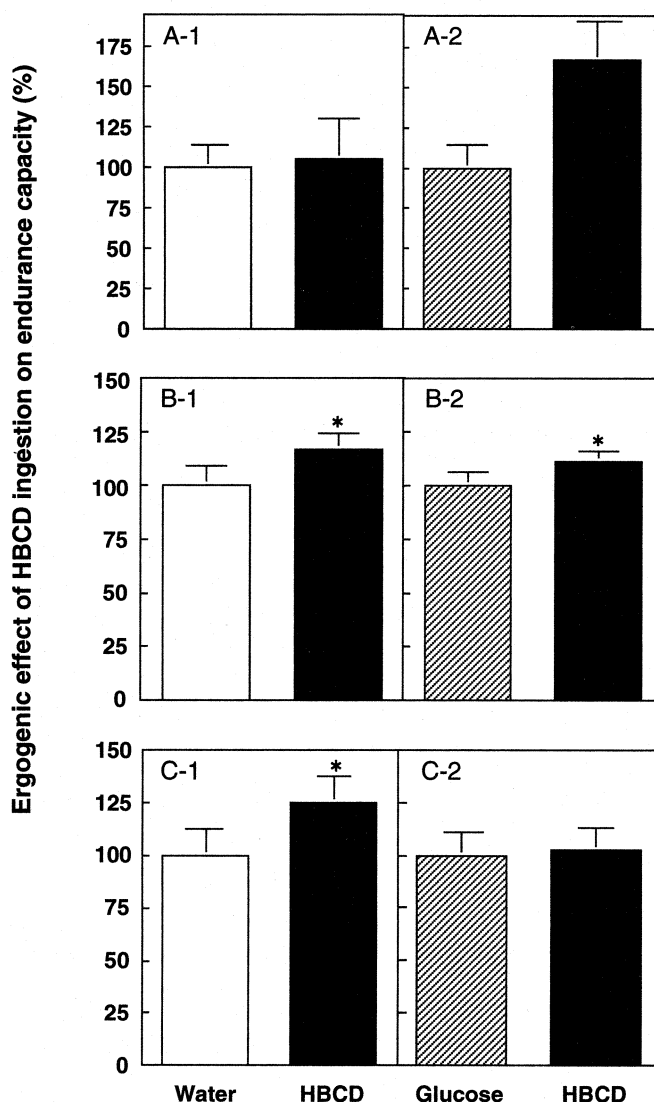


Fig. 4. Relationship between the Swimming Endurance and CHO Ingestion A) 10 min before ( $n=9$ ), B) 10 min after ( $n=18$ ), and C) 30 min after the Onset of Swimming Exercise ( $n=14$ ).

Values in A-1, B-1 and C-1 represent the percentage (mean  $\pm$  SEM) of the swimming time for the mice that had ingested HBCD to that of the mice that had ingested water (as 100%). Values in A-2, B-2 and C-2 represent the percentage of the swimming time for the mice that had ingested HBCD to that of the mice that had ingested glucose (as 100%).

\*Significantly different ( $p<0.05$ ).

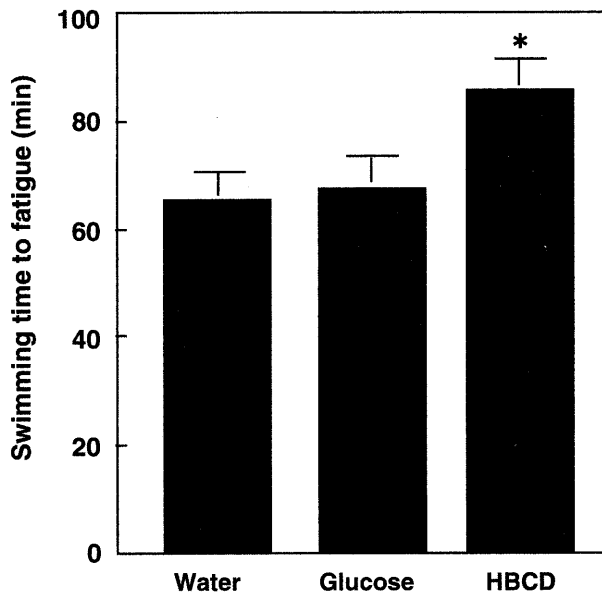


Fig. 5. Relationship between the Mean Swimming Time to Fatigue and CHO Ingestion 10 min after the Onset of Swimming through a Cross-experiment.

