Mechanisms underlying impaired GLUT-4 translocation in glycogen-supercompensated muscles of exercised rats

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LARGE INCREASES IN MUSCLE GLYCOGEN are generally associated with development of insulin resistance of the glucose transport process (16, 25, 29, 46). Carbohydrate feeding after glycogen-depleting exercise results in an increase in muscle glycogen concentration to a higher level than is found in the sedentary fed state (4, 10, 12, 40). This phenomenon has been called glycogen supercompensation (4, 27). Exercise training induces an increase in the GLUT-4 isoform of the glucose transporter in muscle (6, 17, 19, 42, 43, 48). This increase in GLUT-4 results in a proportional increase in maximally insulin-stimulated muscle glucose transport (28, 31, 42, 43, 47). Glycogen supercompensation is markedly enhanced in muscles that have undergone an adaptive increase in GLUT-4 (20, 26, 28, 40).

We have found that glycogen supercompensation is associated with a decrease in insulin responsiveness that is so severe that it completely masks the effect of a training-induced twofold increase in GLUT-4 on insulin-stimulated muscle glucose transport (28, 30). The smaller increase in glucose transport in response to a maximal insulin stimulus in glycogen-supercompensated muscle is due to translocation of less GLUT-4 to the cell surface (30). The present study had two goals. The first was to determine whether a step in the insulin-signaling pathway is impaired in glycogen-supercompensated muscle. The second was to ascertain whether the increase in contraction-stimulated glucose transport that is mediated by an exercise training-induced increase in muscle GLUT-4 protein is also prevented by glycogen supercompensation.

MATERIALS AND METHODS

Materials. 3-O-methyl-d-[3H]glucose (3-MG) was obtained from American Radiolabeled Chemicals (St. Louis, MO) and d-[1-14C]mannitol was obtained from NEN Life Science Products (Boston, MA). Purified porcine insulin was purchased from Eli Lilly (Indianapolis, IN). Polyclonal antisera specific for the GLUT-4 (F349 and 1F8) were the generous gift of Dr. John O. Holloszy (Washington University). The antiphosphoprotein kinase B (PKB, Ser473) polyclonal antibody was from Upstate Biotechnology (Lake Placid, NY). Horseradish peroxidase-conjugated donkey anti-rabbit IgG was purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). Reagents for enhanced chemiluminescence were obtained from Amersham (Arlington Heights, IL). The exofacial label 2-N-[4-(1-azi-2,2,2-trifluoroethyl)benzonyl]-1,3-bis(d-mannos-4-bagai)-2-propylamine (ATB-[2-3H]BMPA) was purchased from Toronto Research Chemicals (Ontario, Canada). All other reagents were obtained from Sigma (St. Louis, MO).

Animal care and incubation of muscles. This research was approved by the Animal Studies Committee of Washington University School of Medicine. Male Wistar rats (body wt 114 ± 5 g) were housed in individual cages and fed a diet of Purina rodent laboratory chow and water ad libitum. Animals were randomly assigned to either an exercise group or
a sedentary control group. Rats in the exercise group were accustomed to swimming for 10 min/day for 2 days. They then were exercised using a two-day swimming training protocol that has been described previously (43). Briefly, rats swam in groups of six in plastic barrels filled with water maintained at 34–35°C for two 3-h-long swimming sessions separated by a 45-min-long rest period during which the rats were kept warm and were provided with drinking water. After completion of the swimming on the 2nd day, food was withheld from one-half of the trained animals (exercised-fasted) and the remaining trained animals were fed ad libitum (exercised-fed), whereas all of the sedentary controls were fasted (sedentary-fasted). Approximately 18 h after the last exercise bout, which is long enough for the acute effect of exercise on insulin responsiveness to wear off (7), the animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body wt), and the epitrochlearis muscles were dissected out.

