Effect of High-Intensity Intermittent Swimming Training on Fatty Acid Oxidation Enzyme Activity in Rat Skeletal Muscle

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Abstract: We previously reported that high-intensity exercise training significantly increased citrate synthase (CS) activity, a marker of oxidative enzyme, in rat skeletal muscle to a level equaling that attained after low-intensity prolonged exercise training (Terada *et al.*, J Appl Physiol 90: 2019–2024, 2001). Since mitochondrial oxidative enzymes and fatty acid oxidation (FAO) enzymes are often increased simultaneously, we assessed the effect of high-intensity intermittent swimming training on FAO enzyme activity in rat skeletal muscle. Male Sprague-Dawley rats (3 to 4 weeks old) were assigned to a 10-day period of high-intensity intermittent exercise training (HIT), low-intensity prolonged exercise training (LIT), or sedentary control conditions. In the HIT group, the rats repeated fourteen 20 s swimming sessions with a weight equivalent to 14–16% of their body weight. Between the exer-

cise sessions, a 10 s pause was allowed. Rats in the LIT group swam 6 h/day in two 3 h sessions separated by 45 min of rest. CS activity in the triceps muscle of rats in the HIT and LIT groups was significantly higher than that in the control rats by 36 and 39%, respectively. Furthermore, 3- β hydroxyacyl-CoA dehydrogenase (HAD) activity, an important enzyme in the FAO pathway in skeletal muscle, was higher in the two training groups than in the control rats (HIT: 100%, LIT: 88%). No significant difference in HAD activity was observed between the two training groups. In conclusion, the present investigation demonstrated that high-intensity intermittent swimming training elevated FAO enzyme activity in rat skeletal muscle to a level similar to that attained after 6 h of low-intensity prolonged swimming exercise training. [The Japanese Journal of Physiology 54: 47–52, 2004]

Key words: triceps muscle, 3- β hydroxyacyl-CoA dehydrogenase, hexokinase, citrate synthase

Repeated exercise sessions, defined as physical exercise training, increase mitochondrial enzyme activities in skeletal muscle recruited during exercise [1–6]. Among previous studies, the types of exercise included running and cycle ergometer exercises at ~50–70% of maximal oxygen uptake ($\dot{V}O_{2\max}$) for more than 30 min, which is regarded as low to moderate intensity [4, 7–9].

With respect to high-intensity exercise training, Tabata *et al.* [10] demonstrated that exhaustive intermittent training, which consisted of seven to eight sets

of 20 s exercise at an intensity of about 170% of $\dot{V}O_{2\max}$ with a 10 s rest between sessions, improved $\dot{V}O_{2\max}$. Furthermore, we recently found that 8 d of high-intensity intermittent swimming training (fourteen 20 s exercise sessions [total exercise time 4 min and, 40 s]) bearing a weight equivalent to 14–16% of body weight increased the GLUT-4 isoform of the glucose transporter and citrate synthase (CS) activity, a marker of oxidative enzyme, in rat forelimb muscle to a level comparable to that observed after low-intensity prolonged exercise training (total exercise time

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360 min) [11]. Since low-intensity prolonged exercise training is well known to increase skeletal muscle mitochondrial enzymes involved in both tricarboxylic acid cycle (TCA) activity and fatty acid β -oxidation simultaneously [5], these results led us to consider the possibility that extremely high-intensity intermittent swimming training might also elevate the activities of enzymes involved in fatty acid oxidation. 3- β hydroxyacyl-CoA dehydrogenase (HAD) plays an important role in the fatty acid β -oxidation pathway in skeletal muscle [4], and its activity is significantly correlated with muscle fatty acid oxidative capacity [12]. In the present investigation, we therefore assessed the effects of extremely high-intensity intermittent swimming exercise training on HAD activity in rat skeletal muscle with the aim of comparing its effect on this enzyme to that observed after low-intensity prolonged exercise training. We found that after 10 d of short-lasting (only 4 min and 40 s) high-intensity intermittent swimming exercise training, HAD activity in the skeletal muscle of rats was increased to a level comparable to that observed after 6-h of low-intensity prolonged exercise training.

