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Effect of acute high-intensity intermittent swimming on post-exercise insulin responsiveness in epitrochlearis muscle of fed rats

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Abstract

Maximally insulin-stimulated glucose uptake in skeletal muscle, ie, insulin responsiveness, is reduced in fed animals as compared with fasted animals; but acute prior endurance exercise improves insulin responsiveness in the muscles of fed rats. The effect of acute prior sprint interval exercise on insulin responsiveness in the muscles of fed animals has not been clarified, and we therefore compared the effect of short high-intensity swimming as a model of sprint interval exercise on insulin responsiveness in the muscles of fed rats with the effect of prolonged low-intensity swimming as a model of endurance exercise. The fed rats were subjected to an acute bout of high-intensity intermittent swimming (HIS) or low-intensity continuous swimming (LIS). The HIS rats swam for eight 20-second periods with a weight equal to 18% of their body weight. The LIS rats swam with no load for 3 hours. HIS increased ($P < .05$) the phosphorylation of adenosine monophosphate–activated protein kinase (AMPK) Thr^{172} and that of its downstream target acetyl-CoA carboxylase (ACC) Ser^{79} 12.6- and 3.1-fold, respectively, whereas LIS increased them 3.8- and 1.9-fold, respectively, immediately after exercise compared with rested muscle. HIS and LIS increased the insulin responsiveness of 2-deoxyglucose uptake measured 4 hours after exercise by 39% and 41%, respectively, compared with rested muscles. These results show that very short (160 seconds) HIS exercise with greater AMPK activation increases the responsiveness of glucose uptake to insulin in the muscles of fed rats to a similar level observed after prolonged (3 hours) LIS exercise with lower AMPK activation. Therefore, it is suggested that an acute bout of sprint interval exercise that activates AMPK to a sufficiently high level can increase post-exercise insulin responsiveness on muscle glucose uptake irrespective of very short exercise duration.

1. Introduction

A single bout of exercise stimulates muscle glucose uptake independently of the insulin signaling pathway [1-4]. This effect on glucose uptake is evident during and immediately after exercise but reverses progressively, with little or no residual effects measured 3 to 4 hours after the cessation of exercise in rats [5,6]. As the exercise effect on insulin-independent glucose uptake subsides, there is a substantial increase in insulin-stimulated glucose uptake in skeletal muscles [6-8].

Insulin's action on glucose uptake is characterized in terms of insulin sensitivity and responsiveness [9]. *Insulin sensitivity* is defined as the concentration of insulin required to cause 50% of its maximal effect on glucose uptake, whereas *insulin responsiveness* is defined as the increase in glucose uptake induced by the maximally effective insulin concentration. Several previous studies reported that an acute bout of exercise enhanced insulin sensitivity in the skeletal muscles of fasted rats within 4 hours after the cessation of exercise, but that it failed to increase insulin responsiveness [7,10,11]. On the other hand, insulin responsiveness was reduced in the muscles of fed rats as compared with fasted rats. However, a single bout of exercise partially restored insulin responsiveness in the muscles of fed rats within 4 hours after the cessation of exercise [11]. These results suggest that the mechanisms by which acute prior exercise increases insulin sensitivity and responsiveness are different.

Exercise is a significant physiological stimulus known to activate adenosine monophosphate–activated protein kinase (AMPK) in skeletal muscles [12,13], and it was previously reported that the treatment of myotubes or isolated rat skeletal muscle with a pharmacological AMPK

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activator, 5-aminoimidazole 4-carboxamide 1-β-D-ribofuranoside, augmented the ability of insulin to stimulate glucose uptake [14,15]. These findings suggest that AMPK activation mediates enhanced both/either insulin sensitivity and/or responsiveness after an acute bout of exercise in skeletal muscle.

It has been reported that AMPK activity increases in an intensity-dependent manner after exercise or muscle contraction in rat [16,17] and human [18-20] skeletal muscles. Chen et al [21] also reported that the activity of AMPK substantially increases with very high intensity, short-term sprint exercise. As such, in our previous study, we hypothesized that high-intensity sprint interval exercise resulting in a significant and greater increase in the activation of AMPK is more effective in enhancing muscle insulin action compared with low-intensity endurance exercise [22]. However, against our hypothesis, in our previous study, although an acute bout of high-intensity intermittent swimming (HIS) significantly increased insulin sensitivity in the rat epitrochlearis muscle of fasted rats as compared with the resting control, HIS was not more effective in enhancing insulin sensitivity than low-intensity prolonged swimming (LIS) [22]. Although our previous study showed that neither HIS nor LIS affected insulin responsiveness in the muscles of fasted rats, Zorzano et al [11] previously reported that acute prolonged endurance exercise increased insulin responsiveness in the muscles of fed rats. Because no studies have examined the effect of an acute bout of sprint interval exercise on insulin responsiveness in the muscles of fed animals, we examined the effect of an acute bout of HIS in the muscles of fed animals and compared it with the effect of LIS.

