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Effect of high-intensity intermittent swimming on postexercise insulin sensitivity in rat epitrochlearis muscle

Keiichi Koshinaka[a], Akiko Sano[a], Kirsten F. Howlett[b,c], Takako Yamazaki[a], Maiko Sasaki[a], Kei Sakamoto[c], Kentaro Kawanaka[a,*]

[a]*Department of Health and Nutrition, Niigata University of Health and Welfare, 1398 Shimami-cho, Kita-ku, Niigata City, Niigata 950-3198, Japan*
[b]*School of Exercise and Nutrition Sciences, Deakin University, Burwood, Australia*
[c]*MRC Protein Phosphorylation Unit, College of Life Sciences, University of Dundee, Dundee, Scotland, United Kingdom*

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Abstract

A bout of prolonged aerobic exercise can enhance the sensitivity of muscle glucose uptake to insulin, and this may be mediated by activation of 5′-adenosine monophosphate–activated protein kinase (AMPK). The aim of this study was to examine whether high-intensity short-term exercise resulting in a significantly greater increase in the activation of AMPK is more effective in enhancing muscle insulin sensitivity compared with low-intensity prolonged aerobic exercise. We measured insulin sensitivity after high-intensity intermittent swimming (HIS) or low-intensity continuous swimming (LIS) exercise in rat epitrochlearis muscle. During HIS, the rats underwent eight 20-second bouts of swimming with a weight equal to 18% of body weight. The LIS rats swam with no load for 3 hours. High-intensity intermittent swimming increased ($P < .05$) 2-deoxyglucose uptake ~8-fold, whereas LIS increased it ($P < .05$) ~2-fold immediately after exercise compared with rested muscle. This response was associated with an increase ($P < .05$) in phosphorylation of AMPK Thr^{172} and its downstream target acetyl-coenzyme A carboxylase (ACC) Ser^{79} in HIS (13- and 6-fold, respectively) and LIS (2.8- and 2-fold, respectively) immediately after exercise. In contrast, submaximal (30 μU/mL) insulin-stimulated 2-deoxyglucose uptake measured 4 hours after exercise was 73% and 46% higher ($P < .05$) in LIS and HIS, respectively, compared with rest. The HIS exercise resulted in a greater activation of AMPK compared with LIS, but insulin sensitivity was higher after LIS compared with HIS. The results suggest that HIS is not more effective in enhancing insulin sensitivity than LIS. Thus, AMPK activation immediately after exercise may not be the only factor that determines the magnitude of the exercise-induced increase in insulin sensitivity in rat epitrochlearis muscle.

1. Introduction

5′-Adenosine monophosphate–activated protein kinase (AMPK) functions as a fuel gauge in cells. When cells sense low fuel (ie, reduced adenosine triphosphate [ATP] level) in response to energy-reducing cellular stresses, including hypoxia, metabolic poisoning, or exercise, AMPK acts to switch off ATP-consuming pathways and turn on ATP-generating pathways. Exercise is a significant physiological stimulus known to activate AMPK in skeletal muscle, and this activation can subsequently stimulate muscle glucose uptake (reviewed by Hardie and Sakamoto [1] and by Winder [2]). The exercise-induced increase in muscle glucose uptake can occur independently of the insulin signaling pathway [3-6]. This effect on glucose uptake is evident during and immediately after exercise but reverses progressively, with little or no residual effects measured 3 hours after exercise in rats [7,8]. Despite this decline in muscle glucose uptake after exercise, there is a marked increase in the sensitivity of muscle glucose uptake to insulin [8-10]. In addition to exercise, insulin sensitivity is also increased when AMPK is activated by incubating isolated rat skeletal muscle with a pharmacological AMPK activator, 5-aminoimidazole 4-carboxamide 1-β-D-ribofuranoside [11]. These findings suggest that AMPK plays a key role in stimulating muscle glucose uptake in an insulin-independent mechanism and may also enhance skeletal muscle insulin sensitivity.

* Corresponding author. Tel.: +81 25 257 4479; fax: +81 25 257 4479.
E-mail address: kawanaka@nuhw.ac.jp (K. Kawanaka).