MATERIALS AND METHODS

Materials. All chemicals were purchased from Sigma Chemical (St. Louis, MO, USA).

Animal care. Three- to 4-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 A.M. to 7:00 P.M. and maintained on an ad libitum diet of standard chow and water. The room temperature was maintained at 20 to 22°C.

Training protocol. The rats were randomly assigned to a high-intensity intermittent training group (HIT; $n=8$) or a low-intensity prolonged training group (LIT; $n=8$). Age-matched sedentary rats served as controls ($n=8$).

During HIT, the rats repeated a 20 s swimming session 14 times while bearing a weight equivalent to 14, 15, and 16% of their body weight for the first 4, the next 4, and the last 2 d, respectively [11]. A 10 s pause was allowed between exercise sessions. Each rat performed the swimming exercise alone in a barrel filled to a depth of 50 cm. The water temperature was maintained at 35°C during the swimming exercise. This high-intensity exercise was shown to increase blood lactate concentration to approximately 11–12 mM [11]. LIT rats swam without a load for 6 h in two 3 h sessions separated by 45 min of rest [11, 13]. Five days of training involving this low-intensity swim-

ming exercise has been shown to induce substantial increases in δ -aminolevulinate (ALA) synthase, citrate synthase, cytochrome oxidase, subunit I, cytochrome c, and GLUT-4 protein concentrations, suggesting that this exercise is a potent stimulus for mitochondrial biogenesis [14]. Eight rats swam simultaneously in a barrel filled to a depth of 50 cm and with an average surface area of 190 cm²/rat. The water temperature was maintained at 35°C during the swimming exercise. The rats performed the swimming training once a day from ~11:00 A.M. to 6:00 P.M. for 10 d.

On the last training day, all exercises were finished before 6:00 P.M. Between 10:00 A.M. and 12:00 P.M. the next day (16–18 h after the last exercise), the rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg body weight), and the heart, epididymal adipose tissue, and triceps muscle were quickly excised. The heart and epididymal adipose tissue were weighed. The triceps muscle was clamp-frozen in liquid nitrogen and stored at –80°C until analysis. The rat triceps muscle was used for the present investigation because it was used extensively during the swimming exercises, as evidenced by glycogen depletion and an adaptive increase in GLUT-4 content and CS activity [7].

All procedures were conducted in accordance with the guidelines of the National Institute of Health and Nutrition for the care and use of laboratory animals.

Measurements of citrate synthase, 3- β hydroxyacyl-CoA dehydrogenase, and hexokinase activities in skeletal muscle. For enzyme activity measurements, 10% homogenates were made from the triceps muscles in 175 mM KCl, 10 mM GSH, and 2 mM EDTA, pH 7.4. This homogenate was frozen and thawed four times and mixed thoroughly before the enzyme activities were measured. For the HAD assay, an aliquot of the homogenate was centrifuged at 700 $\times$ g for 10 min at 4°C. The remainders of the assay were performed on aliquots and dilutions of the mixed whole homogenate. As a marker of oxidative enzyme, the CS activity in the triceps muscle was measured by using Srere's method [15]. The HAD and hexokinase (HK) activities in the triceps muscles were also assayed by Bass [16] and Uyeda's [17] methods, respectively.

Statistics. The data are presented as the mean $\pm$ SD. A statistical analysis was performed by using a one-way analysis of variance (ANOVA) (Jandel Sigma Stat). Whenever the ANOVA indicated significant differences, the Tukey test was used for post hoc analysis. The statistical significance was defined as $p<0.05$.

RESULTS

Effects of two different intensity levels of swimming training on the body, heart, and epididymal adipose tissue weights of rats

The body weight of the LIT rats was significantly lower than that of the HIT rats and the control group rats ($p<0.001$) (Table 1). However, the body weight of the HIT rats did not differ from that of the control rats.

