Voluntary exercise training enhances glucose transport in muscle stimulated by insulin-like growth factor I

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Hokama, J. Y., Ryan S. Streeper, and Erik J. Henriksen. Voluntary exercise training enhances glucose transport in muscle stimulated by insulin-like growth factor I. J. Appl. Physiol. 82(2): 508–512, 1997.—Skeletal muscle glucose transport can be regulated by hormonal factors such as insulin and insulin-like growth factor I (IGF-I). Although it is well established that exercise training increases insulin action on muscle glucose transport, it is currently unknown whether exercise training leads to an enhancement of IGF-I-stimulated glucose transport in skeletal muscle. Therefore, we measured glucose transport activity (by using 2-deoxy-D-glucose [2-DG] uptake) in the isolated rat epitrochlearis muscle stimulated by submaximally and maximally effective concentrations of insulin (0.2 and 13.3 nM) or IGF-I (5 and 50 nM) after 1, 2, and 3 wk of voluntary wheel running (WR). After 1 wk of WR, both submaximal and maximal insulin-stimulated 2-DG uptake rates were significantly (P < 0.05) enhanced (43 and 31%) compared with those of sedentary controls, and these variables were further increased after 2 (86 and 57%) and 3 wk (71 and 70%) of WR. Submaximal and maximal IGF-I-stimulated 2-DG uptake rates were significantly enhanced after 1 wk of WR (82 and 61%), and these increases did not expand substantially after 2 (71 and 58%) and 3 wk (96 and 70%) of WR. This enhancement of hormone-stimulated 2-DG uptake in WR muscles preceded any alteration in glucose transporter (GLUT-4) protein level, which increased only after 2 (24%) and 3 wk (54%) of WR. Increases in GLUT-4 protein were significantly correlated (r = 0.844) with increases in citrate synthase. These results indicate that exercise training can enhance both insulin-stimulated and IGF-I-stimulated muscle glucose transport activity and that these improvements can develop without an increase in GLUT-4 protein.

wheel running; rat epitrochlearis muscle; 2-deoxy-D-glucose uptake; insulin; GLUT-4 protein; citrate synthase

INSULIN STIMULATES GLUCOSE TRANSPORT into skeletal muscle, primarily through the translocation of the glucose transporter protein isoform GLUT-4 from an intracellular site to the sarcolemma (6, 9, 24). In skeletal muscle, glucose transport activity can be stimulated by insulin-like growth factor I (IGF-I) (4, 5, 14, 16, 18, 20), and this process is also associated with a translocation of GLUT-4 protein (2, 18). The exogenous administration of IGF-I increases glucose disposal in healthy human subjects (30). IGF-I is a 70-amino acid peptide that shares a high degree of homology with insulin (16, 22), and IGF-I and insulin probably mediate their effects through some common intracellular mechanisms because the maximal activation of glucose transport activity by these peptides is not additive (20, 25; unpublished observations). However, skeletal muscle expresses a significant number of IGF-I receptors (5, 17, 31), and insulin and IGF-I are thought to mediate their effects on glucose transport in this tissue through their respective receptor systems (5).

Numerous investigations have demonstrated that an acute bout of intense exercise enhances insulin-stimulated glucose transport activity in rodent skeletal muscle (3, 14, 21, 29). Similarly, Henriksen et al. (14) have shown that, after an acute bout of prolonged swim exercise, the actions of IGF-I on glucose transport activity in rat epitrochlearis muscle is also significantly enhanced. Insulin action on skeletal muscle glucose transport activity is also increased by chronic exercise training, whether by treadmill running (7, 26), voluntary activity wheel running (WR) (12, 23), or swim training (19). In most cases, a concomitant increase in GLUT-4 protein level is observed after training (7, 9, 12, 19, 23, 26), consistent with the concept that the expansion of the intracellular GLUT-4 pool is functionally related to the augmented insulin-stimulated glucose transport capacity.

It is, however, currently unknown whether IGF-I action on glucose transport activity in skeletal muscle is increased after exercise training. Therefore, the primary purpose of the present study was to determine the effect of exercise training, by using 1–3 wk of voluntary WR, on insulin- and IGF-I-stimulated glucose transport activity in the isolated rat epitrochlearis muscle. We hypothesized that the actions of insulin and IGF-I on glucose transport activity would be enhanced with increasing exercise training intensity and duration. In addition, muscle GLUT-4 protein level, total hexokinase activity, and citrate synthase activity were measured to determine whether alterations in hormone-stimulated glucose transport activity are associated with changes in these variables. We hypothesized that the increases in insulin- and IGF-I-stimulated glucose transport activity would be temporally related to an expansion of the GLUT-4 protein pool and that the GLUT-4 protein level and citrate synthase activity would increase in parallel, consistent with the previous observation of coregulation of these two proteins in the plantaris muscle during voluntary WR (13).

