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Effects of high-intensity swimming training on GLUT-4 and glucose transport activity in rat skeletal muscle

SHIN TERADA,¹ TOSHIKO YOKOZEKI,² KENTARO KAWANAKA,⁴ KISHIKO OGAWA,² MITSURU HIGUCHI,² OSAMU EZAKI,³ AND IZUMI TABATA¹

¹Department of Physiology and Biomechanics, National Institute of Fitness and Sports, Kanoya, Kagoshima 891-2393; ²Division of Health Promotion and ³Clinical Nutrition, National Institute of Health and Nutrition, Shinjuku Tokyo, 162-8636 Japan; and ⁴Department of Medicine, Washington University School of Medicine, St. Louis, Missouri 63110

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Terada, Shin, Toshiko Yokozeki, Kentaro Kawanaka, Kishiko Ogawa, Mitsuru Higuchi, Osamu Ezaki, and Izumi Tabata. Effects of high-intensity swimming training on GLUT-4 and glucose transport activity in rat skeletal muscle. *J Appl Physiol* 90: 2019–2024, 2001.—This study was performed to assess the effects of short-term, extremely high-intensity intermittent exercise training on the GLUT-4 content of rat skeletal muscle. Three- to four-week-old male Sprague-Dawley rats with an initial body weight ranging from 45 to 55 g were used for this study. These rats were randomly assigned to an 8-day period of high-intensity intermittent exercise training (HIT), relatively high-intensity intermittent prolonged exercise training (RHT), or low-intensity prolonged exercise training (LIT). Age-matched sedentary rats were used as a control. In the HIT group, the rats repeated fourteen 20-s swimming bouts with a weight equivalent to 14, 15, and 16% of body weight for the first 2, the next 4, and the last 2 days, respectively. Between exercise bouts, a 10-s pause was allowed. RHT consisted of five 17-min swimming bouts with a 3-min rest between bouts. During the first bout, the rat swam without weight, whereas during the following four bouts, the rat was attached to a weight equivalent to 4 and 5% of its body weight for the first 5 days and the following 3 days, respectively. Rats in the LIT group swam 6 h/day for 8 days in two 3-h bouts separated by 45 min of rest. In the first experiment, the HIT, LIT, and control rats were compared. GLUT-4 content in the epitrochlearis muscle in the HIT and LIT groups after training was significantly higher than that in the control rats by 83 and 91%, respectively. Furthermore, glucose transport activity, stimulated maximally by both insulin (2 mU/ml) (HIT: 48%, LIT: 75%) and contractions (25 10-s tetani) (HIT: 55%, LIT: 69%), was higher in the training groups than in the control rats. However, no significant differences in GLUT-4 content or in maximal glucose transport activity in response to both insulin and contractions were observed between the two training groups. The second experiment demonstrated that GLUT-4 content after HIT did not differ from that after RHT (66% higher in trained rats than in control). In conclusion, the present investigation demonstrated that 8 days of HIT lasting only 280 s elevated both GLUT-4 content and maximal glucose transport activity in

rat skeletal muscle to a level similar to that attained after LIT, which has been considered a tool to increase GLUT-4 content maximally.

epitrochlearis muscle; high-intensity exercise; intermittent training

SKELETAL MUSCLE IS RESPONSIBLE for at least 80% of glucose uptake in humans (7), and, under most physiological conditions, glucose transport is the rate-limiting step in skeletal muscle glucose metabolism (32). Furthermore, maximal insulin- and contraction-stimulated glucose transport activity is reported to be linearly related to the content of the GLUT-4 isoform of the glucose transporter in muscle (17, 21). Therefore, the level of GLUT-4 in skeletal muscle may be an important determinant of whole body glucose disposal.

