Glycogen supercompensation masks the effect of a training-induced increase in GLUT-4 on muscle glucose transport

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Host, Helen H., Polly A. Hansen, Lorraine A. Nolte, May M. Chen, and John O. Holloszy. Glycogen supercompensation masks the effect of a training-induced increase in GLUT-4 on muscle glucose transport. J. Appl. Physiol. 85(1): 133–138, 1998.—Endurance exercise training induces a rapid increase in the GLUT-4 isoform of the glucose transporter in muscle. In fasted rats, insulin-stimulated muscle glucose transport is increased in proportion to the increase in GLUT-4. There is evidence that high muscle glycogen may decrease insulin-stimulated glucose transport. This study was undertaken to determine whether glycogen supercompensation interferes with the increase in glucose transport associated with an exercise-induced increase in GLUT-4. Rats were trained by means of swimming for 6 h/day for 2 days. Rats fasted overnight after the last exercise bout for an approximately twofold increase in epitrochlearis muscle GLUT-4 and an associated approximately twofold increase in maximally insulin-stimulated glucose transport activity. Epitrochlearis muscles of rats fed rodent chow after exercise were glycogen supercompensated (86.4 ± 4.8 µmol/g wet wt) and showed no significant increase in maximally insulin-stimulated glucose transport above the sedentary control value despite an approximately twofold increase in GLUT-4. Fasting resulted in higher basal muscle glucose transport rates in both sedentary and trained rats but did not significantly increase maximally insulin-stimulated transport in the sedentary group. We conclude that carbohydrate feeding that results in muscle glycogen supercompensation prevents the increase in maximally insulin-stimulated glucose transport associated with an exercise training-induced increase in muscle GLUT-4.

ACTIVATION OF GLUCOSE TRANSPORT by insulin in striated muscle is mediated by translocation of the GLUT-4 isoform of the glucose transporter from intracellular sites to the cell surface (see Ref. 19 for review). The GLUT-1 isoform is also expressed in skeletal muscle, and it is primarily located in the plasma membrane where it appears to play a role in basal, but not insulin-stimulated, glucose transport (25). GLUT-4 protein content varies markedly between skeletal muscle fiber types (17, 22), and there is a good correlation between GLUT-4 content and maximally stimulated glucose transport (17, 24).

Endurance exercise training induces an increase in skeletal muscle GLUT-4 (10, 13, 32, 35, 36). This adaptation occurs rapidly (33). A number of studies have shown that maximally insulin-stimulated glucose transport is increased in proportion to the increase in GLUT-4 in trained muscle (32, 33, 35). The experiments showing that insulin-stimulated glucose transport increases in proportion to the increase in GLUT-4 were done in muscles of fasted animals in which the concentration of glycogen was not elevated. There is considerable evidence that high muscle glycogen levels are associated with decreased insulin-stimulated glucose transport activity (4, 9, 18, 21). Feeding exercise-trained rats a high-carbohydrate diet after glycogen-depleting exercise results in massive glycogen supercompensation (26). In this context, it seemed possible that the increase in insulin-stimulated glucose transport activity in trained muscle with a high GLUT-4 content might be masked in the fed, glycogen-supercompensated state.

In a study in which they used a training protocol that we have found to result in twofold increases in GLUT-4 and maximally insulin-stimulated glucose transport, Reynolds et al. (34) observed only 30–50% increases in GLUT-4 and glucose transport in rat epitrochlearis muscle. The only obvious difference in experimental design was that their rats were fed and ours were fasted. This difference suggested the possibility that feeding and the associated glycogen supercompensation might result in a reduction of insulin-stimulated glucose transport by partially reversing the exercise-induced increase in GLUT-4. The purpose of this study was to determine whether glycogen supercompensation, or a process that occurs concomitantly with the increase in glycogen, interferes with the increase in maximally insulin-stimulated muscle glucose transport induced by exercise training and, if it does, to determine whether glycogen supercompensation masks the effect of an increase in GLUT-4 on glucose transport or results in a lower muscle GLUT-4 content.

METHODS

Materials. 2-[1-3H]deoxy-o-glucose (2-DG) was obtained from American Radiolabeled Chemicals (St. Louis, MO) and mannitol, d-[1-14C] was obtained from NEN Life Science Products (Boston, MA). Purified porcine insulin (Iletin II) was purchased from Eli Lilly. Polyclonal antisera specific for the GLUT-4 (F349) and GLUT-1 (R674) glucose transporters were the generous gift of Dr. Mike Mueckler (Washington University, St. Louis). 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). All other reagents were obtained from Sigma Chemical (St. Louis, MO).