Incubation of muscles with insulin. The epitrochlearis muscles were incubated with shaking for 60 min at 30°C in 1 ml of oxygenated Krebs-Henseleit buffer (KHB) containing 8 mM glucose, 32 mM mannitol, 0.1% RIA-grade BSA, with or without 2 mM/ml of purified porcine insulin. The flask activities and GLUT-4 translocation, we electrically stimulated muscles to contract. After dissection, the epitrochlearis muscles were allowed to recover for 60 min at 35°C in KHB containing 8 mM glucose, 32 mM mannitol, and 0.1% RIA-grade BSA. The flasks were continuously gassed with 95% O2-5% CO2 during incubation. After recovery incubation, the distal end of the muscle was attached to a vertical Lucite rod containing two platinum electrodes. The proximal end was clipped to a jeweler’s chain that was connected to a Grass S48 electrical stimulator. Ten tetanic contractions were produced by stimulating the muscle at 100 Hz for 250-ms-long trains at a rate of 60/min for 10 min. It was confirmed that this contraction protocol has a maximal effect on glucose transport (24). After electrical stimulation, muscles were washed for 10 min at 30°C in KHB containing 40 mM mannitol to remove glucose.

Measurement of glucose transport activity. Glucose transport activity was measured using the glucose analog 3-MG as described previously (56). After the wash, muscles were incubated at 30°C for 10 min in 1.0 ml of KHB containing 8 mM [3-3H]MG (2 μCi/ml) and 32 mM [14C]mannitol (0.2 μCi/ml). Extracellular space and intracellular 3-MG concentration (μmol/ml intracellular water \( \times 10^{-11} \text{min}^{-1} \)) were determined as described previously (56).

Photolabeling of epitrochlearis muscles. After the 10-min wash to remove glucose, some of the muscles were used for determination of the quantity of GLUT-4 at the cell surface with the use of the ATB-[2-3H]BMPA exofacial photolabeling technique as described previously (22), except that the labeled GLUT-4 was precipitated with a rabbit polyclonal antibody followed by protein A-Sepharose.

Muscle glycogen. Percollar acid extracts of muscle were assayed for glycogen by the amyloglucosidase method (41).

Measurement of muscle GLUT-4 protein content. Muscle GLUT-4 glucose transporter content was determined by Western blotting with a rabbit polyclonal antibody directed against the COOH terminus of GLUT-4 as previously described (43).

Assessment of insulin signaling. After dissection, the epitrochlearis muscles were incubated at 30°C in 2 ml of oxygenated KHB containing 8 mM glucose and 32 mM mannitol in the absence or presence of 2 nM/ml of insulin for 60 min, i.e., the same protocol used for insulin stimulation of glucose transport. After incubation, muscles were blotted and then clamp frozen. Muscle samples were 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 Na2PO4, 100 mM NaF, 2.0 mM Na3VO4, aprotinin (10 μg/ml), leupeptin (10 μg/ml), pepstatin (0.5 μg/ml), and phenylmethylsulfonyl fluoride (PMSF, 2 mM) (37). Homogenates were incubated with end-over-end rotation at 4°C for 60 min and then centrifuged at 200,000 g for 50 min at 4°C.

Analysis of PI 3-kinase activity associated with phosphorylated tyrosine, 1-mg aliquots of supernatant were immunoprecipitated overnight with end-over-end rotation at 4°C in the presence of 40 μl of monoclonal antiphosphotyrosine antibody coupled to protein A-Sepharose (Sigma). Immunocomplexes were collected by centrifugation and washed three times with phosphate-buffered saline containing 1% NP-40 and 100 μM Na3VO4, three times with 100 mM Tris-HCl (pH 7.5) containing 500 mM LiCl and 100 μM Na3VO4, and two times with 10 mM Tris-HCl (pH 7.5) containing 100 mM NaCl, 1 mM EDTA, and 100 μM Na3VO4. The pellets were resuspended in 50 μl of the Tris-HCl buffer (pH 7.5) containing 1 mM EDTA, and 17 mM MgCl2 and PI (20 μg) were added. PI 3-kinase activity was measured as described by Goodyear et al. (18). The radioactivity in the spots corresponding to PI 3-phosphate was quantitated by densitometry (Bio-Rad GS-670).

For quantification of phosphorylated PKB, aliquots of the 200,000-g supernatant were treated with 2× Laemmli sample buffer containing 100 mM dithiothreitol (DTT) and boiled for 5 min. Samples (80 μg protein) were subjected to SDS-PAGE (10% resolving gel) and then transferred to nitrocellulose membranes. The membranes were blocked in 5% nonfat dry milk in TBS containing 0.1% Tween 20 (TBST), pH 7.5, overnight. The membranes were rinsed in TBST and incubated with either antiphospho-PKB (Ser473) antibody or antiphospho-PKB (Thr388) antibody (Upstate Biotechnology) for 4 h. The membranes were rinsed in TBST and incubated with horseradish peroxidase-conjugated donkey anti-rabbit IgG (Jackson ImmunoResearch) for 60 min. Antibody-bound protein was visualized by ECL (Amersham). The intensity of the bands corresponding to phosphorylated PKB was assessed by densitometry (Bio-Rad GS-670).