Previous studies in humans showed that low-intensity prolonged (exercise time >30 min) exercise training increased GLUT-4 content in specifically recruited skeletal muscle during exercise (5, 8, 9, 18, 26, 27, 28, 37, 38). Among these previous studies, types of exercise included running and cycle ergometer exercises at ~50–70% of maximal oxygen uptake ($\dot{V}O_{2\max}$), which is regarded as low to moderate intensity. Exercise training at this intensity has been used as a tool for preventing diabetes.

Recently, we found that high-intensity resistance training significantly increased GLUT-4 content in the muscle of humans exposed to 19 days of bed rest (44). The training consisted of thirty 3-s maximal voluntary isometric contractions for a net exercise time of only 90 s. This very short-lasting high-intensity exercise successfully increased GLUT-4 content in vastus lateralis muscle, whereas GLUT-4 content in the same muscle of the control subjects was decreased by 16%. This result led us to consider the possibility that GLUT-4 content in skeletal muscle may be elevated after training at an exercise intensity far higher than that used in the previous studies. Furthermore, previ-

Address for reprint requests and other correspondence: I. Tabata, Dept. of Physiology and Biomechanics, National Institute of Fitness and Sports, 1 Shiromizu-cho, Kanoya City, Kagoshima Prefecture, 891-2393 Japan (E-mail: tabata@nifs-k.ac.jp).

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ous animal studies have shown that not only low-intensity but also relatively high-intensity running exercise training increases GLUT-4 content in rat skeletal muscle in the hindlimb (1, 4). These studies had already raised the possibility that the higher the exercise intensity, the higher the GLUT-4 content after training. Although the exercise intensities in the previous studies were higher than that normally used for animal studies, intensity was still relatively low, e.g., 80% of the rat $\dot{V}O_{2\max}$ (1, 4).

Therefore, for the purpose of further studying the effects of exercise intensity on training-induced GLUT-4 expression, we undertook to observe the effects of extremely high-intensity swimming exercise training on GLUT-4 content in the rat forelimb muscle (epitrochlearis) and to compare its effects on GLUT-4 content with that observed after low-intensity prolonged exercise training, which has been used as a tool to increase GLUT-4. Consequently, we found that, after high-intensity intermittent training [14 20-s exercise bouts (total exercise time: only 4 min and 40 s) bearing a weight equivalent to 14% body wt], GLUT-4 content in the muscle was increased to a level comparable to that observed after low-intensity prolonged exercise training (total exercise time of 360 min), which has been regarded as the strongest stimulus for GLUT-4 expression in the rat epitrochlearis muscle (25).

MATERIALS AND METHODS

Materials. Purified human insulin was purchased from Eli Lilly (Indianapolis, IN). All other biochemicals were purchased from Sigma Chemical (St. Louis, MO).

Animal care. Three- to four-week-old male Sprague-Dawley rats (Crea Japan, Tokyo, Japan) with initial body weight ranging from 45 to 55 g were used for this study. All animals were housed in rooms lighted from 7:00 AM to 7:00 PM and maintained on an ad libitum diet of standard chow and water. Room temperature was maintained at 20–22°C.

Training protocol. This study consisted of two training experiments. In *experiment 1*, training rats were randomly assigned to an extremely high-intensity (HIT; $n = 16$) or low-intensity (LIT; $n = 15$) training group. Age-matched sedentary rats served as controls ($n = 16$). For all three groups of rats, GLUT-4 content and glucose transport activity in the epitrochlearis muscle were determined. *Experiment 2* was performed for the purpose of comparing GLUT-4 content after HIT ($n = 8$) with that after a relatively high-intensity training (RHT; $n = 8$), the latter of which had been used in a high-intensity exercise model in previous studies (1, 4). Age-matched sedentary rats served as the control ($n = 8$).