Heart weight, expressed relative to body weight, was significantly higher in the HIT group rats compared with that in the LIT and control group rats ($p<0.01$) (Table 1). No significant difference in the relative heart weight was observed between rats in the LIT and control groups.

When the weight of epididymal adipose tissue was expressed relative to body weight, the adipose tissue weight of the LIT group rats was significantly lower than that of the control rats ($p<0.05$) (Table 1). The relative weight of the epididymal adipose tissue of HIT group rats was not significantly different from that of the LIT group rats or the control group rats.

Effects of two different intensity levels of swimming training on enzyme activities in rat triceps muscle

CS activities in the triceps muscle of the HIT and LIT rats were significantly higher than in the same muscle of the control rats by 36 and 39%, respectively ($p<0.001$) (Fig. 1). No significant difference in CS activity was observed between rats in the HIT and LIT groups. These results were consistent with those of our previous experiment [11].

Table 1. Effects of two different intensity levels of swimming training on the body, heart, and epididymal adipose tissue weights of rats.

	Control ($n=8$)	HIT ($n=8$)	LIT ($n=8$)
Body weight (g)	143 ± 7	137 ± 8	$121 \pm 5^{***}$
Heart weight ($\text{mg} \cdot \text{g}^{-1}$ Bw)	4.7 ± 0.2	$5.2 \pm 0.5^{\ddagger}$	4.7 ± 0.3
Epididymal adipose tissue weight ($\text{mg} \cdot \text{g}^{-1}$ Bw)	5.8 ± 1.0	5.5 ± 0.5	$5.0 \pm 0.5^{\#}$

Values are means $\pm$ SD; n , number of rats; HIT, high-intensity intermittent training; LIT, low-intensity prolonged training; Bw, body weight. *** Significant difference from HIT and Control groups at a level of $p<0.001$; ‡ significant difference from Control and LIT groups at a level of $p<0.01$; $^{\#}$ significant difference from Control group at a level of $p<0.05$.

After HIT and LIT, the HAD activities in rat triceps muscle were significantly higher than in control group rats by 100 and 88%, respectively ($p<0.001$) (Fig. 2). There was no significant difference in HAD activity between rats in the HIT and LIT groups.

HIT and LIT induced 61 and 45% increases in HK activity in triceps muscle, compared to the activity in control rats, respectively ($p<0.001$) (Fig. 3). No significant difference in HK activity was observed be-

Fig. 1. Effects of two different intensity levels of swimming training on citrate synthase activity in rat triceps muscle. Values are means $\pm$ SD. *** Indicates a significant difference at a level of $p<0.001$. HIT, high-intensity intermittent training; LIT, low-intensity prolonged training.

Fig. 2. Effects of two different intensity levels of swimming training on 3-β hydroxyacyl CoA dehydrogenase activity in rat triceps muscle. Values are means $\pm$ SD. *** Indicates a significant difference at a level of $p<0.001$. HIT, high-intensity intermittent training; LIT, low-intensity prolonged training.

Fig. 3. Effects of two different intensity levels of swimming training on hexokinase activity in rat triceps muscle. Values are means $\pm$ SD. *** Indicates a significant difference at a level of $p<0.001$. HIT, high-intensity intermittent training; LIT, low-intensity prolonged training.

tween rats in the HIT and LIT groups.

DISCUSSION

The present investigation demonstrated that 10 d of short-lasting (only 280 s), extremely high-intensity intermittent exercise training induces an increase in HAD activity in rat skeletal muscle to a level comparable to that observed after low-intensity prolonged (6 h) exercise training.