Each value represents the swimming time to fatigue (mean  $\pm$  SEM) of mice that had ingested CHO 10 min after the onset of swimming through a cross-experiment ( $n=36$ ).

\*Significantly different ( $p<0.05$ ).

#### Analysis of the metabolic parameters during exercise

In order to elucidate the mechanism for the higher endurance from HBCD, we further investigated the postprandial blood glucose and insulin levels after HBCD ingestion during exercise. The mice were made to swim in the current pool at a water flow rate of 7 liter/min, and each solution was administered to the mice 10 min after beginning swimming. Ten min later, *i.e.* 20 min after beginning swimming, blood was taken from the neck vein by decapitation, since the mean glycemic response was statistically different ( $p<0.05$ ) at 10 min after CHO ingestion between the HBCD and glucose groups (Fig. 2). The changes in blood glucose, insulin, and L-lactic acid concentration from the sedentary level are shown in the Table 1. The mean blood glucose rise in the glucose group was significantly higher than that in the other groups (relative to water group,  $p=0.001$ ; relative to the HBCD group,  $p=0.027$ ). The blood insulin rise in the glucose group was also significantly higher than that in the other groups (relative to the water group,  $p=0.005$ ; relative to the HBCD group,  $p=0.030$ ). The changes in the postprandial blood glucose and insulin responses during exercise were similar to those during resting, which is considered to support our hypothesis. The blood L-lactic acid concentration after 20 min of swimming increased above its sedentary level (2.7 mM) in all groups. No significant difference was apparent among all groups. Although the glycogen concentration in the gastrocnemius muscle after swimming in all groups had decreased from its sedentary level, no significant difference was apparent among all groups (data not shown).

Table 1. Changes to Metabolic Parameters in the Serum from the Sedentary Levels after 20 min of Swimming<sup>1,2</sup>

	Treatment		
	Water	HBCD	Glucose
Glucose (mg/dl)	74.1 $\pm$ 10.9 <sup>a</sup>	109.2 $\pm$ 12.5 <sup>b</sup>	147.7 $\pm$ 10.8 <sup>c</sup>
Insulin (pg/ml)	77.5 $\pm$ 41.3 <sup>a</sup>	250.9 $\pm$ 55.3 <sup>a</sup>	437.5 $\pm$ 67.5 <sup>b</sup>
Lactic acid (mM)	2.1 $\pm$ 0.5	1.2 $\pm$ 0.3	1.6 $\pm$ 0.4

<sup>1</sup> Each solution was administered to the mice 10 min after the onset of swimming.

<sup>2</sup> Each value represents the mean  $\pm$  SEM for 5–8 mice.

<sup>a-c</sup> Means in the same row with no common superscripts are statistically significant.

#### Gastric emptying rate of the CHO solutions

To improve the endurance by CHO ingestion, it must be rapidly transferred from the stomach to the small intestine. Therefore, it is important to evaluate the gastric emptying rate of the ingested CHO solution. The gastric emptying rate of the ingested solution is represented by the solution volume transferred from the stomach to the small intestine. The transferred volumes of water, 5% HBCD solution and 5% glucose solution in 5 min following a 300  $\mu$ l solution loading were 264  $\pm$  11, 237  $\pm$  16 and 198  $\pm$  12  $\mu$ l (mean  $\pm$  SEM), respectively. The fluid volume transferred from the stomach to the small intestine within 5 min after administering the solution was significantly less with 5% glucose than with nothing (water,  $p=0.015$ ) and the 5% HBCD ( $p=0.042$ ).