During HIT, rats repeated a 20-s swimming bout 8 and 10 times while bearing a weight equivalent to 14% of their body weight for the first 2 and the following 6 days, respectively. Between exercise bouts, a 10-s pause was allowed. After the 14th bout of this exercise, blood lactate concentration had increased up to 11.4 ± 2.7 mM ($n = 4$, mean \pm SD). RHT consisted of five repetitions of 17-min swimming bouts with 3 min of rest between bouts. During the first bout, the rat swam without weight, whereas during the following four bouts, the rat was tied to a weight of 4 and 5% of their body weight for the first 5 days and the following 3 days, respectively. LIT rats swam 6 h/day in two 3-h bouts separated by 45 min of rest. After the first 30-min bout, a weight equal to 2% of body weight was tied to the body of the rat. The rat

swam with the weight attached for the remaining three exercise bouts. All rats swam in a barrel filled to a depth of 50 cm and an average surface area of 190 cm²/rat. Water temperature was maintained at 35°C during swimming training. The rats performed the swimming training once a day from ~2:00 PM to 9:30 PM for 8 days.

Muscle preparation. On the last training day, all exercise was finished before 6:00 PM, and food was restricted to 8 g after 7:00 PM the night before the experiment. Between 11:00 AM and 1:00 PM the next day (17–19 h after the last bout of exercise; Ref. 2), rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body wt), and the epitrochlearis muscle was excised. The order of anesthetization and excision was randomized among the three groups (i.e., two training groups and one nonexercise control group). The suitability of the epitrochlearis muscle for in vitro incubation experiments has been demonstrated previously (22, 23, 36). Epitrochlearis muscles isolated from animals of this size weigh ~10–15 mg wet wt.

Insulin treatment. After dissection, muscles were allowed to recover for 30 min in oxygenated Krebs-Henseleit bicarbonate buffer (KHBB) containing 8 mM glucose and 32 mM mannitol. The muscles for measurement of insulin-stimulated maximal glucose transport activity were placed in 3 ml of oxygenated KHBB containing 8 mM sodium pyruvate, 24 mM mannitol, 0.1% radioimmunoassay-grade bovine serum albumin, and 2 mU/ml insulin. The gas phase in the flasks was 95% O₂-5% CO₂ at 35°C.

Electrical stimulation. After dissection, muscles for measurement of contraction-stimulated maximal glucose transport activity were incubated for 30 min in 25-ml stoppered Erlenmeyer flasks containing 3 ml of oxygenated KHBB with 8 mM glucose and 32 mM mannitol. For electrical stimulation, the distal end of the muscle was attached to a vertical Lucite rod containing two platinum electrodes. The proximal end was clipped to a jeweler's chain that was connected to an isometric force transducer (TB-653t, Nihon Kohden, Tokyo, Japan), and resting tension was adjusted to 5×10^{-3} N. The mounted muscle was immersed in 100 ml KHBB containing 32 mM mannitol and 8 mM glucose. The muscles were continuously oxygenated with 95% O₂-5% CO₂ at 35°C. For activation of glucose transport by tetanic contractions, the muscles were stimulated with supramaximal square-wave pulses of 0.2-ms duration with an electrical stimulator (SEN-7203, Nihon Kohden). Tetanic contractions were produced by stimulating the muscle at 100 Hz for 10 s. Tetanic contractions were repeated 25 times at a rate of 1 contraction/min.

Detection of GLUT-4 protein. Epitrochlearis muscles were homogenized with a glass homogenizer (Kontes, Vineland, NJ) in 9 ml of buffer containing 0.25 M sucrose, 1 mM EDTA, and 10 mM Tris·HCl, pH 7.5, at 4°C. The homogenate thus obtained was centrifuged at 175,000 *g* for 60 min at 4°C. This pellet (the membrane fraction) was suspended in 490 μ l of buffer containing 1 mM EDTA and 10 mM Tris·HCl, pH 7.5, at 4°C, and blended vigorously in a vortex mixer until the visible pellets had completely disappeared. Then this solution was solubilized by adding 10 μ l of 0.35 M (10% wt/vol) SDS, mixed well in a vortex mixer, and kept for 10 min at room temperature. This solution was transferred to a microcentrifuge tube and centrifuged at 10,000 *g* for 15 min to remove unsolubilized materials. These solubilized membranes were used for the assay for protein and GLUT-4 concentration. The solubilized membranes were incubated for 10 min at 37°C in a solution containing 2.5% SDS, 75 mM dithiothreitol, 1.7 M (12.5% vol/vol) glycerol, 361 mM (0.025% wt/vol) bromophenol blue, and 12.5 mM Tris·HCl, pH 7.0. SDS-PAGE was performed according to Laemmli (33) with a