2. Materials and methods

2.1. Materials

Antibodies against *p*-AMPK (Thr^{172}), *p*-acetyl-CoA carboxylase (ACC) (Ser^{79}), *p*-Akt (Ser^{473}), *p*-Akt (Thr^{308}), and total Akt were from Cell Signaling Technology (Beverly, MA). Antibody against glucose transporter (GLUT) 4 was from Biogenesis (Poole, United Kingdom). Horseradish peroxidase-conjugated anti-rabbit immunoglobulin G was from Biosource International (Camarillo, CA). Enhanced chemiluminescence reagents (ECL and ECL plus) were obtained from Amersham Biosciences (Buckinghamshire, United Kingdom). All other reagents were obtained from Sigma (St Louis, MO).

2.2. Treatment of animals

This research was approved by the Animal Studies Committee of Niigata University of Health and Welfare. Male Wistar rats were obtained from CLEA (Tokyo, Japan). The animals were maintained in individual cages and fed a standard rodent chow diet and water ad libitum. Two days before the experiment, all the rats (100-120 g) were accustomed to swimming for 10 minutes and then were divided into 4 groups: fasted resting control (Fast-Rest), fed control (Fed-Rest), high-intensity intermittent swimming (Fed-HIS), or low-intensity prolonged swimming (Fed-LIS). Rats in the Fast-Rest group were fasted for 24 hours from 8:00 PM of the day preceding the experiment, whereas rats in the Fed-Rest, Fed-HIS, and Fed-LIS groups were fed normally until the Fed-LIS group started swimming. Rats in the Fed-HIS group underwent eight 20-second bouts of swimming carrying a weight equal to 18% of their body weight, with a 40-second rest between bouts [22]. Single rats swam in a barrel filled to a depth of 30 cm with a surface area of 450 cm^2. Meanwhile, rats in the Fed-LIS group underwent swimming for 3 hours without a weight, with 4 rats swimming simultaneously in a barrel filled to a depth of 40 cm with an average surface area of 240 cm^2 per rat. Water temperature was maintained at a constant temperature of 35°C during the swimming protocol.

After the exercise protocol, the rats were killed by cervical dislocation either immediately or 4 hours after completion of the exercise. In the animals killed immediately after exercise, the epitrochlearis and triceps muscles were dissected. The epitrochlearis muscles were either clamp-frozen in liquid nitrogen for measurement of glycogen concentrations and Western blot analysis, or used for subsequent incubation as described below. The triceps muscles were rapidly frozen in liquid nitrogen (within 40 seconds from cessation of exercise) for measurement of high-energy phosphate compounds. Animals to be killed 4 hours after exercise were returned to their cages and remained fasting for 4 hours. The rats were then killed; and the epitrochlearis muscles were dissected out for subsequent incubation, as described below.

2.3. Muscle incubation

The epitrochlearis muscles were incubated with shaking for 20 minutes at 30°C in 3 mL of oxygenated Krebs-Henseleit buffer containing 40 mmol/L mannitol and 0.1% radioimmunoassay-grade bovine serum albumin, in the absence or presence of purified human insulin (100 or 10,000 μU/mL). Flasks were gassed continuously with 95% O_2-5% CO_2 during incubation. After incubation, the epitrochlearis muscles either were used either for measurement of 2-deoxyglucose (2DG) uptake or were blotted, clamp-frozen in liquid nitrogen, and then processed for Western blot analysis.

2.4. Measurement of 2DG uptake

The rate of muscle glucose uptake was determined by the method described by Ueyama et al [23]. After the 20-minute incubation described above, the epitrochlearis muscles were incubated for 20 minutes at 30°C in 3 mL of Krebs-Henseleit buffer containing 8 mmol/L 2DG, 32 mmol/L mannitol, and 0.1% bovine serum albumin

with or without insulin at the same concentration as the initial 20-minute incubation described above. The flasks were gassed continuously with 95% O_2-5% CO_2 during incubation. After incubation, the muscles were blotted, clamp-frozen in liquid nitrogen, weighed, homogenized in 0.3 mol/L perchloric acid, and then centrifuged at 1000*g*. After centrifugation, the supernatant was collected and neutralized by the addition of 2 N KOH, followed by fluorometric measurement of 2-deoxyglucose-6-phosphate (2DG6P) [24]. Under the above conditions, the intracellular accumulation of free 2DG in muscles is negligible, whereas the intracellular accumulation of 2DG6P is linear [25]. The intracellular accumulation rate of 2DG6P therefore reflects muscle glucose transport activity [25].