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Previous studies reported that prolonged aerobic exercise increases muscle insulin sensitivity [8-10], whereas the effect of high-intensity short-term exercise on muscle insulin sensitivity is not known. It has been reported that AMPK activity increases in an intensity-dependent manner after exercise or muscle contraction in rat [12,13] and human [14-16] skeletal muscle. Chen et al [17] also reported that the activity of AMPK substantially increases with very high intensity, short-term exercise. As such, we hypothesized that high-intensity short-term exercise resulting in a significant and greater increase in the activation of AMPK is more effective in enhancing muscle insulin sensitivity compared with low-intensity prolonged aerobic exercise. To examine this, we measured insulin sensitivity after short (160 seconds) high-intensity intermittent swimming (HIS) or prolonged (3 hours) low-intensity continuous swimming (LIS) exercise in rat epitrochlearis muscle.

2. Materials and methods

2.1. Materials

Purified human insulin was purchased from Eli Lilly Japan (Kobe, Japan). Antibodies against *p*-AMPK (Thr^{172}), *p*-acetyl coenzyme A carboxylase (ACC) (Ser^{79}), *p*-p38 mitogen-activated protein kinase (MAPK) (Thr^{180}/Tyr^{182}), *p*-Akt (Ser^{473}), *p*-Akt (Thr^{308}), total Akt, and total p38 MAPK were from Cell Signaling Technology (Beverly, MA). Anti–glucose transporter (GLUT) 4 antibody was from Biogenesis (Poole, United Kingdom). Horseradish peroxidase (HRP)–conjugated anti-rabbit immunoglobulin (Ig) G was from Biosource International (Camarillo, CA). Horseradish peroxidase–conjugated anti-mouse IgG was from Santa Cruz Biotechnology (Santa Cruz, 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. A total of 180 male Wistar rats were obtained from CLEA Japan (Tokyo, Japan). Animals were maintained in individual cages and fed a standard rodent chow diet and water ad libitum. All rats were accustomed to swimming for 10 minutes 2 days before the experiment. Food was removed, and animals were fasted from 8:00 PM of the day preceding the experiment.

Rats (100-120 g) were divided into 3 groups: resting control (rest, n = 54), HIS (n = 65), or LIS (n = 61). Rats in the HIS group underwent eight 20-second bouts of swimming, with 40 seconds of rest between bouts and with a weight equal to 18% body weight [18]. Single rat swam in a barrel filled to a depth of 30 cm and with a surface area of 450 cm^2. On the other hand, rats in the LIS group underwent swimming for 3 hours without a weight. Four rats swam simultaneously in a barrel filled to a depth of 40 cm and with an average surface area of 240 cm^2 per rat. Water was maintained at a constant temperature of 35°C during the swimming protocol.

After the exercise protocol, rats were killed by cervical dislocation either immediately or 4 hours after completion of the exercise. In animals that were killed immediately after exercise, epitrochlearis and triceps muscles were dissected. 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 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-Hensleit buffer containing 40 mmol/L mannitol and 0.1% radioimmunoassay-grade bovine serum albumin, in the absence or presence of purified human insulin (7.5, 30, or 10 000 μU/mL). Flasks were gassed continuously with 95% O_2-5% CO_2 during incubation. After incubation, epitrochlearis muscles were either used 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

2-Deoxyglucose was used to measure the rate of muscle glucose uptake based on the method described by Ueyama et al [19]. After the 20-minute incubation as described above, epitrochlearis muscles were incubated for 20 minutes at 30°C in 3 mL of Krebs-Hensleit buffer containing 8 mmol/L 2DG, 32 mmol/L mannitol, and 0.1% bovine serum albumin without or with insulin at the same concentration as in 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 and then clamp-frozen in liquid nitrogen. Muscles were weighed, homogenized in 0.3 mol/L perchloric acid, and 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) [20]. Under the above condition, intracellular accumulation of free 2DG in muscles is negligible, whereas intracellular accumulation of 2DG6P is linear [21]. The intracellular accumulation rate of 2DG6P therefore reflects muscle glucose transport activity [21].