It has been generally believed that fatty acid oxidation enzyme activity increases in response to low- to moderate-intensity prolonged exercise training, during which lipid fuel sources are important energy substrates for skeletal muscle. But there has been a study showing that not only low-intensity, but also high-intensity interval exercise training increases fatty acid oxidation capacity in rat skeletal muscle [18]. This finding has already raised the possibility that high-intensity interval training could be beneficial for elevating the capacity for fatty acid oxidation in skeletal muscle. Although the exercise intensity in the previous study was higher than that normally used for training study, the intensity was still relatively low (near or at $\dot{V}O_{2\max}$ level), and the exercise duration (16–30 min/d) was relatively longer [18]. To our knowledge, this is the first study demonstrating that extremely high-intensity intermittent exercise training lasting only 280 s (20 s $\times$ 14 sessions) elevated mitochondrial fatty acid oxidation enzyme activity as well as oxidative enzymes in rat skeletal muscle.

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 enzyme activities after the 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), which has been considered to induce the maximal effect on GLUT-4 content and HK activity in rat forelimb muscles [13]. After 2 d of this training, GLUT-4 content and HK activity in the epitrochlearis muscle were shown to be elevated by $\sim 100\%$. For comparison, as a model of high-intensity training for rats we used a high-intensity intermittent swimming training protocol (HIT) that was originally developed for elite sportsmen [10]. In the present investigation, the HIT induced a massive increase in HAD activity in rat triceps muscle (Fig. 2). As a result, no significant difference in HAD activity was observed between the groups that underwent HIT and the group that underwent LIT. There-

fore the present study demonstrated that the HIT induced an elevation of HAD activity to a level similar to that induced by the LIT, which was previously regarded as maximal stimuli in terms of physical training induced HAD elevation. These results also suggest that no matter how high the exercise intensity is, HAD activity increases to the same level if the stimuli induced by different training protocols are satisfactorily saturated.

Mitochondrial enzyme activities in skeletal muscle can be stimulated by several physiological factors, including, for example, the presence of thyroid hormone [19] and the presence of sex hormone [20]. Among these stimuli that elevate mitochondrial enzyme activity, two candidates specific to exercise training induced increase in mitochondrial enzyme activity have been postulated: Ca^{2+} [21–23] and 5' AMP-activated protein kinase (AMPK) [24]. Ojuka *et al.* [23] hypothesized that the adaptive increase in muscle mitochondria induced by exercise training is mediated by both the increase in cytosolic Ca^{2+} and the decrease in high-energy phosphates ($\sim P$), resulting in the activation of AMPK. If this hypothesis is correct, it seems reasonable that during the low-intensity prolonged exercise that results in modest perturbations in $\sim P$ concentrations and minimal activation of AMPK, the primary adaptive stimulus is the increase in cytosolic Ca^{2+} . On the other hand, by substantially activating both Ca^{2+} and AMPK pathways within a few minutes, the high-intensity exercise would result in a more powerful stimulus to mitochondrial biogenesis.

In the present investigation, we assessed the effects of 10 d of high-intensity intermittent and low-intensity prolonged swimming training on mitochondrial enzyme activities in rat skeletal muscle. Neuffer and Dohm [25] found a significantly elevated gene transcription for CS 3 h after acute exercise that was still present 24 h after the exercise. Further, Ren *et al.* [13] reported that 2 d of 6-h low-intensity prolonged swimming exercise could induce an increase in HK and CS activity in rat epitrochlearis muscle by ~ 100 and $\sim 40\%$, respectively. These findings provide the possibility that only a single session period of high-intensity intermittent and low-intensity prolonged swimming exercise is able to enhance gene expression for HAD and thereby increase HAD activity in rat skeletal muscle. Since triceps muscles were dissected out 16–18 h after the last exercise, enhanced enzyme activities observed in HIT and LIT group rats may reflect chronic and acute effects of the exercises. Extensive investigations are expected to examine the effects of a single period of high- and low-intensity exercise on mitochondrial enzyme activities in skeletal muscle.