METHODS

Animals and exercise training. Female Wistar rats (Harlan, Indianapolis, IN) with initial body weights of ~100 g were randomly assigned to either sedentary control groups or exercise training groups. Sedentary animals were housed individually in hanging wire mesh cages (18 × 26 × 20 cm) for 1, 2, or 3 wk. Exercising animals were housed individually in side cages of similar dimensions and had free access to vertical stainless steel activity wheels (1.13 m in circumference; Lafayette Instruments, West Lafayette, IN) for 1, 2, or 3 wk. Animals had free access to chow and water. Running distances were assessed daily, and body weights were mea-
for 1 h at 37°C. After the final wash, papers were dried and exposed to Kodak XAR-5 film at −70°C for 48–72 h. Autoradiographs were analyzed by scanning densitometry (model GS300 with GS370v2.3 software, Hoefer, San Francisco, CA). GLUT-4 protein levels in muscles from WR groups were expressed relative to the average of age-matched sedentary controls (arbitrarily set at 1.0) run on the same gel.

Citrate synthase (27) and hexokinase (28) activities were assayed spectrophotometrically on the same homogenates that were used for determination of GLUT-4 protein.

Statistical analysis. All data are expressed as means ± SE. Differences between groups were tested by analysis of variance, with Dunnett’s post hoc test used to locate the source of significant differences (StatView II, Abacus Concepts, Berkeley, CA). Correlations were analyzed by using univariate linear regression. Probability levels of < 0.05 were considered significant.

**RESULTS**

Running activity and body weights. The average running activity after 1, 2, and 3 wk was 5.8 ± 0.6, 9.5 ± 1.1, and 13.9 ± 0.9 km/day, respectively. Body weights were not significantly different between sedentary controls and WR groups at the end of 1 wk (142.2 ± 1.2 vs. 140.8 ± 1.7 g), 2 wk (171.1 ± 1.9 vs. 169.4 ± 1.5 g), or 3 wk (199.1 ± 2.6 vs. 200.8 ± 2.2 g).

Hormone-stimulated glucose transport activity. 2-DG uptake in the absence of hormone was not significantly altered by exercise training (Figs. 1 and 2). However, after 1 wk of WR, submaximal and maximal insulin-
stimulated 2-DG uptake rates were 43 and 31% greater ($P < 0.05$), respectively, compared with rates in the age-matched sedentary control groups (Fig. 1). Submaximal and maximal IGF-I-stimulated glucose transport activities were also greater ($P < 0.05$) than activities of sedentary controls after 1 wk of WR (82 and 61%, respectively; Fig. 2). After 2 wk of WR, the effects of submaximal and maximal doses of insulin were 86 and 57% greater ($P < 0.05$) than in age-matched sedentary controls. At this same time point, the effects of submaximal and maximal doses of IGF-I on 2-DG uptake in muscles from trained animals were 71 and 57% greater ($P < 0.05$) compared with values from the corresponding sedentary groups. After 3 wk of exercise training, 2-DG uptake stimulated by submaximal and maximal doses of insulin were 71 and 70% greater ($P < 0.05$) compared with age-matched sedentary controls. In response to submaximal and maximal IGF-I doses, 2-DG uptake in 3-wk WR groups was 96 and 70% greater ($P < 0.05$) compared with controls.

Glycogen, hexokinase activity, GLUT-4 protein level, and citrate synthase activity. Epitrochlearis muscle glycogen levels after 1 wk (27.7 ± 2.1 vs. 28.0 ± 0.7 nmol/mg muscle), 2 wk (31.8 ± 1.7 vs. 32.7 ± 1.0 nmol/mg muscle), and 3 wk (24.0 ± 2.0 vs. 25.1 ± 1.0 nmol/mg muscle) were not different between the sedentary control groups and the WR animals. Therefore, the potential modulation of hormone-stimulated glucose transport activity by glycogen (12) was not a confounding factor in the present study.

After 1 and 2 wk of exercise training, total hexokinase activity was significantly elevated ($P < 0.05$) compared with sedentary controls (28 and 43% greater, respectively; Fig. 3A). In contrast, after 1 wk, citrate synthase activity and GLUT-4 protein level in WR muscles were no different compared with sedentary controls (Fig. 3, B and C). However, after 2 wk, GLUT-4 protein level and citrate synthase activity were 24 and 23% greater ($P < 0.05$), respectively, in muscles from WR animals compared with sedentary controls. GLUT-4 protein level and citrate synthase activity were 54 and 35% greater ($P < 0.05$) after 3 wk of WR. Linear regression analysis showed a significant correlation ($r = 0.844$, $P < 0.05$) between the level of GLUT-4 protein and citrate synthase activity in the epitrochlearis muscle over the 3-wk training period (Fig. 4).