#### Discussion

The results of the present study with an adjustable-current swimming pool, which is new forced-swimming apparatus for measuring the maximum swimming time, indicate that HBCD ingestion during medium-intensity prolonged exercise could improve the endurance of mice. It was also found that ingestion early after starting the exercise (10 min after starting swimming) was best. We used this apparatus because of the many advantages offered in evaluating the endurance of mice. Namely, the data show higher reproducibility than those obtained from treadmill running or forced swimming with a weight attached to the tail.<sup>13</sup> As shown in Fig. 5, we also confirmed the reproducibility among several experiments. The flow rate is changeable, and the intensity of the swimming exercise can be adjusted to the purpose of each experiment. In this study, the flow rate of 7 liter/min corresponds to a surface current of about 19 cm/s at the center of the pool. The intensity of the exercise in which mice swim against the current is estimated to be about 50% of  $\dot{V}O_2$ max from the blood L-lactic acid concentration.<sup>13</sup>

The first purpose of this study was to examine whether HBCD supplementation as a drink could enhance the endurance of mice. HBCD was thought to be suitable to an ergogenic sports drink, because it has many original characteristics: a dextrin with a narrow size distribution, high solubility in water, low propensity for retrogradation, no tastes, and no smell, which most commercial dextrans do not have. For example, the

average molecular weight of HBCD is 160,000 containing little glucose or oligosaccharides (<0.15%). The average composition of one commercial dextrin is as follows: 2% free glucose, 8% maltose, 10% maltotriose, 40% between 4 and 7 glucose units, 20% between 8 and 25 glucose units, and 20% >25 glucose units.<sup>16)</sup> The commercial dextrans which have been used in several previous studies have had a broad molecular size distribution. They have usually been mixtures including glucose, maltose and oligosaccharides. The molecular size and composition of dextrans depend on their producers and may differently influence the postprandial blood glucose and insulin response. We thought HBCD might provide a new way to increase endurance capacity with dextrin having a narrow molecular weight distribution in spite of its relatively high molecular weight.

We administered a 5% HBCD solution to mice to investigate the postprandial blood glucose and insulin responses in the resting condition (Fig. 2). Figure 2 shows that HBCD gave a lower postprandial glucose response in the mice, being relatively slowly digested. Figure 2 also seems to show that HBCD may be a resistant dextrin. However, we think that HBCD is not a resistant dextrin because it has been reported that HBCD was, like commercial dextrans, completely digested to glucose by  $\alpha$ -amylase and  $\alpha$ -glucosidase present in the small intestine.<sup>12)</sup> In addition, as more time is necessary for its digestion in the small intestine, HBCD is thought to have been absorbed more slowly than glucose in the small intestine. In other words, the glucose absorbed per unit time in the small intestine is thought to have been greater from glucose ingestion than from HBCD ingestion. As a result, the postprandial blood glucose and insulin responses in mice that had ingested HBCD were lower than those in mice that had ingested glucose in the resting condition. Therefore, we hypothesized that HBCD would particularly produce a lower postprandial insulin response than isocaloric glucose by its structure. In this study, when we administered 15 mg of HBCD (300  $\mu$ l of a 5% CHO solution) to mice during exercise, we found evidence that HBCD supplemented as a drink during exercise could enhance the endurance of mice.

There are several studies that support the advantage of CHO ingestion during prolonged exercise. For example, Christensen and Hansen have reported that a high-CHO diet could delay the onset of hypoglycemia and increase the time to exhaustion during light exercise and that a CHO supplement ingested at the time of exhaustion could rapidly alleviate hypoglycemia and prolong the exercise period for a longer period of time.<sup>17)</sup> Bagby *et al.* have demonstrated that a continuous infusion to rats of glucose during moderate-intensity running could reduce the rate of liver and muscle glycogen utilization and delay the onset of fatigue.<sup>18)</sup> Yaspelkis *et al.* have suggested that CHO supplementation could enhance aerobic endurance during prolonged continuous exercise of varying low to moderate intensity in part by reducing the dependency on muscle glycogen as a fuel source.<sup>19)</sup>