Measurement of AMP-activated protein kinase activity. For contraction-stimulated AMP-activated protein kinase (AMPK) activity determination, rat epitrochlearis muscles were stimulated to contract indirectly via the nerve. Square-wave pulses (0.1 ms) were delivered with a Grass S48 stimulator at 100 Hz to give 250-ms-long trains at a rate of 60/min for 5 min. After a 1-min rest period, the muscles were stimulated for a second 5-min interval by use of the same protocol. This in situ muscle contraction protocol increases glucose transport maximally. Immediately after contractions, muscles were freeze clamped and stored at −80°C. For measuring AMPK activity, muscles were weighed and then homogenized in buffer (1:19 wt/vol) containing 20 mM Tris-HCl (pH 7.4), 250 mM sucrose, 50 mM NaF, 1 mM EDTA, 5 mM sodium...
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RESULTS

Insulin-stimulated 3-MG transport. We have found in previous studies that our 2-day swimming program induces an approximately twofold increase in GLUT-4 protein that is associated with a proportional increase in maximally insulin-stimulated glucose transport in epimysial muscles (28, 30, 43). In the present study, GLUT-4 protein (data not shown) and maximally insulin-stimulated 3-MG transport (Fig. 1) were again approximately twofold higher in muscles from rats that were fasted overnight after the last exercise bout than they were in muscles of sedentary controls. As was also observed previously (28, 30), when rats were fed rodent chow ad libitum after the exercise, their muscles became so insulin resistant that insulin-stimulated 3-MG transport was not significantly different from that of the sedentary control group (Fig. 1). This effect is not due to a decrease in GLUT-4, because GLUT-4 protein concentration was increased to the same extent, approximately twofold, in the muscles of fed and fasted swimmers (data not shown) (28). It therefore seemed possible that the muscle insulin resistance induced by feeding a high-carbohydrate diet after exercise might be mediated by impaired insulin signaling.

Insulin signaling. To evaluate a possible role of impairment of insulin signaling in the decrease in muscle insulin responsiveness in rats fed carbohydrate after exercise, we measured PI 3-kinase activity and PKB phosphorylation. To obtain information regarding the activation of insulin signaling at the time that 3-MG transport was measured, we used the same insulin stimulation protocol that was used for stimulation of 3-MG transport. This involved incubation of muscles in the presence of 2 mM/ml insulin. Representative autoradiogram showing 32P incorporation into PI (P13P) in epimysial muscles. Results are in arbitrary optical density (OD) units, with the fasting insulin set at 100.0, and are presented as means ± SE for 6 muscle groups.

Fig. 2. Insulin-stimulated phosphatidylinositol (PI) 3-kinase activity in epimysial muscles of exercised-fasted rats and in glycogen-supercompensated muscles of exercised-fed rats. (See Fig. 1 legend for description of exercise protocol.) The insulin stimulation protocol was the same as that used for maximal stimulation of glucose transport: muscles were incubated for 60 min in the presence of 2 mM/ml insulin. Representative autoradiogram showing 32P incorporation into PI (P13P) in epimysial muscles. Results are in arbitrary optical density (OD) units, with the fasting insulin set at 100.0, and are presented as means ± SE for 6 muscle groups.

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Fig. 1. 3-O-methyl-D-glucose (3-MG) transport in maximally insulin-stimulated epimysial muscles of overnight-fasted sedentary and exercised rats and in glycogen-supercompensated muscles of exercised fed rats. Exercised rats performed two 3-h-long bouts of swimming separated by a 45-min rest period on 2 successive days. One group of exercised rats was fasted, and the other group was fed rat chow for 18 h, before muscles were used for measurement of glucose transport. Values are means ± SE of actual transport rates, i.e., including basal, for 5–8 muscles/group. *P < 0.01, exercised fasted vs. sedentary-fasted or exercised-fed rats.