2.5. Western blot analysis

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) [22]. 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 resolved proteins were then transferred to polyvinylidene difluoride membranes 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 and incubated overnight with appropriate antibody at 4°C, followed by rinsing in TBST and incubation for 120 minutes with HRP-conjugated anti-rabbit IgG or HRP-conjugated anti-mouse IgG. 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

Epitrochlearis muscles were weighed and homogenized in 0.3 mol/L perchloric acid, and the extracts were assayed for glycogen by the amyloglucosidase method [23]. Triceps muscles were weighed, homogenized in 0.3 mol/L perchloric acid, and centrifuged at 1000*g*. After centrifugation, the supernatant was collected and neutralized by the addition of 2 N KOH, followed by fluorometric measurement of ATP and phosphocreatine (PCr) [20].

2.7. Statistical analysis

Data are expressed as means ± SE. Differences 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 $P < .05$.

3. Results

3.1. Skeletal muscle glucose uptake

The 2DG uptake in epitrochlearis muscle measured immediately after LIS and HIS exercise was increased significantly by 2- and 7-fold, respectively, compared with rest (Fig. 1A). The increase in glucose uptake after exercise was greater ($P < .05$) with HIS compared with LIS. When

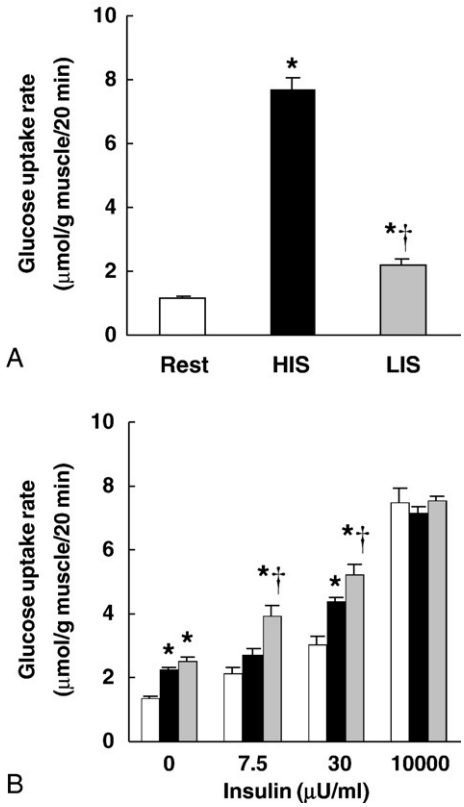

Fig. 1. (A) Glucose uptake in rat epitrochlearis muscles at rest and immediately after HIS or LIS. All muscles were incubated in glucose-free medium for 20 minutes, followed by measurement of 2DG uptake. Values are expressed as means ± SE (n = 11-14). *$P < .05$ vs rest; †$P < .05$ vs HIS. (B) Basal and insulin-stimulated glucose uptake in rat epitrochlearis muscles 4 hours after rest or after HIS or LIS. All muscles were incubated in glucose-free medium in the absence or presence of insulin (7.5, 30, or 10 000 μU/mL) for 20 minutes, followed by measurement of 2DG uptake. Open bars, rest; solid bars, HIS; gray bars, LIS. Values are expressed as means ± SE (n = 7-8). *$P < .05$ vs rest with same insulin concentration; †$P < .05$ vs HIS with same insulin concentration.

skeletal muscle glucose uptake was measured 4 hours after exercise, a small but significant insulin-independent increase in muscle glucose uptake was still evident in both HIS and LIS compared with rest (Fig. 1B). In response to insulin stimulation (7.5 μU/mL) 4 hours after rest or exercise, skeletal muscle glucose uptake was increased 85% after LIS compared with the resting control, while the insulin-stimulated effect after HIS was similar to rest. With a higher submaximal dose of insulin (30 μU/mL), glucose uptake was 46% and 73% higher after HIS and LIS, respectively, compared with the resting control trial. At both submaximal insulin doses (7.5 and 30 μU/mL), insulin-stimulated glucose uptake was higher ($P < .05$) after LIS than HIS exercise. In contrast, maximal (10 000 μU/mL) insulin-stimulated 2DG uptake was similar either after rest or LIS and HIS exercise. Taken together, both HIS and LIS exercise significantly enhanced insulin sensitivity of glucose uptake, but not responsiveness, in rat skeletal muscle. Low-intensity continuous swimming was more effective in improving muscle insulin sensitivity.

