Increased GLUT-4 translocation mediates enhanced insulin sensitivity of muscle glucose transport after exercise

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Hansen, Polly A., Lorraine A. Nolte, May M. Chen, and John O. Holloszy. Increased GLUT-4 translocation mediates enhanced insulin sensitivity of muscle glucose transport after exercise. J. Appl. Physiol. 85(4): 1218-1222, 1998.—The purpose of this study was to determine whether the increase in insulin sensitivity of skeletal muscle glucose transport induced by a single bout of exercise is mediated by enhanced translocation of the GLUT-4 glucose transporter to the cell surface. The rate of 3-O-[3H]methyl-D-glucose transport stimulated by a submaximally effective concentration of insulin (30 µU/ml) was approximately twofold greater in the muscles studied 3.5 h after exercise than in those of the sedentary controls (0.89 ± 0.10 vs. 0.43 ± 0.05 µmol·ml⁻¹·10 min⁻¹; means ± SE for n = 6/group). GLUT-4 translocation was assessed by using the ATB-[2-3H]BMPA exofacial photolabeling technique. Prior exercise resulted in greater cell surface GLUT-4 labeling in response to submaximal insulin treatment (5.36 ± 0.45 dpm × 10⁶/g in exercised vs. 3.00 ± 0.38 dpm × 10⁶/g in sedentary group; n = 10/group) that closely mirrored the increase in glucose transport activity. The signal generated by the insulin receptor, as reflected in the extent of insulin receptor substrate-1 tyrosine phosphorylation, was unchanged after the exercise. We conclude that the increase in muscle insulin sensitivity of glucose transport after exercise is due to translocation of more GLUT-4 to the cell surface and that this effect is not due to potentiation of insulin-stimulated tyrosine phosphorylation.

It is presently not known whether the exercise-induced increase in the sensitivity of glucose transport activation is mediated by translocation of a greater number of GLUT-4 to the cell surface or by an increase in GLUT-4 intrinsic activity. As a first step in trying to elucidate the mechanism by which exercise increases insulin sensitivity, we have used a cell-surface photoaffinity labeling technique to assess whether a greater increase in sarcolemmal GLUT-4 occurs in response to a submaximal insulin stimulus. We also determined whether prior exercise results in an increase in the magnitude of the signal generated by the same submaximal concentration of insulin.

MATERIALS AND METHODS

Treatment of animals. Male Wistar rats (120–140 g) were given free access to Purina chow and water until the night before an experiment, when food was removed at 5:00 PM. The following morning, rats performed four 30-min bouts of swimming exercise in stainless steel barrels filled to a depth of ~60 cm with water maintained at 34–36°C, as described previously (2). All procedures employed in this study were approved by the Washington University Animal Studies Committee.

Muscle preparation. Immediately after cessation of exercise, rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body wt), and the epitrochlearis muscles were excised. The epitrochlearis is a small, thin muscle in the foreleg, suitable for studies of glucose transport in vitro (24). Muscles of exercised rats and sedentary controls were incubated with shaking for 3 h at 35°C in 2 ml of oxygenated Krebs-Henseleit buffer (KHB) supplemented with 8 mM glucose, 32 mM mannitol, and 0.1% BSA. Muscles were then transferred to KHB containing 40 mM mannitol and 0.1% BSA, with or without 30 µU/ml insulin, for 30 min at 30°C. The gas phase throughout the incubations was 95% O₂-5% CO₂.

Measurement of glucose transport activity. Muscle glucose transport activity was assayed by using 1.0 ml of KHB containing 8 mM 3-O-[3H]methyl-D-glucose (3-MG; 206 µCi/mmol; New England Nuclear), 32 mM [¹⁴C]mannitol (9 µCi/mmol; New England Nuclear), 0.1% BSA, and insulin, if present during the previous incubation. Incubations were performed at 30°C with shaking for 10 min with a gas phase of 95% O₂-5% CO₂. Extracellular space and intracellular 3-MG concentration (µmol·ml intracellular water⁻¹·10 min⁻¹) were determined as previously described (24).

Photolabeling of epitrochlearis muscles. Cell-surface GLUT-4 was assessed by using the 2-N-4-(1-azi-2,2,2-trifluoroethyl)-benzoyl-1,3-bis-(D-mannose-4-yloxy)-2-propylamine (ATB-[2-3H]BMPA) exofacial photolabeling technique as described previously (6), except that the labeled GLUT-4 was immunoprecipitated by using a rabbit polyclonal antibody.