Pyrophosphate, 2 mM DTT, 1 mM benzamidine, 4 mg/l leupeptin, 1 mg/l aprotinin, 1 mg/l pepstatin, 50 mg/l trypsin inhibitor and 0.6 mM PMSF. The homogenates were centrifuged at 48,000 g for 30 min. AMPK was precipitated from a measured volume of the supernatant by the addition of an equal volume of the homogenate buffer containing 288 mg/ml of ammonium sulfate, followed by incubating tubes for 30 min on ice (54). The precipitate was collected by centrifugation at 48,000 g for 30 min, and the pellet was dissolved in 10% of the original volume of the homogenate buffer. AMPK activity was determined on this extract as described by Winder and Hardie (54), by use of the SAMS peptide, which has the amino acid sequence HMRSAMSGLHLVKR, as substrate (14).

Statistics. The results are expressed as means ± SE. Significant differences among the three treatment groups (sedentary-fasted, exercised-fasted, exercised-fed) were evaluated using a one-way ANOVA. Post hoc analyses were done with the Student-Newman-Keuls method with a significance level set at P < 0.05.

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Insulin signaling. To evaluate a possible role of impairment of insulin signaling in the decrease in muscle insulin responsiveness in rats fed carbohydrate after exercise, we measured PI 3-kinase activity and PKB phosphorylation. To obtain information regarding the activation of insulin signaling at the time that 3-MG transport was measured, we used the same insulin stimulation protocol that was used for stimulation of 3-MG transport. This involved incubation of muscles in the presence of 2 mM/ml insulin. As shown in Fig. 2, the increase in phosphotyrosine-associated PI 3-kinase activity in response to insulin was slightly, but not statistically significantly, lower in muscles of the exercised-fed compared with the exercised-fasted rats. PKB is activated by phosphorylation of Thr308 in the activation loop in the core of the kinase and of Ser473 in the carboxy terminus of the enzyme (1, 52). As shown in Fig. 3, phosphorylation of both Thr308 and Ser473 was ~50% lower in muscles of the exercised-fasted compared with the exercised-fasted rats. The total amount of PKB protein was similar in the two groups: 48 ± 4 optical density (OD) units in the exercised-fed and 47 ± 4 OD units in the exercised-fasted groups (means ± SE for 6 muscle groups). This finding...
of a reduction in insulin-stimulated PKB phosphorylation suggested that the resistance of glucose transport to stimulation might be limited to the insulin-signaling pathway in muscles of rats fed carbohydrate after exercise. To evaluate this possibility, we examined the response of glucose transport to contractile activity.

**Contraction-stimulated 3-MG transport.** As shown in Fig. 4, contraction-stimulated 3-MG transport was markedly reduced in muscles of exercised rats that were fed after the last exercise bout compared with those of the exercised-fasted group. The resistance to contractile activity in these muscles was so severe that the effect of the exercise-induced increase in GLUT-4 protein on glucose transport activity was completely masked. Thus the contraction-stimulated pathway for activation of glucose transport is inhibited at least as severely (~64% lower than in fasted groups) as the insulin-stimulated pathway (~52% lower than in fasted groups) in muscles of the 2-day-exercised rats fed rodent chow after exercise.

**Increase in cell surface GLUT-4.** The increase in cell surface GLUT-4 (evaluated by labeling with ATB-[2-3H]BMPA) induced by contractions was approximately twofold greater in muscles of the fasted exercisers than in those of the fasted sedentary controls (Fig. 5). This greater increase in cell surface GLUT-4 explains the
twofold greater increase in glucose transport in response to contractile activity in muscles of the fasted-exercised compared with the fasted-sedentary rats. However, the contraction-induced increase in cell surface GLUT-4, as reflected in ATB-[2-3H]BMPA labeling, was ~66% smaller in the fed exercisers’ muscles than in those of the fasted exercisers. This smaller increase in cell surface GLUT-4 accounts for the lower contraction-stimulated glucose transport activity in muscles of the fed compared with those of the fasted-exercised rats.