2.5. Western blot analysis

The epitrochlearis muscles were homogenized in ice-cold buffer containing 50 mmol/L HEPES (pH 7.4), 150 mmol/L NaCl, 10% glycerol, 1% Triton X-100, 1.5 mmol/L $MgCl_2$, 1 mmol/L EDTA, 10 mmol/L $Na_4P_2O_7$, 100 mmol/L NaF, 2 mmol/L Na_3VO_4, 2 mmol/L phenylmethylsulfonyl fluoride, aprotinin (10 μg/mL), leupeptin (10 μg/mL), and pepstatin (5 μg/mL) [26]. The homogenates were then rotated end-over-end at 4°C for 60 minutes and centrifuged at 4000*g* for 30 minutes at 4°C. Aliquots of the supernatants were treated with 2× Laemmli sample buffer containing 100 mmol/L dithiothreitol. All samples were subjected to 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis with the exception of ACC phosphorylation. For measurement of ACC phosphorylation, the samples were run on 5% sodium dodecyl sulfate polyacrylamide gel electrophoresis. The same number of samples (2 samples) for each group (Fast-Rest, Fed-Rest, Fed-HIS, and Fed-LIS) was always run on the same gel. The resolved proteins were then transferred to polyvinylidene difluoride membranes and blocked in 5% nonfat dry milk in Tris-buffered saline containing 0.1% Tween 10 (TBST), pH 7.5. After blocking, the membranes were rinsed in TBST, incubated overnight with appropriate antibody at 4°C, rinsed in TBST, and incubated for 120 minutes with horseradish peroxidase–conjugated anti-rabbit immunoglobulin G. Antibody bound protein was visualized by enhanced chemiluminescence (ECL or ECL plus, Amersham), with the intensity of the bands being quantified using densitometry.

2.6. Measurement of muscle metabolites

The epitrochlearis muscles were weighed and homogenized in 0.3 mol/L perchloric acid, and the extracts were assayed for glycogen by the amyloglucosidase method [27]. The triceps muscles were weighed, homogenized in 0.3 mol/L perchloric acid, and centrifuged at 1000g. After centrifugation, the supernatant was collected and neutralized by the addition of 2 N KOH, followed by the fluorometric measurement of adenosine triphosphate (ATP) and phosphocreatine (PCr) [24].

2.7. Statistical analysis

Data are expressed as means ± SE. Differences between the groups were determined using a 1-way analysis of variance, with a subsequent Fisher least significant difference method. Differences between groups were considered statistically significant when P was less than .05.

3. Results

3.1. Skeletal muscle glucose uptake

The 2DG uptake in the epitrochlearis muscle was measured immediately after exercise (Fig. 1A). At this time point, the insulin-independent glucose uptake was not significantly lower in the muscles of the fed resting rats than in the fasted resting rats (Fig. 1A). The insulin-independent 2DG uptake measured immediately after HIS and LIS exercise was increased by 10.1- and 2.9-fold, respectively, compared with the fed resting control (Fig. 1A). The increase in glucose uptake after exercise was greater ($P < .05$) with HIS than with LIS.

Furthermore, the uptake of 2DG in the epitrochlearis muscles was measured 4 hours after exercise (Fig. 1B). At this time point, the basal (without insulin) and both the submaximal (100 μU/mL) and maximal (10,000 μU/mL) insulin-stimulated glucose uptakes in the muscles of fed resting rats were decreased by 71%, 58%, and 43%, respectively, compared with those in the muscles of fasted resting rats ($P < .05$). When the fed rats were subjected to exercise 4 hours before these measurements, a small but significant ($P < .05$) increase in basal glucose uptake was observed in the muscles of both the Fed-HIS and Fed-LIS rats compared with the muscles of the Fed-Rest rats. Compared with the muscles of the fed resting control rats, the submaximal (100 μU/mL) insulin-stimulated glucose uptake was 102% and 110% higher after HIS and LIS, respectively ($P < .05$). Similarly, the maximal (10,000 μU/mL) insulin-stimulated glucose uptake was 37% and 41% higher after HIS and LIS, respectively, compared with the muscles of the fed resting control rats ($P < .05$). The submaximal and maximal insulin-stimulated glucose uptakes were not significantly different in the Fed-HIS and Fed-LIS groups.