3.2. AMPK regulation and ACC phosphorylation

Exercise resulted in a significant increase in AMPK Thr^{172} phosphorylation compared with rest (Fig. 2A). However, the increase in AMPK Thr^{172} phosphorylation was significantly greater immediately after HIS (13-fold) than when compared with LIS (2.8-fold). Allosteric regulators of AMPK were also examined in triceps muscle immediately after exercise (Table 1). Compared with the resting control, ATP and PCr concentrations were similar after LIS exercise; however, HIS resulted in a significant reduction in ATP and PCr. Similarly to AMPK Thr^{172} phosphorylation, ACC Ser^{79} phosphorylation in epitrochlearis muscle was also increased ($P < .05$) immediately after exercise (Fig. 2B), with a greater ($P < .05$) increase demonstrated after HIS (6-fold) than when compared with LIS (2-fold).

3.3. Akt phosphorylation

Akt Ser^{473} phosphorylation in skeletal muscle was significantly increased by approximately 3.5-fold immediately after HIS exercise, while LIS exercise did not increase Akt Ser^{473} phosphorylation when compared with rest (Fig. 3A). Insulin stimulation of increasing concentrations

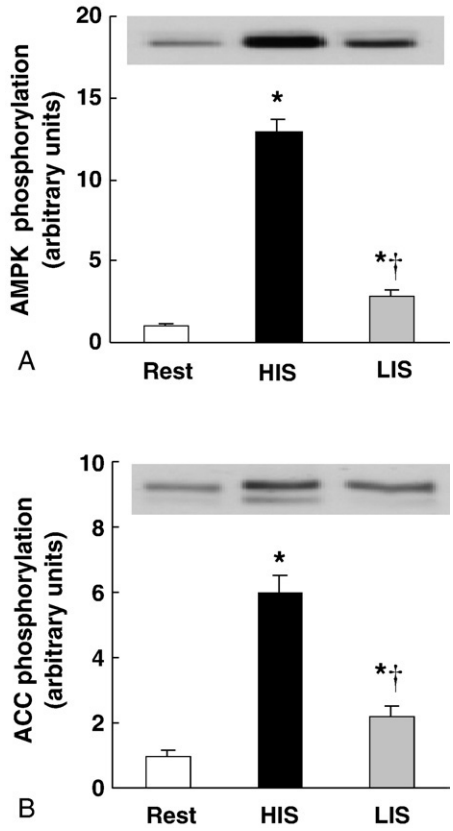

Fig. 2. Phosphorylation of AMPK and ACC in rat epitrochlearis muscles at rest and immediately after HIS or LIS. Muscles were removed immediately after HIS or LIS, and after a rest period in control animals (rest). All muscles were clamp-frozen, followed by measurement of phosphorylation of AMPK Thr^{172} (A) and ACC Ser^{79} (B). Values are expressed as means ± SE (n = 7-24). *$P < .05$ vs rest; †$P < .05$ vs HIS.

Table 1
High-energy phosphate concentrations in triceps muscles immediately after rest or exercise

	Rest	HIS	LIS
ATP (μmol/g muscle)	6.94 ± 0.28	4.63 ± 0.40*	6.88 ± 0.11†
PCr (μmol/g muscle)	18.38 ± 1.02	7.08 ± 0.96*	17.78 ± 1.40†

Triceps muscles were dissected out after a rest period and immediately after HIS or LIS, and clamp-frozen. High-energy phosphates were determined as described in Materials and methods. Values are expressed as means ± SE (n = 7-8).

* $P < .05$ vs rest.

† $P < .05$ vs HIS.

resulted in a concomitant greater increase ($P < .05$) in Akt Ser^{473} phosphorylation 4 hours after exercise or rest (Fig. 3C). However, the insulin-stimulated increase in Akt Ser^{473} phosphorylation was similar in the rest, HIS, and LIS exercise trials (Fig. 3C). A similar effect in response to exercise and/or insulin was also observed for Akt Thr^{308} phosphorylation (Fig. 3B, D). Total Akt protein levels were similar at rest and after HIS or LIS exercise (Table 2).