**AMPK activity.** There is considerable evidence that activation of AMPK during contractile activity plays a role in the stimulation of glucose transport by muscle contractions (3, 23, 33). We therefore measured the activation of AMPK by contractions in muscles of fed- and fasted-exercised rats to evaluate the possibility that a reduction in AMPK activity might be involved in the decreased responsiveness of glucose transport and GLUT-4 translocation to contractions in the glycogen-supercompensated muscles. As shown in Fig. 6, the increase in AMPK activity in response to contractions was markedly (~80%) smaller in the muscles of the fed-compared with those of the fasted-exercised rats.

**Muscle glycogen.** The glycogen content of epitrochlearis muscles of the fasted-sedentary and -exercised rats was low (~9 μmol/g wet wt) and decreased further (to ~4 μmol/g) in response to the tetanic stimulation protocol. In the fed-exercised group, there was muscle glycogen supercompensation (~80 μmol/g), and the electrical stimulation protocol resulted in an ~40% decrease in glycogen concentration (Fig. 7).

**DISCUSSION**

The two-day swimming program used in this study induces a twofold increase in GLUT-4 protein expression in rat epitrochlearis muscles (28, 30, 43). Insulin- and contraction-stimulated muscle glucose transport is increased in proportion to the increase in GLUT-4 in muscles of exercise-trained rats (28, 31, 42, 43). The results of the present study show that the enhancement of contraction-stimulated glucose transport in muscles that have adapted to exercise with an increase in GLUT-4 is entirely explained by translocation of GLUT-4 to the cell surface. It was previously found that the greater increase in glucose transport in response to a maximal insulin stimulus in muscles of rats adapted to exercise is also mediated by translocation of more GLUT-4 to the cell surface (30, 44).

Ingestion of a high-carbohydrate diet after glycogen-depleting exercise results in a large increase in muscle glycogen, above normal fed levels. This increase in glycogen, which has been termed glycogen supercompensation, (4) (see also Ref. 27 for review), is markedly enhanced in muscles that have adapted to exercise with an increase in GLUT-4 (20, 26, 28, 40). We previously found that glycogen supercompensation is associated with a decrease in muscle insulin responsiveness so marked that the effect of an exercise-induced twofold increase in GLUT-4 on insulin-stimulated glucose transport is completely prevented (28, 30) (Fig. 1). This reduction in insulin-stimulated glucose transport activity in glycogen-supercompensated muscles is accounted for by a proportionally smaller increase in GLUT-4 at the cell surface in response to insulin (30).

One purpose of this study was to evaluate the possibility that impaired insulin signaling is involved in the decrease in insulin-stimulated glucose transport in glycogen-supercompensated muscle. We measured insulin-stimulated PI 3-kinase activity and PKB phosphorylation and found that, although PI 3-kinase activity was not significantly reduced, insulin-stimulated PKB phosphorylation of both Ser<sup>473</sup> and Thr<sup>308</sup> was decreased by ~50%. Although the finding of a decrease in PKB phosphorylation in the absence of a reduction in PI 3-kinase activity seems surprising, it is not unprecedented. Kurowski et al. (32) have reported a very similar finding in muscles incubated with 25 mM glucose for 4 h, and Song et al. (51) found that the activity of PKB, but not of PI 3-kinase, is reduced in muscles of diabetic GK rats.

The second goal of this study was to determine whether contraction-stimulated glucose transport is...
also reduced in glycogen-supercompensated muscle. Our findings show that contraction-stimulated GLUT-4 translocation to the cell surface and glucose transport activity are decreased to at least as great an extent as are insulin-stimulated glucose transport and GLUT-4 translocation. As in the case of insulin, the smaller increase in cell surface GLUT-4 completely explains the blunted response of glucose transport to contractile activity in glycogen-supercompensated muscle.

Although insulin and contractions both increase glucose transport by translocation of GLUT-4 to the cell surface, they mediate this process via separate pathways. The earliest evidence for two pathways was provided by the finding that the maximal effects of insulin and contractions on glucose transport are additive (11, 57). Subsequently, it was shown that inhibition of PI 3-kinase with wortmannin completely blocks the effect of insulin-stimulated, but has no effect on contraction-stimulated, glucose transport (34, 35, 55). It has also been demonstrated that PKB is not activated by contractile activity (5, 36).