0.56 M (4% wt/vol) stacking gel and a 1.4 M (10% wt/vol) resolving gel. Immunoblotting of electrophoresis gels was performed as described previously (12). Proteins in gels were electrophoretically transferred to polyvinylidene difluoride sheets in a transfer buffer. The sheets were incubated successively with antibodies to glucose transporters for 20 h, and with ^{125}I -labeled protein A for 24 h at 4°C. Autoradiography was performed with Kodak XAR film at -70°C for 4–12 h. To quantify the glucose transporters, we cut out pieces of sheet containing the GLUT-4 protein and counted the radioactivity in a γ -counter.

Measurement of glucose transport activity and glycogen concentration. Glucose transport activity was measured using the glucose analog 2-deoxyglucose (2-DG) and the procedure of Young et al. (47). After electrical stimulation or incubation with insulin, the muscles were transferred to flasks containing 3 ml of KHBB with 40 mM mannitol and then incubated with shaking for 10 min at 29°C to remove glucose. In the case of insulin stimulation, 2 mU/ml insulin were added to this 3 ml of KHBB. The muscles were then incubated for 20 min at 29°C in 2 ml of KHBB containing 1 mM 2-deoxy-[1,2- ^3H]glucose (1.5 mCi/mmol) and 39 mM [^{14}C]mannitol (8 $\mu\text{Ci}/\text{mmol}$; Moravek Biochemicals). The gas phase in the flasks during both the rinse and incubation periods was 95% O_2 -5% CO_2 . After incubation, the muscles were blotted briefly on filter paper dampened with incubation medium, trimmed, and frozen in liquid N_2 . The extracellular space and intracellular 2-DG concentrations were determined as described previously (47). The samples were weighed and then homogenized in 0.3 M perchloric acid and stored for later determination of glycogen concentration. Glycogen concentrations were determined by enzymatic methods according to Lowry and Passonneau (34) after acid hydrolysis. The remainder of the homogenate was centrifuged at 1,000 g. Aliquots of the muscle extracts and incubation media were placed in scintillation vials containing 4 ml of Aquasol-2 (DuPont-NEN) and counted in a liquid scintillation counter with channels preset for simultaneous ^3H and ^{14}C counting. The amount of each isotope present in the samples was determined, and this information was used to calculate the extracellular space and the intracellular concentration of 2-DG. The intracellular water content of the muscles was calculated by subtracting the measured extracellular space water from total muscle water. Glucose transport activity is expressed as micromoles 2-DG per milliliter of intracellular water per 20 min.

Measurement of citrate synthase activity. As a marker of oxidative enzymes, citrate synthase activity was measured (42) in the epitrochlearis muscle, which was also used for the GLUT-4 assay.

Statistics. All values are expressed as means \pm SD. Statistical comparisons among the three groups were made by one-way ANOVA (Jandel Sigma Stat). Statistical significance was defined as $P < 0.05$.

Table 2. GLUT-4 content of control, HIT, and LIT rat in experiments 1 and 2

	GLUT-4 Content, cpm/ μg membrane protein		
	Control	HIT	LIT
Experiment 1	36.0 \pm 8.8	66.0 \pm 7.2 \ddagger	68.6 \pm 19.2 \ddagger
Experiment 2	25.0 \pm 4.1	42.5 \pm 13.0 \dagger	41.5 \pm 8.3*

Values are means \pm SD; $n = 8$ rats per group. *, \dagger , and \ddagger Significant differences from the value obtained in their respective control group at levels of $P < 0.05$, 0.01, and 0.001, respectively.