3.4. p38 MAPK phosphorylation

p38 MAPK Thr^{180}/Tyr^{182} phosphorylation in skeletal muscle was significantly increased by approximately 3-fold immediately after HIS exercise, while LIS exercise did not increase p38 MAPK Thr^{180}/Tyr^{182} phosphorylation when compared with rest (Fig. 4A). Insulin stimulation (7.5 μU/mL) 4 hours after rest or exercise had no effect on p38 MAPK Thr^{180}/Tyr^{182} phosphorylation (Fig. 4B). We also observed that maximal concentration of insulin (10 000 μU/mL) did not increase p38 MAPK Thr^{180}/Tyr^{182} phosphorylation in muscles of the resting control (data not shown). Therefore, we did not test the effects of exercise on p38 MAPK phosphorylation in muscles stimulated by 30 and 10 000 μU/mL insulin. Total p38 MAPK protein levels were similar at rest and after HIS and LIS exercise (Table 2).

3.5. GLUT4 protein and muscle glycogen concentration

Skeletal muscle GLUT4 protein concentration was similar 4 hours after rest or exercise (Table 2). Exercise significantly decreased muscle glycogen levels compared with rest (11.7 ± 0.8 μmol/g), although muscle glycogen level was lower ($P < .05$) immediately after HIS (0.9 ± 0.2 μmol/g) compared with LIS (3.6 ± 0.6 μmol/g).

4. Discussion

It is well established that a bout of prolonged aerobic exercise can enhance insulin-stimulated skeletal muscle glucose uptake in the period after exercise [8-10]. Because it was reported that a master cellular energy–sensing enzyme, AMPK, is a key mediator of enhanced postexercise insulin sensitivity [11], we hypothesized that high-intensity short-term exercise resulting in a greater increase in the activation