3.2. AMPK regulation and ACC phosphorylation

Phosphorylation of AMPK Thr^{172} and ACC Ser^{79} in the epitrochlearis muscle was measured immediately after exercise (Fig. 2A and B). The activation of AMPK requires phosphorylation of the catalytic subunit of AMPK at Thr^{172} site by an upstream kinase [12,13]. Furthermore, active AMPK phosphorylates ACC at Ser^{79} site [12,13]. Therefore, phosphorylation of AMPK Thr^{172} and ACC Ser^{79} reflects AMPK activation level.

Both HIS and LIS exercises resulted in a significant increase in AMPK Thr^{172} phosphorylation compared with

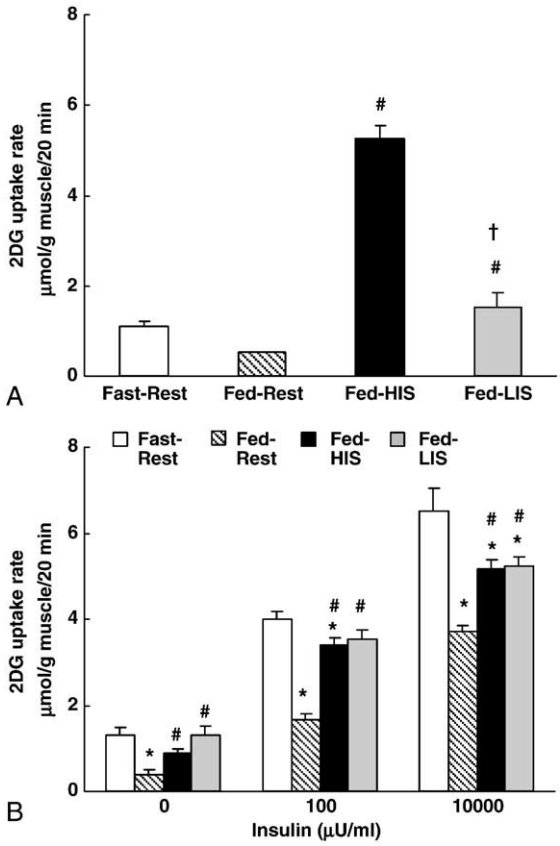

Fig. 1. A, Glucose uptake in rat epitrochlearis muscles at rest and immediately after HIS or LIS. Rats in the Fast-Rest group were fasted for 24 hours, whereas rats in the Fed-Rest, Fed-HIS, and Fed-LIS groups were fed normally. The muscles were dissected immediately after HIS exercise (Fed-HIS), LIS exercise (Fed-LIS), or a time-matched resting period (Fast-Rest, Fed-Rest). All muscles were incubated in glucose-free medium in the absence of insulin for 20 minutes, followed by measurement of 2DG uptake. Values are expressed as means ± SE (n = 4-7). B, Basal and insulin-stimulated glucose uptake in rat epitrochlearis muscles at rest and 4 hours after HIS or LIS. Muscles were dissected 4 hours after HIS exercise (Fed-HIS), LIS exercise (Fed-LIS), or a time-matched resting period (Fast-Rest, Fed-Rest). All muscles were incubated in glucose-free medium in the absence or presence of insulin (100 or 10,000 μU/mL) for 20 minutes, followed by measurement of 2DG uptake. Values are expressed as means ± SE (n = 7-8). Open bars, Fast-Rest; hatched bars, Fed-Rest; solid bars, Fed-HIS; gray bars, Fed-LIS. *P < .05 vs Fast-Rest with same insulin concentration; $^{\#}P$ < .05 vs Fed-Rest with same insulin concentration; $^{\dagger}P$ < .05 vs Fed-HIS with same insulin concentration.

the fed resting control (Fig. 2A). However, the increase in AMPK Thr^{172} phosphorylation was significantly greater immediately after HIS (12.7-fold) when compared with that after LIS (3.8-fold). Allosteric regulators of AMPK were also examined in the triceps muscle immediately after exercise (Table 1). Compared with the fed resting control, the ATP and PCr concentrations were similar after LIS exercise; however, HIS resulted in a significant reduction (P < .05) in ATP and PCr. Similar to AMPK Thr^{172} phosphorylation, ACC Ser^{79} phosphorylation in the epitrochlearis muscle was also increased (P < .05) immediately after exercise (Fig. 2B), with a greater (P < .05) increase demonstrated after HIS (3.1-fold) when compared with that after LIS (1.9-fold). There is no difference in AMPK Thr^{172} phosphorylation, ACC Ser^{79} phosphorylation, ATP, PCr, and glycogen between the fasted resting and fed resting muscles.