The second purpose of this study was to reveal the mechanism for enhancing the mouse's endurance. We

further investigated the ergogenic effect of HBCD by comparing with glucose as a CHO control (Fig. 4). It is well known that the ingestion of CHO has a direct influence on blood glucose concentration, and that blood glucose affects insulin secretion. These phenomena lead to various metabolic changes and would influence the ability in exercise. The timing of CHO supplementation plays a key role in displaying its ergogenic effect on endurance exercise. When a CHO-containing beverage is ingested at rest in the last hour preceding exercise, for example, one may experience rebound hypoglycemia as a result of the high insulin level present in the blood (insulin-induced hypoglycemia) when exercise begins.<sup>20)</sup> This effect can be overcome by postponing ingestion until during prolonged exercise. After the onset of exercise, the insulin response was blunted, possibly as a consequence of catecholamine release.<sup>21)</sup> Galbo *et al.* have reported that the insulin release in blood was suppressed during exercise, since catecholamines were secreted after the onset of exercise.<sup>22)</sup> The timing and frequency of CHO supplementation may need to be determined according to the type or intensity of exercise.<sup>23,24)</sup> Therefore, we administered HBCD or glucose or water to mice immediately before or during prolonged exercise (10 min before, 10 min after, or 30 min after the onset of exercise).

The ingestion of CHO 10 min before the prolonged swimming exercise by mice was unsuccessful in respect of HBCD and glucose (Fig. 4A). The mice that ingested glucose 10 min before swimming might have fallen into the rebound hypoglycemic range as a result of a higher insulin level in the blood when exercise began (Fig. 2B). On the other hand, during prolonged exercise, the ingestion of CHO was successful, especially in respect of HBCD. As shown in Fig. 4B, the mice administered with HBCD 10 min after beginning swimming, an early stage of prolonged exercise, swam significantly longer ( $p < 0.05$ ) than the mice administered with glucose and water. The rise of blood glucose level in the mice given HBCD 10 min after beginning swimming, measured 20 min after the onset of exercise, was significantly higher ( $p = 0.038$ ) than that in the mice given water, but significantly lower ( $p = 0.027$ ) than that in the mice given glucose. The rise of blood insulin level in the mice given HBCD tended to be higher ( $p = 0.057$ ) than that in the mice given water, but significantly lower ( $p = 0.030$ ) than that in the mice given glucose (Table 1). HBCD raised the postprandial blood glucose and insulin responses after its ingestion during exercise, although the rise was significantly less than that by glucose. Our results indicate that HBCD contributed to the enhanced endurance of mice because supplying CHO maintained the energy substrates' oxidation during prolonged exercise with a lower insulin response than that by glucose during prolonged exercise. The mice given HBCD 30 min after the start of swimming, which is the mid or later stage of prolonged exercise, could swim 20% longer than the mice given water, while the mice given glucose swam as long as the mice given HBCD (Fig. 4C).

To summarize our data on the swimming endurance of mice in relation to CHO ingestion and its timing,

HBCD enhanced the swimming endurance of the mice by its ingestion throughout the exercise, whereas glucose enhanced it by its ingestion only in the later stage of the exercise. We think that these phenomena are based on the molecular size and structure of CHO. HBCD is a large molecule and needs to be hydrolyzed to glucose. Since available glucose molecules are gradually produced from HBCD by digestive enzymes in the small intestine, the absorption of glucose there takes a relatively long time and causes lower glycemic and insulin responses. On the other hand, glucose does not need to be hydrolyzed and is ready to be absorbed. When glucose molecules reach the small intestine, they would be absorbed more quickly. This might be why HBCD produced lower glycemic and insulin responses than glucose.