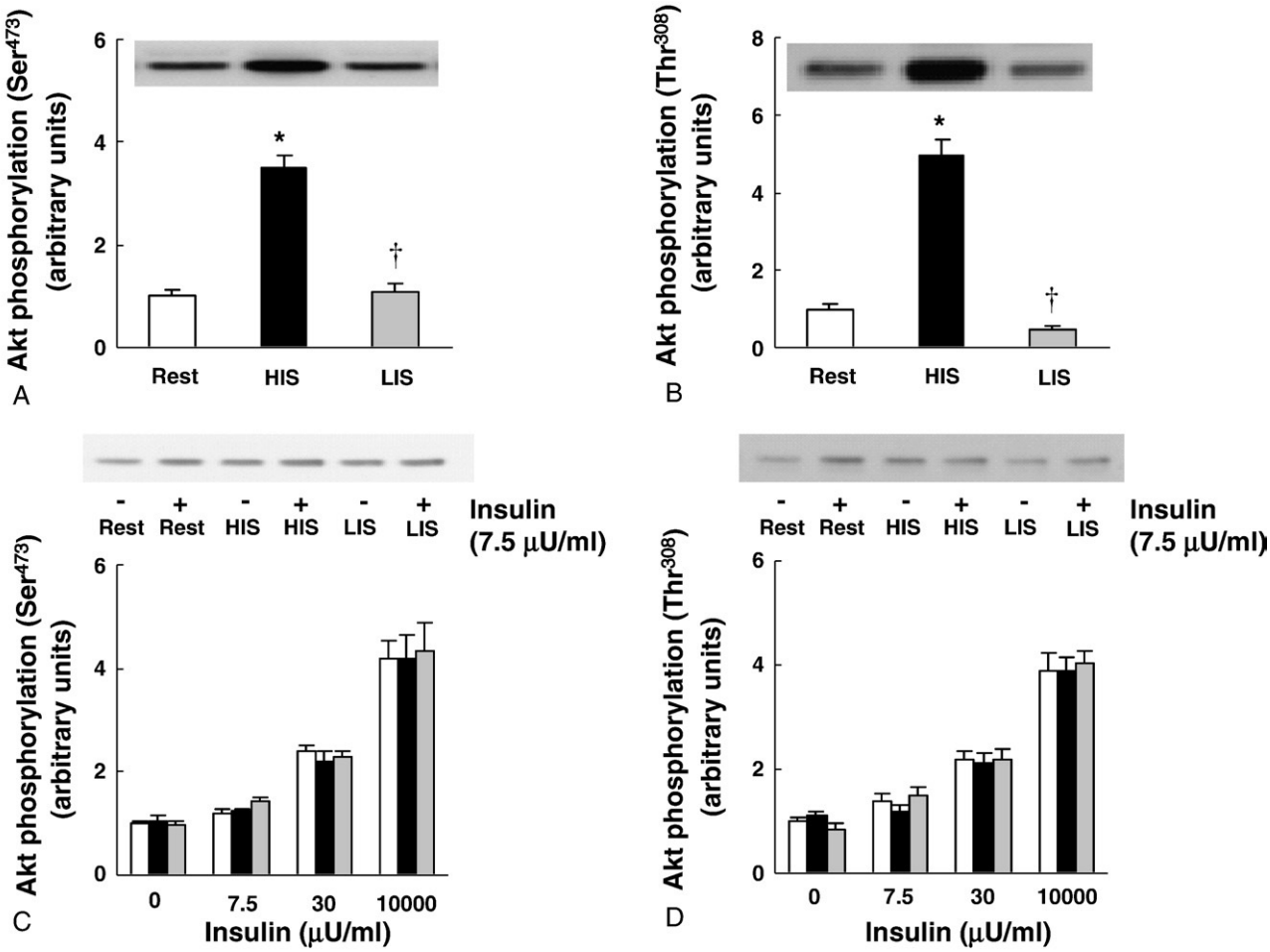

Fig. 3. (A, B) Phosphorylation of Akt Ser^{473} or Thr^{308} in rat epitrochlearis muscles at rest and immediately after HIS or LIS. Muscles were removed immediately after HIS or LIS, and after a rest period in control animals (rest). All muscles were clamp-frozen, followed by measurement of phosphorylation of Akt Ser^{473} or Thr^{308}. Values are expressed as means ± SE (n = 12-18). *P < .05 vs rest; $^{\dagger}P$ < .05 vs HIS. (C, D) Basal and insulin-stimulated Akt Ser^{473} or Thr^{308} phosphorylation in rat epitrochlearis muscles 4 hours after rest or after HIS or LIS. All muscles were incubated in glucose-free medium in the absence or presence of insulin (7.5, 30, or 10000 μU/mL) for 20 minutes. Open bars, rest; solid bars, HIS; gray bars, LIS. Values are expressed as means ± SE (n = 6-8).

of AMPK is more effective in enhancing muscle insulin sensitivity compared with low-intensity prolonged aerobic exercise. The results of this study demonstrate that short (160 seconds) HIS resulted in greater AMPK activation and insulin-independent muscle glucose uptake compared with prolonged (3 hours) low-intensity continuous swimming (LIS). However, in contrast to our hypothesis, insulin sensitivity of glucose uptake was slightly but significantly lower 4 hours after HIS exercise compared with LIS exercise in rat epitrochlearis, although HIS significantly increased insulin sensitivity as compared with resting control.

Table 2
Total protein concentrations of GLUT4, Akt, and p38 MAPK in epitrochlearis muscles 4 hours after rest or exercise

	Rest	HIS	LIS
GLUT4	1.00 ± 0.03	0.99 ± 0.02	0.98 ± 0.02
Akt	1.00 ± 0.05	0.95 ± 0.05	0.98 ± 0.05
p38 MAPK	1.00 ± 0.10	0.95 ± 0.08	0.95 ± 0.08

Epitrochlearis muscles were dissected out 4 hours after a rest period and HIS or LIS, and clamp-frozen. Total protein concentrations of GLUT4, Akt, and p38 MAPK were determined as described in Materials and methods. Values are expressed as arbitrary units, mean ± SE (n = 7-8).