RESULTS

Effect of training on body weight of rats. In experiment 1, body weight of the LIT rats was significantly lower than that of the HIT and that of the control group rats (Table 1). However, body weight of the HIT rats did not differ from that of control rats.

When body weight was compared among the three groups in experiment 2, that of RHT rats was significantly lower than that of the control and that of the HIT rats (Table 1). No difference in body weight was observed between the control and HIT groups.

Effect of exercise training on GLUT-4 content in epitrochlearis muscle. In experiment 1, GLUT-4 content in the epitrochlearis muscle of the HIT and LIT rats was significantly higher than that in the same muscle of control rats (Table 2). However, no significant difference in GLUT-4 content in the muscle was observed between the HIT (83% higher than that of the control rats) and the LIT (91% higher than that of the control rats) training groups.

In experiment 2, GLUT-4 content after HIT and RHT training was higher than that observed in control rats by 77 and 66%, respectively (Table 2). However, GLUT-4 content after RHT did not differ from that after HIT.

Effect of exercise training on maximal insulin-stimulated glucose transport activity in the epitrochlearis muscle. Glucose transport activity stimulated by the maximal dose of insulin (2.0 mU/ml) in the epitrochlearis muscle of the HIT and LIT groups of experiment 1 was higher than that in the same muscle of the control rats by 48 and 75%, respectively (Table 3). In this condition, glucose transport activity in the HIT rats did not differ significantly from that in the LIT rats. Glycogen content in the same muscle of the HIT and LIT rats was higher than that in the same muscle of the

Table 1. Body weight of control, HIT, and LIT rats in experiments 1 and 2

	Body Weight, g		
	Control	HIT	LIT
Experiment 1	128 \pm 11 ($n = 16$)	121 \pm 12 ($n = 16$)	110 \pm 10 ($n = 15$) \ddagger
Experiment 2	118 \pm 5 ($n = 8$)	114 \pm 4 ($n = 8$)	109 \pm 6 ($n = 8$)* \S

Values are means \pm SD; n , no. of rats. HIT, extremely high-intensity training; LIT, low-intensity training; RHT, relatively high-intensity training. * and \dagger Significant differences from the value obtained in their respective control group at levels of $P < 0.01$ and 0.001, respectively; \ddagger significant difference between the HIT and LIT groups at $P < 0.05$; \S significant difference between the HIT and RHT groups at $P < 0.05$.

Table 3. 2-Deoxyglucose transport activity stimulated maximally by insulin and contraction and glycogen concentration after measurement of glucose transport activity in epitrochlearis muscle of control rats and rats in the two training groups of experiment 1

	Control	HIT	LIT
2-Deoxyglucose transport activity, $\mu\text{mol}\cdot\text{ml}^{-1}\cdot 20\text{ min}^{-1}$			
2.0 mU/ml Insulin	1.42 \pm 0.20	2.10 \pm 0.52*	2.49 \pm 0.63†
25 10-s Tetani	1.35 \pm 0.28	2.09 \pm 0.57*	2.28 \pm 0.55†
Glycogen concentration, $\mu\text{mol/g}$			
2.0 mU/ml Insulin	21.3 \pm 4.1	34.4 \pm 4.0†	40.6 \pm 12.5†
25 10-s Tetani	8.4 \pm 3.7	13.3 \pm 7.2	13.9 \pm 3.9

Values are means \pm SD; $n = 8$ rats per group. * and † Significant differences from the value obtained in their respective control group at levels of $P < 0.01$ and 0.001 , respectively.

control rats (Table 3). No difference was observed between the two training groups.