3.3. Akt phosphorylation 4 hours after exercise

Akt is a key enzyme in insulin signaling that stimulates glucose uptake in skeletal muscles [28-30]. Furthermore, the phosphorylation of Akt at both Ser^{473} and Thr^{308} is required for maximal enzyme activation [31]. Therefore, we estimated Ser^{473} and Thr^{308} phosphorylation of Akt in the epitrochlearis muscles 4 hours after exercise.

Akt Ser^{473} phosphorylation in the muscles of the fed resting rats was deceased significantly in the basal (without insulin, 29%) and both the submaximal (100 μU/mL, 23%) and maximal (10 000 μU/mL, 19%) insulin stimulations compared with the fasted resting rats (P < .05) (Fig. 3A). The same tendency was observed for Akt Thr^{308} phosphorylation

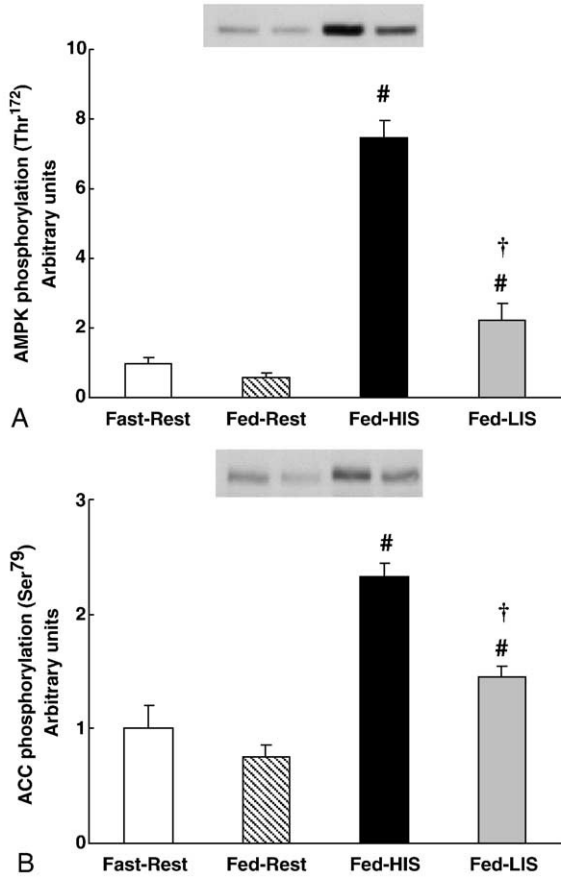

Fig. 2. Phosphorylation of AMPK and ACC in rat epitrochlearis muscles at rest and immediately after HIS or LIS. The muscles were dissected immediately after HIS exercise (Fed-HIS), LIS exercise (Fed-LIS), or a time-matched resting period (Fast-Rest, Fed-Rest). All muscles were clamp-frozen, followed by measurement of the phosphorylation of AMPK Thr^{172} (A) and ACC Ser^{79} (B). Values are expressed as means ± SE (n = 6-8). Open bars, Fast-Rest; hatched bars, Fed-Rest; solid bars, Fed-HIS; gray bars, Fed-LIS. $^{\#}P$ < .05 vs Fed-Rest; $^{\dagger}P$ < .05 vs Fed-HIS.

Table 1
Glycogen and high-energy phosphate concentrations in epitrochlearis and triceps muscles immediately after exercise

	Fast-Rest	Fed-Rest	Fed-HIS	Fed-LIS
Glycogen (μmol/g muscle)	10.27 ± 0.36	12.04 ± 0.79*	2.53 ± 0.10*,†	6.45 ± 0.48*,†,‡
ATP (μmol/g muscle)	6.35 ± 0.28	6.14 ± 0.56	3.45 ± 0.30*,†	6.61 ± 0.98‡
PCr (μmol/g muscle)	18.38 ± 0.92	16.51 ± 2.22	7.77 ± 0.50*,†	15.91 ± 1.56‡

Epitrochlearis and triceps muscles were dissected out immediately after HIS or LIS and were clamp-frozen. Glycogen (epitrochlearis muscle) and high-energy phosphate (triceps muscle) concentrations were determined as described in "Materials and methods." Values are expressed as means ± SE (n = 4-8).

* $P < .05$ vs Fast-Rest.

† $P < .05$ vs Fed-Rest.

‡ $P < .05$ vs Fed-HIS.