5′-Adenosine monophosphate–activated protein kinase is stimulated by allosteric modification via an increase in AMP/ATP ratio and a decrease in PCr (reviewed by Hardie and Sakamoto [1] and by Winder [2]). However, full activation of this enzyme requires Thr^{172} phosphorylation in the activation loop of AMPKα catalytic subunit by an upstream AMPK kinase(s) (reviewed by Hardie and Sakamoto [1] and by Winder [2]). Furthermore, previous studies suggested the possibility that a decrease in muscle glycogen may directly increase the activation state of AMPK in skeletal muscle [24,25]. In the present study, 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

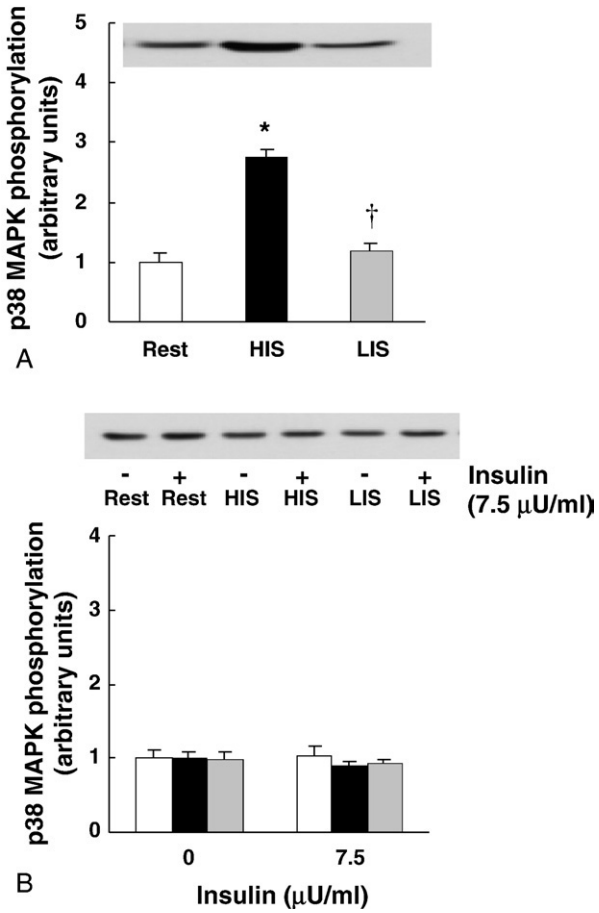

Fig. 4. (A) Phosphorylation of p38 MAPK in rat epitrochlearis muscles at rest and immediately after HIS or LIS. Muscles were removed immediately after HIS or LIS, and after a rest period in control animals (rest). All muscles were clamp-frozen, followed by measurement of phosphorylation of p38 MAPK Thr^{180}/Tyr^{182}. Values are expressed as means ± SE (n = 9-15). *$P < .05$ vs rest; †$P < .05$ vs HIS. (B) Basal and insulin-stimulated p38 MAPK Thr^{180}/Tyr^{182} phosphorylation in rat epitrochlearis muscles 4 hours after rest or after HIS or LIS. All muscles were incubated in glucose-free medium in the absence or presence of insulin (7.5 μU/mL) for 20 minutes. Open bars, rest; solid bars, HIS; gray bars, LIS. Values are expressed as means ± SE (n = 7-8).

with previous studies demonstrating that AMPK is a key intermediary that regulates insulin-independent glucose uptake in skeletal muscle [26-28], greater AMPK activation immediately after HIS exercise was associated with higher muscle glucose uptake compared with LIS.

However, greater AMPK activation with HIS exercise was not associated with enhanced insulin-stimulated muscle glucose uptake measured 4 hours after exercise. Indeed, less AMPK activation after LIS resulted in greater increase in submaximal insulin-stimulated muscle glucose uptake. These findings suggest that there may be dissociation between the magnitude of AMPK activation immediately after exercise and the improvements in insulin sensitivity. Kim et al [29] examined the effect of various electrical stimulation conditions (ie, frequency, duration, train rate) on the subsequent increase in insulin sensitivity in rat epitrochlearis muscles using an in situ muscle contraction protocol. Under certain conditions, there was no increase in insulin sensitivity after muscle contraction, although AMPK was significantly activated. Taken together, the results from this current study 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 in rat epitrochlearis muscle. Previous studies showed that the intracellular Ca^{2+} signaling is involved in the insulin-independent increase in glucose transport by muscle contractile activity [30-32]. Therefore, it might be possible that the increase in cytosolic Ca^{2+} during exercise is also involved in the postexercise increase in insulin sensitivity.