In the present investigation, HIT substantially elevated HAD activity in skeletal muscle with no effect on epididymal adipose tissue weight (Table 1). This may be partially explained by the lower total energy expenditure during HIT, compared with LIT. Adipose tissue metabolism is under both hormonal and sympathetic nervous regulations, and the rate-limiting step in adipose tissue lipolysis is catalyzed by the enzyme hormone-sensitive lipase (HSL) activity. Enevoldsen *et al.* [26] demonstrated that 6 h of low-intensity swimming training for 5 week increased the amount of HSL and the sensitivity of HSL to stimulation by adrenaline in intra-abdominal adipose tissue, suggesting that this adaptation contributes to enhanced fat utilization in trained individuals. It might be possible that HIT had little effect on HSL activity in intra-abdominal adipose tissue, though we could not measure the HSL activity in the present investigation. Future extensive studies are expected to address this issue.

It is well documented that concurrent with an increase in mitochondrial enzyme activities, an increase in muscle HK activity is also seen in response to endurance exercise training [27, 28]. In the triceps muscle of HIT rats, it was significantly elevated to a level similar to that obtained in LIT rats (Fig. 3). We previously reported that this HIT increased GLUT-4 content in rat skeletal muscle to a level comparable to that observed after LIT [11]. On the basis of the finding in the previous study and the result in the present study, we conclude that HIT seems to be an effective training program to increase the capacities of muscle both to take up and to phosphorylate glucose.

In conclusion, the present investigation demonstrated that 10 d of high-intensity intermittent swimming training, lasting only 4 min and 40 s, elevated fatty acid oxidation enzyme activity in rat skeletal muscle to a level similar to that attained after 6 h of low-intensity exercise training.

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REFERENCES

1. Essig DA: Contractile activity-induced mitochondrial biogenesis in skeletal muscle. *Exerc Sport Sci Rev* 24: 289–319, 1996
2. Hood DA: Plasticity in skeletal muscle, cardiac, and smooth muscle invited review: Contractile activity-induced mitochondrial biogenesis in skeletal muscle. *J Appl Physiol* 90: 1137–1157, 2001
3. Hood DA: Assembly of the cellular powerhouse: Current issues in mitochondrial biogenesis. *Exerc Sport Sci Rev* 28: 68–73, 2001
4. Holloszy JO: Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. *J Biol Chem* 242: 2278–2282, 1967
5. Holloszy JO and Booth FW: Biochemical adaptations to endurance exercise in muscle. *Annu Rev Physiol* 38: 273–291, 1976
6. Holloszy JO and Coyle EF: Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J Appl Physiol* 56: 831–838, 1984
7. Host HH, Hansen PA, Nolte LA, Chen MM, and Holloszy JO: Rapid reversal of adaptive increases in muscle GLUT-4 and glucose transport capacity after training cessation. *J Appl Physiol* 84: 798–802, 1998
8. Kirwan JP, Costill DL, Flynn MG, Neufer PD, Fink WJ, and Morse WM: Effects of increased training volume on the oxidative capacity, glycogen content and tension development of rat skeletal muscle. *Int J Sports Med* 11: 479–483, 1990
9. Spina RJ, Chi MM, Hopkins MG, Nemeth PM, Lowry OH, and Holloszy JO: Mitochondrial enzymes increase in muscle in response to 7–10 days of cycle exercise. *J Appl Physiol* 80: 2250–2254, 1996
10. Tabata I, Nishimura K, Kouzaki M, Hirai Y, Ogita F, Miyachi M, and Yamamoto K: Effects of moderate-intensity endurance and high-intensity intermittent training on anaerobic capacity and $\dot{V}O_{2\max}$. *Med Sci Sports Exerc* 28: 1327–1330, 1996
11. Terada S, Yokozeki T, Kawanaka K, Ogawa K, Higuchi M, Ezaki O, and Tabata I: Effects of high-intensity swimming training on GLUT-4 and glucose transport activity in rat skeletal muscle. *J Appl Physiol* 90: 2019–2024, 2001
12. Morio B, Hocquette JF, Montaurier C, Boirie Y, Bouteloup-Demange C, McCormack C, Fellmann N, Beaufriere B, and Ritz P: Muscle fatty acid oxidative capacity is a determinant of whole body fat oxidation in elderly people. *Am J Physiol Endocrinol Metab* 280: E143–E149, 2001
13. Ren JM, Semenkovich CF, Gulve EA, Gao J, and Holloszy JO: Exercise induces rapid increases in GLUT4 expression, glucose transport capacity, and insulin-stimulated glycogen storage in muscle. *J Biol Chem* 269: 14396–14401, 1994
14. Baar K, Wende AR, Jones TE, Marison M, Nolte LA, Chen M, Kelly DP, and Holloszy JO: Adaptations of skeletal muscle to exercise: rapid increase in the transcriptional coactivator PGC-1. *FASEB J* 16: 1879–1886, 2002
15. Srere PA: Citrate synthase. *Methos Enzymol* 13: 3–5, 1969
16. Bass A, Brdiczka D, Eyer P, Hofer S, and Pette D: Metabolic differentiation of distinct muscle types at the level of enzymatic organization. *Eur J Biochem* 10: 198–206, 1968
17. Uyeda K and Racker E: Regulatory mechanisms in carbohydrate metabolism. IV. Hexokinase and phospho-