($P < .05$), with the exception of the submaximal insulin dose (100 μU/mL, $P = .06$, Fig. 3B). To estimate the possible role of Akt on the enhanced post-exercise insulin action on glucose uptake, we measured Akt phosphorylation in the muscles of fed rats that had exercised previously. Four hours after HIS, no significant effect of exercise on the basal, submaximal, or maximal insulin-stimulated Akt Ser^{473} and Thr^{308} phosphorylation was observed in the muscles of the fed rats (Fig. 3A and B). Four hours after LIS, no significant effect of exercise on the basal, submaximal, or maximal insulin-stimulated Akt Ser^{473} phosphorylation was found in the muscles of the fed rats (Fig. 3A), whereas this mode of exercise caused a significant increase ($P < .05$) in both the submaximal (100 μU/mL) and maximal (10 000 μU/mL) insulin-stimulated Akt Thr^{308} phosphorylation in these animals compared with the fed resting controls (Fig. 3B).

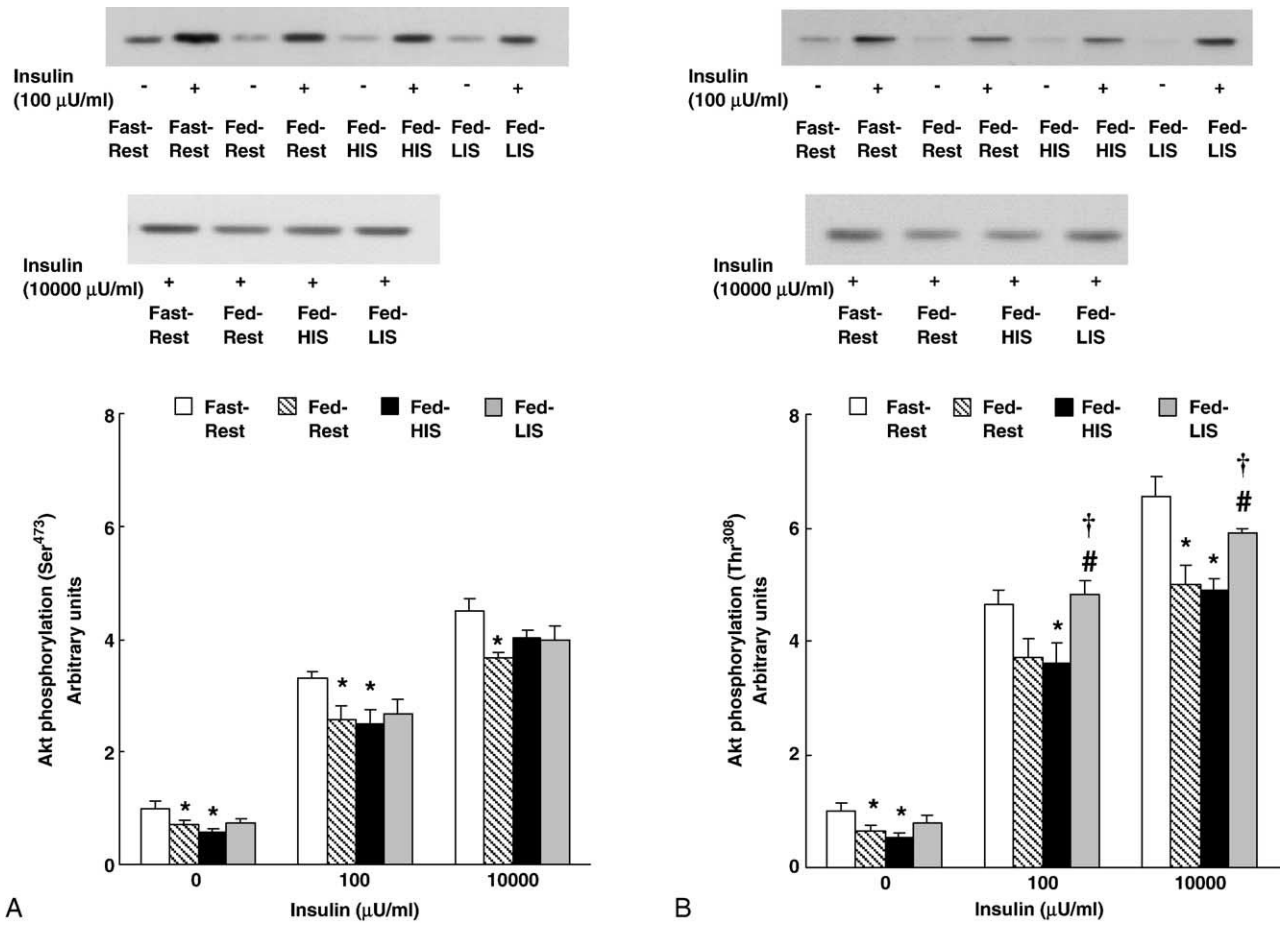

Fig. 3. Basal and insulin-stimulated Akt phosphorylation in rat epitrochlearis muscles at rest and 4 hours after HIS or LIS. Muscles were dissected 4 hours after HIS exercise (Fed-HIS), LIS exercise (Fed-LIS), or a time-matched resting period (Fast-Rest, Fed-Rest). All muscles were incubated in glucose-free medium in the absence or presence of insulin (100 or 10,000 μU/mL) for 20 minutes and were then clamp-frozen, followed by Western blot analysis with antibodies against phospho-Akt Ser^{473} (A) and phospho-Akt Thr^{308} (B). Values are expressed as means ± SE (n = 7-8). Open bars, Fast-Rest; hatched bars, Fed-Rest; solid bars, HIS-Rest; gray bars, LIS-Rest. *$P < .05$ vs Fast-Rest with same insulin concentration; $^{\#}P < .05$ vs Fed-Rest with same insulin concentration; $^{\dagger}P < .05$ vs Fed-HIS with same insulin concentration.

Table 2
Total GLUT4 protein concentrations in epitrochlearis muscles 4 hours after exercise

	Fast-Rest	Fed-Rest	Fed-HIS	Fed-LIS
Protein concentrations				
GLUT4 (arbitrary units)	1.00 ± 0.05	0.80 ± 0.06*	0.73 ± 0.06*	0.80 ± 0.06*

Epitrochlearis muscles were dissected 4 hours after HIS or LIS and were clamp-frozen. Total GLUT4 protein concentrations were determined as described in "Materials and methods." Values are expressed as means ± SE (n = 8).

* $P < .05$ vs Fast-Rest.

3.4. GLUT4 protein concentrations 4 hours after exercise

As it has been well established that an abundance of GLUT4 protein is a determinant of insulin responsiveness of glucose uptake in skeletal muscles [32,33], we measured the GLUT4 protein expression. The concentration of GLUT4 protein in the epitrochlearis muscle was 20% lower in the muscles of the fed resting rats than in the muscles of the fasted resting rats ($P < .05$) (Table 2). Therefore, decreased insulin responsiveness in fed muscles is a consequence, at least in part, of decreased GLUT4 expression. Although a previous study has demonstrated that a single bout of exercise can increase muscle GLUT4 protein concentration 18 hours after the cessation of exercise [34], we found no significant increase in muscle GLUT4 concentration 4 hours after the cessation of either HIS or LIS compared with the fed resting controls (Table 2).