Effect of exercise training on contraction-stimulated maximal glucose transport activity in the epitrochlearis muscle. Glucose transport activity in the epitrochlearis muscle of the HIT rats of *experiment 1* (48% higher than that in control rats) did not differ from that in the same muscle of the LIT rats (75% higher than that in control rats; Table 3). Glucose transport activity in the control group rats was not stimulated by 25 tetani to the level observed in the rats in the two training groups. After measurement of glucose transport activity stimulated by 25 tetani, glycogen content in epitrochlearis muscle was determined, and no differences in glycogen content were observed among the three groups (Table 3).

Effect of training on citrate synthase activity in the epitrochlearis muscle. Citrate synthase activity in the epitrochlearis muscle of the HIT and LIT groups of rats in *experiment 1* was significantly higher than that in the same muscle of the control rats by 47 and 33%, respectively (Table 4). No significant difference in citrate synthase activity was observed between the HIT and LIT groups.

In *experiment 2*, citrate synthase activity in rat epitrochlearis muscle after HIT and RHT training was significantly higher than that in control group rats by 40 and 45%, respectively (Table 4). No significant difference in citrate synthase activity was observed between the two training groups.

Table 4. Citrate synthase activity in epitrochlearis muscle of control, HIT, and LIT rats in experiments 1 and 2

	Citrate Synthase Activity, $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$		
<i>Experiment 1</i>	Control	HIT	LIT
	14.8 \pm 3.6 ($n = 8$)	21.7 \pm 3.9 ($n = 8$)†	19.7 \pm 2.5 ($n = 7$)*
<i>Experiment 2</i>	Control	HIT	RHT
	15.7 \pm 2.2 ($n = 7$)	22.0 \pm 2.8 ($n = 6$)*	22.7 \pm 5.2 ($n = 7$)†

Values are means \pm SD; n , no. of rats. * and † Significant differences from the value obtained in their respective control group at levels of $P < 0.05$ and 0.01 , respectively.

DISCUSSION

This study demonstrated, for the first time, that 8 days of short-lasting (only 280 s), extremely high-intensity intermittent exercise training induces massive expression of GLUT-4 in rat skeletal muscle to a level comparable to that observed after low-intensity prolonged (6 h) exercise training, which has heretofore been regarded as the highest stimulus for exercise training-induced GLUT-4 expression.

Insulin- and contraction-stimulated glucose transport activity is linearly related to the content of GLUT-4 in muscle (17, 21). Moreover, exercise training increases GLUT-4 content and improves insulin-stimulated glucose uptake (13, 30, 46). Therefore, the physiological mechanism explaining the enhanced GLUT-4 expression after physical training is of great interest. From a physiological point of view, the overall effect of a specific type of training depends on exercise intensity and exercise time. Because maximal exercise time is dependent on exercise intensity, we compared changes in GLUT-4 after training of different intensities, during which the training exercises were continued to exhaustion. As a model for low-intensity training, we adopted a regimen of 6-h low-intensity prolonged swimming (LIT training), which has been considered to induce the maximal effect on GLUT-4 content in rat epitrochlearis muscle (25). After 2 days of this training, GLUT-4 content in the epitrochlearis muscle was shown to be elevated by $\sim 100\%$ (25). For comparison, as a model of high-intensity training for rats, we used a high-intensity intermittent swimming training protocol (HIT training) that was originally developed for elite sportsmen (43). This extremely high-intensity exercise training induced massive effects on GLUT-4 content in rat epitrochlearis muscle. As a result, no difference in GLUT-4 content in rat skeletal muscle was observed between the groups that underwent HIT training and the group that underwent LIT training. Therefore, the present study demonstrated that high-intensity intermittent exercise training induces GLUT-4 expression at a level equal to that induced by the low-intensity training that has previously been regarded as maximal stimuli in terms of physical training (25). Furthermore, we compared GLUT-4 content after HIT training to that observed after RHT training, which was developed as a high-intensity (80% of the rat's $\dot{V}O_{2\text{max}}$) training model in previous studies (1, 4). As shown in Table 2, GLUT-4 content after HIT train-

ing did not differ from that observed after RHT training. These results suggest that, no matter how high the exercise intensity is, GLUT-4 content increases to the same level if the stimuli induced by different training protocols are satisfactorily saturated.