Although we have no direct evidence with HBCD, the findings on the mechanism for enhancing endurance by other glucose polymers would help to learn that by HBCD. Coyle *et al.* have studied the effect of glucose polymer ingestion during bicycle ergometer exercise.<sup>1)</sup> The result of their exercise test, in which the subjects were given glucose polymer 20 min after beginning the exercise, shows that the blood glucose level in the glucose polymer-fed group was maintained well above the preexercise level for the last 90 min of a 180-min exercise. The blood insulin level in the glucose polymer-fed group was also significantly higher than that in the placebo group for the last 120 min of the 180-min exercise. They concluded that the delayed onset of fatigue induced by the glucose polymer administration during prolonged exercise depended on maintaining the blood glucose and insulin levels above the preexercise level, especially in the later stage of prolonged exercise. This might have resulted in the increased utilization of blood glucose with a proportional slowing of muscle glycogen depletion.<sup>1)</sup> Similar findings have also been reported from studies using a cycle ergometer,<sup>25)</sup> motor-driven treadmill running<sup>26)</sup> and during ultra-endurance bicycling.<sup>27)</sup> The intake of CHO during exercise could elevate and maintain the blood glucose level during the later stage of prolonged exercise. The maintenance of blood glucose level is thought to allow CHO oxidation to continue until the end of exercise when the body's endogenous stores, such as muscle and liver glycogen, have been depleted.<sup>28-30)</sup>

On the other hand, the intake of glucose before exercise decreased the endurance of the mice (Fig. 4A). When glucose was given 10 min before exercise, the blood glucose reached its highest level at the beginning of the exercise (Fig. 2A). This triggered insulin secretion (Fig. 2B), and the subsequent insulin-induced hypoglycemia might have decreased the endurance by inhibiting the energy substrates' oxidation. In contrast, the postprandial blood glucose rise in the mice that had ingested HBCD was significantly smaller than that in mice that ingested glucose at the start of the exercise (Fig. 2A) and at an early stage of the exercise (Table 1), which blunted insulin secretion in the HBCD-fed mice (Fig. 2B, Table 1). The low insulin responsiveness of HBCD might have had an influence on maintaining the blood

glucose concentration and on the energy substrates' oxidation in mice.

The gastric emptying rate of the HBCD solution was significantly faster than that of the glucose solution by 20%. Rapid supply of a CHO substance as HBCD might have contributed to the improvement in CHO oxidation. It has been reported that gastric emptying and, consequently, fluid and caloric delivery might have been faster with a glucose polymer solution than with an isocaloric glucose solution, because of the lower osmotic pressure developed.<sup>16)</sup> Neuffer *et al.* have suggested that only the initial gastric emptying was probably accelerated with a glucose polymer solution.<sup>31)</sup> In our study, the HBCD solution was transferred from the stomach to the small intestine faster than the glucose solution. This might have provided fast availability of the glucose supply in mice during exercise. On the other hand, the glucose polymer took longer to be absorbed in the small intestine than glucose itself. Since HBCD was hydrolyzed into glucose as an available energy source, glucose was supplied rapidly and continuously by HBCD ingestion. The available glucose was produced gradually from HBCD with hydrolysis by  $\alpha$ -amylase and  $\alpha$ -glucosidase in the small intestine, which might have contributed to maintaining the CHO supply during prolonged exercise by mice.

These results indicated that the improvement of endurance by HBCD depended on three factors: the fast gastric emptying rate, the gradual digestion in the small intestine and the low postprandial blood glucose response. When HBCD was ingested, it was transferred quickly from the stomach to the small intestine. There, HBCD is thought to have been gradually digested by  $\alpha$ -amylase and  $\alpha$ -glucosidase. This might have enabled the HBCD-fed mice to maintain a continuous supply of glucose as an energy substrate to the muscles and other tissues through the blood flow. Consequently, the endurance would have been improved.

In conclusion, HBCD enhanced the endurance of mice by the administration of 500 mg/kg of body wt. during exercise. This was the result of the supplied CHO maintaining the energy substrates' oxidation during prolonged exercise with lower insulin responsiveness that caused a slower and smaller rise in the postprandial blood glucose level after its ingestion. The most appropriate timing for ingestion when HBCD exhibited its best ergogenic effect was in the early stage of exercise (10 min after starting).

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