Assessment of insulin signaling. Epitrochlearis muscles were incubated as described above for the transport assay. After the 30-min incubation in the presence or absence of insulin, muscles were blotted and then damp frozen. Muscle

AN ACUTE BOUT OF EXERCISE has two separate effects on skeletal muscle glucose transport. One effect, evident during exercise and for a relatively short period after exercise, is an insulin-independent stimulation of glucose transport (8, 14, 20). The second effect, which becomes evident as the acute effect of exercise on glucose transport wears off, consists of a large increase in insulin sensitivity after exercise (8, 14, 20). The increase in insulin sensitivity after exercise is likely one of the most important health benefits of exercise.
samples were pulverized, then homogenized in ice-cold buffer containing 50 mM HEPES (pH 7.4), 150 mM NaCl, 10% glycerol, 1% Triton-X-100, 1.5 mM MgCl₂, 1.0 mM EDTA, 10 mM Na₃P₂O₇, 100 mM NaF, 2.0 mM Na₃VO₄, aprotinin (10 µg/ml), leupeptin (10 µg/ml), pepstatin (0.5 µg/ml), and phenylmethylsulfonyl fluoride (2 mM) (13). Homogenates were incubated with end-over-end rotation at 4°C for 60 min, then centrifuged at 200,000 g for 50 min at 4°C. For analysis of tyrosine phosphorylation, aliquots of the supernatant were treated with 2× Laemmli sample buffer containing 100 mM dithiothreitol and boiled for 5 min. Samples (65 µg protein) were subjected to SDS-PAGE (6.25% resolving gel) and then were transferred to polyvinylidene difluoride membranes. The membranes were incubated with an anti-phosphotyrosine antibody (1 µg/ml; Zymed), followed by horseradish peroxidase-conjugated donkey anti-rabbit IgG (Jackson Immunoresearch). Antibody-bound protein was visualized by enhanced chemiluminescence (ECL; Amersham). The intensity of the bands corresponding to insulin receptor substrate-1 (IRS-1) and the ß-subunit of the insulin receptor was assessed by densitometry (Bio-Rad GS-670).

Assessment of GLUT-4 protein content. After a bout of swimming exercise, epitrochlearis muscles were removed and incubated in vitro for 3.5 h, as described above for the transport assay. After the incubation, muscles were blotted and then clamp frozen. Muscle GLUT-4 protein content was determined by Western blotting as described previously (7).

Statistical analysis. Data are presented as means ± SE. Analysis of differences between the exercised and sedentary groups was performed by using a Student’s t-test. P < 0.05 was considered to be significant.

RESULTS

Glucose transport. As in previous studies (2, 5), the stimulation of glucose transport by 30 µU/ml insulin was approximately twofold greater in the muscles studied 3.5 h after exercise than in those of the sedentary controls (Fig. 1A). The magnitude of this increase can be appreciated by comparing it with the effect of 2 µU/ml insulin (a maximally effective concentration); as shown in Fig. 1A, there was no significant difference between the effect of 30 µU/ml insulin in the exercised muscles and 2 µU/ml insulin in the control muscles. [With the protocol used in this study, insulin responsiveness is not increased 3.5 h after exercise (5)].

GLUT-4 translocation. In the muscles studied 3.5 h after exercise, 30 µU/ml insulin resulted in a more than twofold greater increase in ATB-[2-3H]BMPA labeling of GLUT-4 in the exercised compared with the control muscles (Fig. 1B). The enhanced GLUT-4 labeling in the exercised muscles closely mirrored the enhanced 3-MG transport (see Fig. 1A).

Muscle GLUT-4 content. Epitrochlearis muscle total GLUT-4 protein content was not greater in muscles isolated from exercised animals than in those from sedentary controls [4.15 ± 0.53 in exercised vs. 4.34 ± 0.22 (SE) in sedentary; values are in optical density units/mm² for 6 muscles/group].

Insulin-stimulated tyrosine phosphorylation. As shown in Fig. 2, there were small, but detectable, increases in tyrosine phosphorylation of the insulin receptor ß-subunit and IRS-1 after a 30-min exposure to 30 µU/ml insulin. There was no difference in the magnitude of tyrosine phosphorylation of either protein between the exercised and control groups.

DISCUSSION

The results of this study provide the first evidence that the greater increase in muscle glucose transport in response to a given submaximal insulin concentration after exercise is mediated by translocation of a greater number of GLUT-4 to the cell surface. The magnitude of the enhancement of GLUT-4 translocation closely matches the increase in glucose transport activity, indicating that an increase in GLUT-4 intrinsic activity does not play a role in the exercise-induced increase in insulin sensitivity. This increase in GLUT-4 translocation to the cell surface in response to a submaximal insulin stimulus 3.5 h after exercise is not due to an
exercise-induced increase in total muscle GLUT-4 content, as there was no difference in epitrochlearis GLUT-4 levels between the exercised and sedentary groups.