The possible mechanisms related to this phenomenon of exercise training-induced changes in stimuli, which are known to induce GLUT-4 expression, are herein investigated and discussed. GLUT-4 expression in skeletal muscle is known to be stimulated by several physiological factors, including, for example, glucocorticoids (11, 45), growth hormone (6), and so forth. Among these stimuli that induce GLUT-4 expression in muscle, two candidates specific to exercise training-induced GLUT-4 expression have been postulated: neurotrophic factor (15, 35) and AMP kinase (24). We speculate that a large amount of neurotrophic factor was released to the epitrochlearis muscle during the LIT training, although the rate of secretion of neurotrophic factor is far lower than that during the HIT training exercise. We also speculate that, during the HIT training, a fairly large amount of the factor might have been delivered to the muscle in proportion to exercise intensity. It is conceivable that the amount of neurotrophic factor after either of the two types of training was large enough to induce maximal expression of GLUT-4 in rat skeletal muscle.

With regard to AMP kinase, given that Hutber et al. (29) reported that AMP kinase activity during exercise increases gradually, it is possible that, during LIT training in our study, AMP kinase activity increased to a higher level, at which the maximal amount of GLUT-4 expression was induced. On the other hand, given that Rasmussen et al. (39) demonstrated that AMP kinase activity is exercise intensity dependent, it is possible that, during the HIT training, a maximal activation of the kinase was achieved within a few minutes. In any case, we postulate that, relative to regulation of GLUT-4 content after the respective type of training, AMP kinase activity during either type of training may be increased to a level sufficiently high to induce maximal expression of GLUT-4.

Glucose transport in skeletal muscle is stimulated via at least two distinct pathways (19, 20, 23). One pathway is activated by insulin and the other by contractions and exercise or hypoxia. This is evidenced by findings that the maximal effects of insulin and contractions or hypoxia on sugar transport (3, 19) and GLUT-4 translocation to the cell surface (16) are additive. The results of previous studies have suggested that there are two separate pools of GLUT-4 in skeletal muscle (10, 23). Given that maximal glucose transport activity stimulated by tetani and insulin was augmented after the HIT training, the HIT training may have increased both exercise- and contraction- and insulin-responsive GLUT-4 pools in the muscle.

Glycogen content has been suggested to influence glucose transport activity (14, 25, 30, 31, 40, 41). Given that muscle glycogen content is known to increase after training, it is necessary to reduce muscle glycogen content to the same level for the purpose of comparing

the glucose transport activity between training rats and control rats. Because we contracted the muscle tetanically 25 times, muscle glycogen content did not differ among the three groups (i.e., the two training groups and control). Therefore, we consider that, among the three groups, glycogen content exhibited no effect on glucose transport activity stimulated by contractions, and the increased glucose transport activity is a reflection of enhanced expression of GLUT-4 after the two types of training.

As stated previously, the HIT protocol (8–10 exhausting bouts of 20-s high-intensity exercise with 10 s rest between bouts) adopted in this study was originally developed for elite athletes (43). This training induces a very fast increase in $\dot{V}O_{2\max}$ in humans (43). In the present investigation, $\dot{V}O_{2\max}$ was not measured for the rats undergoing this type of training. However, because the observed improvement in citrate synthase activity in HIT rats was comparable to that observed in LIT rats (Table 4), mitochondria development and aerobic energy release seems to have also been significantly improved by the HIT training.

In conclusion, the present investigation demonstrated that high-intensity, intermittent swimming training, lasting only 4 min and 40 s, elevated both GLUT-4 content and maximal glucose transport activity in rat skeletal muscle to a level similar to that attained after 6 h of low-intensity exercise training.

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