Akt is a key enzyme in insulin signaling that stimulates glucose uptake in skeletal muscle [33-36]. In the current study, both Ser^{473} and Thr^{308} phosphorylations of Akt were increased immediately after exercise only in HIS, while insulin further enhanced neither Akt Ser^{473} nor Thr^{308} phosphorylation 4 hours after exercise in either HIS or LIS. Our present results are consistent with the results of previous studies showing that Akt phosphorylation in response to a submaximal insulin stimulus was not increased after a single bout of exercise [11,37,38]. Our present and these previous results suggest that the exercise-induced increase in insulin sensitivity is mediated at a point downstream of Akt or, alternatively, that classic proximal insulin signaling proteins are not involved. In contrast to the present study, a recent study by Arias et al [39] has reported that subjecting rats to a prior swimming exercise (30 minutes × 4 sets) resulted in increases in insulin-stimulated Akt Thr^{308} phosphorylation and Akt substrate of 160 kd phosphorylation as well as insulin sensitivity to glucose uptake in epitrochlearis measured 3 to 4 hours after exercise. Currently, we do not know the reason for this discrepancy; however, it is possible that it could be due to differences in animal treatment (exercise and fasting protocol), insulin concentration, and/or the timing of the signaling measurements.

Previous studies showed that electrically induced contraction activates p38 MAPK in skeletal muscles [40,41]. Furthermore, Thong et al [42] hypothesized that p38 MAPK activation may be involved in mediating the exercise-induced increase in muscle insulin sensitivity. In contrast, Geiger et al [43] showed that activation of p38 does not mediate the exercise-induced increase in insulin sensitivity. In the current study, we only observed a significant increase in p38 MAPK phosphorylation immediately after HIS, but not after LIS exercise. Furthermore, both HIS and LIS exercise resulted in no further increase in p38 MAPK phosphorylation 4 hours postexercise with or without insulin stimulation. These results suggest that activation of p38 MAPK is unlikely to play an important role in mediating the increase in muscle insulin sensitivity after HIS and LIS exercise, which supports the result of Geiger et al [43].

It should be noted that, in the present study, very short HIS exercise (160 seconds) significantly increased insulin sensitivity in rat epitrochlearis muscle when compared with

resting control (Fig. 2B). These present findings demonstrate that short-term exercise at higher intensity may also bring about beneficial effects to improve insulin sensitivity. Indeed, Terada et al [44] have previously reported that 8 days of short (280 seconds) HIS training induced the adaptive increase in GLUT4 protein abundance and maximal insulin-stimulated glucose uptake in rat epitrochlearis muscle to the level observed after prolonged (6 hours) LIS training. In further support of an important role for high-intensity exercise in having significant health implications, a recent study by Gibala et al [45] demonstrated that sprint interval training can induce rapid adaptations in skeletal muscle and exercise performance that are comparable to endurance training in young active men.

In summary, higher AMPK activation immediately after HIS exercise was associated with greater muscle glucose uptake compared with LIS, suggesting that the magnitude of the change in AMPK activation appears to play an important role in regulating the insulin-independent increase in muscle glucose uptake induced by exercise. However, HIS exercise with greater AMPK activation did not increase insulin-stimulated muscle glucose uptake measured 4 hours after exercise to a level higher than LIS. Thus, the level of AMPK activation immediately after exercise does not appear to be a significant determinant of the magnitude of the exercise-induced increase in insulin sensitivity in rat epitrochlearis muscle. Further studies will be required to elucidate how exercise improves insulin sensitivity in skeletal muscle because such information will be critical in developing strategies aimed at the prevention and treatment of type 2 diabetes mellitus.

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