4. Discussion

It was previously reported that a bout of prolonged endurance exercise can enhance maximally insulin-stimulated glucose uptake, ie, insulin responsiveness, in the skeletal muscle of fed animals after exercise [11]. Our present data showed that very short (160 seconds) HIS as a model of sprint interval exercise increases the insulin responsiveness of glucose uptake in the muscles of fed rats to a similar level as that observed after prolonged (3 hours) LIS as a model of endurance exercise.

AMPK is stimulated by allosteric modification via an increase in the AMP/ATP ratio and a decrease in PCr [12,13]. However, full activation of this enzyme requires Thr^{172} phosphorylation in the activation loop of the AMPKα catalytic subunit by an upstream AMPK kinase(s) [12,13]. Furthermore, previous studies suggested the possibility that a decrease in muscle glycogen may directly increase the activation state of AMPK in skeletal muscle [35,36]. In the present study, the muscle ATP, PCr, and glycogen concentrations were lower immediately after HIS compared with LIS exercise; and HIS increased AMPK phosphorylation to a significantly higher level than LIS. These changes lead to HIS-induced greater activation of AMPK. Consistent with previous studies demonstrating that AMPK is a key intermediary that regulates insulin-independent glucose uptake in skeletal muscle [37-39], greater AMPK activation immediately after HIS exercise was associated with higher insulin-independent muscle glucose uptake compared with LIS.

It was also previously reported that the treatment of myotube or isolated rat skeletal muscle with a pharmacological AMPK activator, 5-aminoimidazole 4-carboxamide 1-β-D-ribofuranoside, augmented the ability of insulin to stimulate glucose uptake [14,15]. These findings suggest that AMPK also plays a key role in the post-exercise enhancement of insulin-stimulated muscle glucose uptake. In our present study, both very short (160 seconds) HIS and prolonged (3 hours) LIS exercise increased the insulin responsiveness of glucose uptake in the epitrochlearis muscles of fed rats to a similar level 4 hours after cessation of either form of exercise. Because HIS resulted in greater AMPK activation compared with LIS, it is probable that exercise that activates AMPK to a sufficiently high level can increase post-exercise insulin action on muscle glucose uptake irrespective of how short the exercise duration is.