Considerable evidence has accumulated that activation of AMPK during contractile activity is involved in mediating the stimulation of muscle glucose transport by contractions (3, 23, 33). Our finding that the increase in AMPK activity induced by contractions is markedly reduced in glycogen-supercompensated muscles may provide an explanation of the mechanism by which contraction-stimulated glucose transport is decreased. That the activation of both PKB and AMPK is inhibited is surprising in view of the strong evidence that the insulin- and contraction-stimulated pathways are activated independently. The currently available information regarding the regulation of the activities of these enzymes provides no clues regarding a possible mechanism for this inhibitory effect, making it an interesting topic for future research.

It is not yet clear whether the resistance of glucose transport in muscle to stimulation is a direct consequence of the increase in glycogen or whether it is caused by “glucose toxicity” resulting from the large amount of glucose that floods into muscle during development of glycogen supercompensation. One hypothesis regarding the mechanism for the reduced insulin responsiveness of glycogen-supercompensated muscle is that GLUT-4 vesicles become associated with glycogen particles, so that, as glycogen increases, the number of GLUT-4 vesicles available for translocation decreases (8, 9). Although this hypothesis is appealing, experimental evidence supporting it has been elusive. It is interesting, relative to this concept, that the decrease in contraction-stimulated glucose transport is as great as the reduction in insulin-stimulated transport in glycogen-supercompensated muscle despite the large, contraction-induced decrease in glycogen (Fig. 7).

Muscles exposed to high concentrations of glucose become insulin resistant (13, 21, 32, 45, 50). This glucose-induced insulin resistance, which has been termed glucose toxicity, can develop without a large increase in muscle glycogen (21, 45). Glucose toxicity insulin resistance has been documented only in muscles exposed to a high concentration of glucose (13, 21, 32, 45, 50). Plasma glucose levels are in the normal fed range during the development of muscle glycogen supercompensation in response to carbohydrate ingestion after glycogen-depleting exercise (10, 20, 26, 40, 53). Therefore, if the resistance of glucose transport to stimulation that develops in glycogen-supercompensated muscle is due to glucose toxicity, it would be the only condition under which this form of insulin resistance has been demonstrated to occur in the absence of hyperglycemia. Although it seems reasonable that, rather than exposure to high concentration of glucose, flooding of glucose into the cell would be responsible for inducing glucose toxicity, this may actually not be the case. It has been reported that exposure of muscles to a high glucose concentration in the absence of insulin, a condition under which glucose uptake is relatively slow, causes insulin resistance (21, 32, 45).

Clearly, more research is needed to elucidate the mechanisms responsible for glucose toxicity and for the resistance of glucose transport to stimulation in glycogen-supercompensated muscle, as well as to determine whether they are due to the same or different mechanisms. However, we have been able to rule out one potential mechanism. It is widely believed, on the basis of considerable evidence, that accumulation of UDP-N-acetylglucosamine and other UDP-hexosamines is responsible for the glucose toxicity type of insulin resistance (2, 38, 39, 49). This mechanism is not responsible for the resistance of glucose transport to stimulation in glycogen-supercompensated muscles, because UDP-N-acetylhexosamine concentrations are not higher in glycogen-supercompensated muscles than in muscles with a low glycogen content (30).

While the paper was in preparation, Derave et al. published a study (15) in which they related muscle glycogen content to contraction-stimulated glucose transport and cell surface GLUT-4. Muscle glycogen was lowered by 2 h of swimming and was kept low by fat feeding or raised by feeding a high-carbohydrate diet. 2-Deoxyglucose uptake correlated negatively with muscle glycogen content in perfused fast-twitch plantar muscles. Cell surface GLUT-4 in the plantaris, measured in vitro, was also negatively correlated with glycogen level. Although Derave et al. and we were addressing somewhat different questions, it seems clear that we are dealing with the same phenomenon and that our results are in agreement.

In conclusion, our results show that both insulin-stimulated and contraction-stimulated glucose transport are reduced in glycogen-supercompensated muscles of 2-day-exercised rats fed a high-carbohydrate diet after their last exercise bout. As shown previously for insulin stimulation and in the present study for contraction stimulation, the decrease in glucose transport activity is the result of translocation of less GLUT-4 to the cell surface. A large decrease in the activation of PKB by insulin suggests a possible explanation for this reduction in insulin-stimulated glucose transport activity.
transport, whereas a large decrease in contraction-stimulated AMPK activity provides a possible explanation for the reduction in contraction-stimulated transport.

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