- fructokinase. *J Biol Chem* 240: 4682–4688, 1965
18. Chilibeck PD, Bell GJ, Farrar RP, and Martin TP: Higher mitochondrial fatty acid oxidation following intermittent versus continuous endurance exercise training. *Can J Physiol Pharmacol* 76: 891–894, 1998
19. Winder WW, Baldwin KM, Terjung RL, and Holloszy JO: Effects of thyroid hormone administration on skeletal muscle mitochondria. *Am J Physiol* 228: 1341–1345, 1975
20. Campbell SE and Febbraio MA: Effect of ovarian hormones on mitochondrial enzyme activity in the fat oxidation pathway of skeletal muscle. *Am J Physiol Endocrinol Metab* 281: E803–E808, 2001
21. Freyssenet D, Di Carlo M, and Hood DA: Calcium-dependent regulation of cytochrome c gene expression in skeletal muscle cells. Identification of a protein kinase c-dependent pathway. *J Biol Chem* 274: 9305–9311, 1999
22. Lawrence JC Jr and Salsgiver WJ: Levels of enzymes of energy metabolism are controlled by activity of cultured rat myotubes. *Am J Physiol* 244: C348–C355, 1983
23. Ojuka EO, Jones TE, Han DH, Chen M, and Holloszy JO: Raising Ca^{2+} in L6 myotubes mimics effects of exercise on mitochondrial biogenesis in muscle. *FASEB J* 17: 675–681, 2003
24. Winder WW, Holmes BF, Rubink DS, Jensen EB, Chen M, and Holloszy JO: Activation of AMP-activated protein kinase increases mitochondrial enzymes in skeletal muscle. *J Appl Physiol* 88: 2219–2226, 2000
25. Neufer PD and Dohm GL: Exercise induces a transient increase in transcription of the GLUT-4 gene in skeletal muscle. *Am J Physiol* 265: C1597–C1603, 1993
26. Enevoldsen LH, Stallknecht B, Langfort J, Petersen LN, Holm C, Ploug T, and Galbo H: The effect of exercise training on hormone-sensitive lipase in rat intra-abdominal adipose tissue and muscle. *J Physiol (Lond)* 536: 871–877, 2001
27. Holloszy JO and Hansen PA: Regulation of glucose transport into skeletal muscle. *Rev Physiol Biochem Pharmacol* 128: 99–193, 1996
28. Ivy JL: Role of exercise training in the prevention and treatment of insulin resistance and non-insulin-dependent diabetes mellitus. *Sports Med* 24: 321–336, 1997