DISCUSSION

The primary finding of the present study was that exercise training, as performed by voluntary WR, significantly enhanced the in vitro action of IGF-I on glucose transport activity in rat skeletal muscle. The action of IGF-I on glucose transport activity was increased after exercise training at both submaximally and maximally effective in vitro concentrations (Fig. 2). The increase in
IGF-I-stimulated glucose transport activity occurred within 1 wk and remained enhanced throughout the 3-wk period of exercise training.

Another important finding was the early and marked increase in insulin action on glucose transport activity in exercise-trained skeletal muscle (Fig. 1). It is noteworthy that the increases in hormone-stimulated muscle glucose transport activities due to exercise training preceded any detectable augmentation of the total GLUT-4 protein pool (Fig. 3B). These results suggest that an increase in total pool of GLUT-4 protein is likely not an absolute requirement for initial increases in hormone-stimulated glucose transport activity after exercise training and that other components of the glucose transport system in skeletal muscle may be enhanced in the early stages of exercise training. There may be increased cycling of GLUT-4 protein from intracellular sites to the sarcolemmal membrane, resulting in an increase in surface GLUT-4 protein level without an increase in total GLUT-4 protein level. This concept requires further experimental verification.

As with previous studies (10, 12, 23), we assessed hormone-stimulated glucose transport activity 9–12 h after the animals were denied access to the running wheel. Therefore, we cannot exclude the possibility that the observed enhancement of hormone action was not due to the last bout of running. This experimental design was used to accurately assess the metabolic state of the muscle in the resting state before the next running session, which always occurred during the dark cycle (1800–0600).

Although the initial training-induced enhancement of insulin-stimulated muscle glucose transport activity was not associated with an increase in total GLUT-4 protein level, the increase in insulin action after 2 and 3 wk of training relative to that at the end of week 1 (Fig. 1) was accompanied by significantly greater total GLUT-4 protein level (Fig. 3B). This is consistent with the idea that an augmented GLUT-4 pool resulting from increased neuromuscular activity can induce a relative enhancement of insulin-stimulated glucose transport activity (7, 9, 12, 19, 23, 26). However, it is clear from the data in the present study (Fig. 2) that, despite the increase in GLUT-4 protein after 2 and 3 wk of training, no further enhancement of IGF-I action on glucose transport activity is induced. This observation provides further indirect evidence for a distinctness, at least in the very proximal elements, between the insulin and IGF-I pathways for activation of glucose transport in skeletal muscle (14).

Henriksen and Halseth (13) have previously observed a coordinated upregulation of GLUT-4 protein level and citrate synthase activity in the fast-twitch plantaris muscle over the course of 4 wk of voluntary WR. The apparent coordinated upregulation of these two proteins involved in glucose transport and oxidation was also reported to occur in the plantaris muscle undergoing chronic low-level electrical stimulation over a 90-day period (8). In the present study, we observed that increases in the GLUT-4 protein level in the fast-twitch epitrochlearis muscle over the 3-wk period of exercise training were strongly correlated with increases in citrate synthase activity (Fig. 4). Interestingly, it has recently been seen that decreases in GLUT-4 protein level in the slow-twitch soleus muscle during 96 h of denervation, a model of decreased neuromuscular activity, are associated with a parallel decline in citrate synthase activity (8a). Collectively, these results are supportive of a coordinated regulation of the levels of GLUT-4 protein and citrate synthase under conditions of altered neuromuscular activity. In contrast, because total hexokinase activity was enhanced after just 1 wk of voluntary exercise training, the regulation of the level of this enzyme appears to be uncoupled from that of citrate synthase and GLUT-4 protein (13).

Henriksen and Halseth have reported that voluntary WR induces greater glycogen storage in soleus (12) and plantaris (13). However, it has also previously been reported that this enhanced glycogen storage does not occur in the epitrochlearis muscle after 5 wk of voluntary WR (23). We have confirmed this latter observation in the present study. These findings indicate that the training-induced glycogen supercompensation seen after voluntary WR is muscle specific.

In summary, we have demonstrated that IGF-I-stimulated glucose transport activity is significantly enhanced in the isolated rat epitrochlearis muscle after voluntary exercise training. Insulin-stimulated glucose transport activity was also enhanced after exercise training. Furthermore, this enhanced hormonal stimulation of glucose transport occurs early (within 1 wk) during the exercise training and is apparent even in the absence of an increase in GLUT-4 protein level. The present findings support the concept that increased IGF-I action may contribute to enhanced glucose regulation after exercise training.

Fig. 4. Relationship between GLUT-4 protein level and citrate synthase activity in epitrochlearis muscles during 3 wk of voluntary wheel running. ●, 1 wk of wheel running; ▲, 2 wk of wheel running; △, 3 wk of wheel running. Data from Fig. 3 were analyzed by linear regression. Correlation coefficient for this data set was 0.844 (P < 0.05).
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