In our previous study using fasted animals, although we hypothesized that HIS exercise resulting in a significant and greater increase in the activation of AMPK is more effective in enhancing muscle insulin sensitivity compared with LIS exercise, HIS was not more effective in enhancing insulin sensitivity than LIS [22]. In our present study using fed animals, HIS also did not increase post-exercise muscle insulin responsiveness to higher level than LIS. Taken together, the results from our current and previous studies indicate that AMPK activation immediately after exercise may not be the only factor that determines the magnitude of the exercise-induced increase in insulin sensitivity and responsiveness in rat epitrochlearis muscle. It was reported that AMPK is not the sole mediator of insulin-independent increase in glucose uptake, which suggests that multiple pathways such as calmodulin-dependent protein kinases, protein kinase C family, nitric oxide, and reactive oxygen species are also associated with an insulin-independent signaling mechanism [40,41]. Therefore, it might be possible that these pathways other than AMPK are also involved in the increased insulin-stimulated muscle glucose uptake after exercise.

In this study, we showed that the insulin responsiveness of glucose uptake was lower in the epitrochlearis muscles of fed rats compared with those of fasted rats. Furthermore, insulin-stimulated Akt (Ser^{473} and Thr^{308}) phosphorylation was lower in the muscles of fed rats than in those of fasted rats. As Akt is a key enzyme in insulin signaling that stimulates glucose uptake in skeletal muscles [28-30], our results strongly suggest that the decrease in the insulin responsiveness of glucose uptake in fed muscles is attributable to an impairment in Akt signaling. These results are consistent with findings from a previous study in which Akt Ser^{473} phosphorylation was lower in the muscles of fed animals than in the muscles of fasted animals [42].

Interestingly, in fed rats subjected to LIS, the insulin responsiveness of glucose uptake and maximal insulin-stimulated Akt Thr^{308} phosphorylation were increased in muscles measured 4 hours after exercise, whereas insulin-stimulated Akt Ser^{473} phosphorylation remained unchanged. In contrast, HIS exercise increased the insulin responsiveness of glucose uptake without changing either Akt Ser^{473} or Thr^{308} phosphorylation. The phosphorylation of Akt at both Ser^{473} and Thr^{308} is required for maximal enzyme activation, but Akt activity can reportedly be increased by phosphorylation only at the Thr^{308} site [43]. It is therefore possible that enhanced Akt Thr^{308} phosphorylation has a potential role in improving the responsiveness of glucose uptake to insulin after LIS exercise, but not after HIS exercise, in the muscles of fed rats. Therefore, HIS and LIS exercises may increase the insulin responsiveness of glucose uptake by different mechanisms in fed animals.

It was previously reported that the insulin responsiveness of glucose uptake in skeletal muscle is increased in proportion to the increase in total GLUT4 protein [34,44]. Moreover, in a study by Ren et al [34], it was found that the total GLUT4 protein expression was increased by 50% in epitrochlearis muscles 16 hours after a bout of swimming. However, in the present study, both HIS and LIS increased insulin responsiveness at 4 hours after the cessation of exercise without an increase in GLUT4 protein expression. Thus, the increased muscle insulin responsiveness observed in the present study within a relatively short period after the cessation of exercise is not due to the increased GLUT4 protein expression. Hansen et al [45] showed that prior exercise resulted in greater cell surface GLUT4 in response to insulin treatment 3.5 hours after exercise despite no change in total GLUT4 protein content in rat epitrochlearis muscle. This result is consistent with our present result.

In summary, a single bout of very short (160 seconds) HIS with greater AMPK activation increased the insulin responsiveness of glucose uptake in the muscles of fed rats to a similar level observed after prolonged (3 hours) LIS with lower AMPK activation. This provides evidence that acute exercise that activates AMPK to a sufficiently high level can increase post-exercise insulin action on muscle glucose uptake even if the exercise duration is very short. We also demonstrated that LIS, but not HIS, increased Akt Thr^{308} phosphorylation in response to insulin stimulation in fed rats. This LIS-induced increase in Akt Thr^{308} phosphorylation may be relevant to the increase in insulin responsiveness caused by LIS. Thus, it is possible that HIS and LIS exercises increase the insulin responsiveness of glucose uptake by different mechanisms in fed animals.

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