In previous studies of muscle GLUT-4 translocation, insulin responsiveness, i.e., the response to a maximally effective insulin stimulus, was examined. In these studies, it was found that in the absence of insulin resistance the increases in glucose transport induced by various stimuli (including insulin, exercise, hypoxia, insulin + exercise) can be explained by increases in GLUT-4 at the cell surface (6, 11, 12, 21). In addition, differences in maximally insulin-stimulated glucose transport between trained and untrained muscles can be explained by differences in GLUT-4 content and in a proportional difference in the amount of GLUT-4 translocated to the cell surface (15, 16). The present study provides the new information that the quantity of GLUT-4 translocated to the cell surface in response to a given submaximal insulin stimulus can vary, despite no change in total GLUT-4 content, i.e., that the sensitivity of the GLUT-4 movement induced by a low concentration of insulin can be altered and, thus, mediate a change in the sensitivity of muscle to insulin.

The insulin-mediated signal that regulates the uptake and disposal of glucose is initiated by the hormone binding to its cell-surface receptor and activating the receptor tyrosine kinase, resulting in autophosphorylation of the receptor on tyrosine residues and rapid phosphorylation of an immediate downstream substrate molecule IRS-1 (9). One mechanism by which an increased translocation of GLUT-4 to the cell surface by a given submaximal insulin stimulus could be mediated is an amplification of the signal generated by the binding of insulin to its receptor. Our finding in the present study that tyrosine phosphorylation of the insulin receptor and IRS-1 in response to 30 µU/ml insulin was not altered by a previous bout of exercise provides evidence that the sensitivity to insulin of these steps in the signaling pathway is unaffected. The absence of enhanced sensitivity of tyrosine phosphorylation after exercise in the present study is consistent with the earlier findings of Treadway et al. (19) showing that prior exercise had no effect on submaximal insulin-stimulated autophosphorylation or exogenous kinase activity of insulin receptors purified from muscle. Our findings are also consistent with the recent observation of Wojtaszewski et al. (22) that IRS-1 tyrosine phosphorylation induced by a submaximal insulin stimulus was not enhanced in human skeletal muscle after a bout of exercise that increased insulin sensitivity of glucose uptake. Taken together, our results and the results of these two studies provide strong evidence that the increase in insulin sensitivity after exercise is not due to potentiation of the tyrosine phosphorylation events generated by a submaximal insulin concentration.

In two other studies, the maximal effect of insulin on activation of the insulin-signaling pathway immediately after contractile activity was examined. In one study, Goodyear et al. (4) found that the responsiveness of tyrosine phosphorylation and phosphatidylinositol 3-kinase (PI 3-kinase) activity to a maximally effective insulin stimulus was decreased in rat hindlimb muscle after muscle contractions. In the other, Zhou and Dohm (25) found that exercise increased insulin-stimulated activation of PI 3-kinase, although there were no differences in insulin-stimulated tyrosine phosphorylation of IRS-1 between exercised and rested muscles. Because it is well documented that the maximal effects of insulin and the acute effect of exercise on glucose transport are additive (14, 26), it seems unlikely that any changes in the response of the insulin-signaling pathway to a maximal insulin stimulus immediately after exercise have a significant effect on the activation of glucose transport. In any case, findings regarding the maximal effect of insulin superimposed on the acute effect of exercise do not have direct relevance to the phenomenon investigated in the present study, i.e., the increase in insulin sensitivity that occurs after the acute stimulatory effect of exercise on muscle glucose transport wears off.
The evidence that the early phosphorylation steps in the insulin-signaling pathway are not activated to a greater extent by the same submaximal insulin concentration in exercised than in control muscles suggests a number of possibilities. One is that steps beyond IRS-1 in the signaling cascade are activated to a greater extent in the exercised than in the control muscle. In its tyrosine phosphorylated form, IRS-1 acts as a docking protein that forms signaling complexes with several proteins, including PI 3-kinase (18). There is strong evidence that activation of PI 3-kinase is essential for the stimulation of glucose transport by insulin in skeletal muscle (10, 12, 23). However, it was recently shown in human skeletal muscle that after a bout of exercise that increased the sensitivity of glucose uptake, PI 3-kinase activation in response to a submaximal insulin stimulus was ~30–50% lower than in nonexercised control muscle (22), suggesting that exercise increases insulin sensitivity at some point downstream of PI 3-kinase. Another possibility is that exercise induces changes in constituents of the glucose transporter vesicles and/or plasma membrane that enhance the translocation response to a submaximal insulin signal. The alternative possibility that more GLUT-4 vesicles become available for translocation seems less likely because, under the experimental conditions used in the present study, insulin responsiveness is not increased 3.5 h after exercise (5), as would be expected if the pool of GLUT-4 available for translocation by the action of insulin were increased.

The results of this study show that the increase in muscle insulin sensitivity of glucose transport after a single bout of exercise is completely accounted for by muscle insulin sensitivity of glucose transport after a single bout of exercise is completely accounted for by the action of insulin were increased. More transporter vesicles and/or plasma membrane that enhance the translocation process to a submaximal insulin signal. The alternative possibility that more GLUT-4 vesicles become available for translocation seems less likely because, under the experimental conditions used in the present study, insulin responsiveness is not increased 3.5 h after exercise (5), as would be expected if the pool of GLUT-4 available for translocation by the action of insulin were increased.

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