Animal care and exercise program. This research was approved by the Animal Studies Committee of Washington University. Female specific-pathogen-free Wistar rats (body weight 126 ± 3 g) were housed in individual cages and fed a diet of Purina rodent laboratory chow and water ad libitum. Animals were randomized to either an exercise group or a sedentary control group. The exercise group was trained by using a 2-day swimming protocol that has been described previously (33). Rats swam in groups of five or six in steel
The rats were accustomed to swimming for 10 min/day on the 2 days before the start of the training protocol. The swim-training protocol consisted of two 3-h-long swimming sessions separated by a 45-min-long rest period, during which the rats were kept warm and provided with drinking water. After completion of the exercise on the second day, food was withheld from one-half of the trained animals (trained-fasted) and one-half of the sedentary controls (sedentary-fasted), while the remaining animals were fed ad libitum (trained-fed and sedentary-fed). Approximately 18 h after the second exercise bout, which is long enough for the acute effect of exercise on insulin responsiveness to wear off (4), the animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (5 mg/100 g of body wt), and the epitrochlearis and triceps muscles were dissected out. The triceps muscles were immediately frozen with clamps precooled in liquid nitrogen and stored at -80°C. The anesthetized rats were killed by exsanguination.

Muscle incubations. The epitrochlearis muscles were incubated with shaking for 60 min at 30°C in 2 ml of oxygenated Krebs-Henseleit buffer (KHB) in erlenmeyer flasks gassed continuously with 95% O2-5% CO2. The epitrochlearis is a small, thin muscle of the forelimb that, in rats of the size used in this study, is suitable for measurement of sugar transport in vitro (43). The KHB was supplemented with 8 mM glucose, 32 mM mannitol, and 0.1% radioimmunoassay-grade BSA, in the presence or absence of 2 mU/ml purified porcine insulin. This concentration of insulin maximally activates glucose transport in this muscle preparation (38). To remove glucose, muscles were then washed for 10 min at 30°C in KHB containing 40 mM mannitol, 0.1% BSA, and insulin if present in previous incubations. Extracellular space and intracellular 2-DG concentration (µmol·ml⁻²·20 min⁻¹) were determined as previously described (43).

Measurement of immunoreactive GLUT-1 and GLUT-4 proteins. Epitrochlearis and triceps muscles glucose transporter content was determined by Western blotting as described previously (16), by using rabbit polyclonal antibodies directed against either the COOH terminus of GLUT-4 (F349) or GLUT-1 (R674), followed by horseradish peroxidase-conjugated anti-rabbit IgG. Antibody-bound transporter protein was visualized by using enhanced chemiluminescence. Films were scanned by using an imaging densitometer.

Analytic methods. Glycogen was measured in perchloric acid extracts of the epitrochlearis muscle by using the amyloglucosidase method (30). Glycogen synthase was measured in 60% glycerol extracts of the triceps muscles by using a fluorometric indirect assay (31). Muscle hexokinase activity was determined at 30°C as described by Uyeda and Racker (37) on the 700-g supernatant fraction of epitrochlearis muscle homogenates prepared in 10 mM HEPES, 1 mM EDTA, and 250 mM sucrose, pH 7.4.

Statistics. The results are expressed as means ± SE. Significant differences among the four treatment groups (sedentary-fasted, sedentary-fed, trained-fasted, trained-fed) were evaluated by using a two-way ANOVA, with exercise (untrained vs. trained) and dietary manipulation (fasted vs. fed) as the main effects. Post hoc analyses were done with Tukey’s honestly significant difference test with a significance level set at P < 0.05.

RESULTS

Insulin-stimulated glucose transport activity. Basal 2-DG transport in epitrochlearis muscles was ~1.7-fold higher in the sedentary-fasted group than in the sedentary-fed group (0.80 ± 0.11 vs. 0.47 ± 0.06 µmol·ml⁻¹·20 min⁻¹; P < 0.05) (Fig. 1). Feeding rats after their last bout of swimming also affected basal muscle glucose transport activity, with the trained-fed rats having a basal 2-DG uptake rate that was ~50% lower than that of the trained-fasted animals (0.53 ± 0.06 vs. 1.13 ± 0.13 µmol·ml⁻¹·20 min⁻¹; P < 0.001). The 2 days of swim training resulted in an approximately twofold increase in insulin-stimulated glucose transport activity in epitrochlearis muscles of the rats that were not fed after their last exercise session (Fig. 1). Most of this increase in maximally insulin-stimulated glucose transport activity was prevented by feeding the swimmers after their last exercise session. As shown in Fig. 1, insulin-stimulated 2-DG uptake in epitrochlearis muscles of the fed swimmers was significantly lower than in the muscles of the fasted swimmers and was not significantly different than in muscles of the sedentary animals.

Muscle GLUT-4 protein. As shown in Fig. 2, the 2 days of swim training induced an approximately twofold increase in epitrochlearis muscle GLUT-4 content. This increase in GLUT-4 was not affected by feeding the animals after the last exercise bout.

Muscle GLUT-1 protein. Because of the differences in basal glucose transport activity between the fed and fasted rats, we also measured muscle GLUT-1 content...
to evaluate the possibility that adaptive increases in GLUT-1 might account for the higher basal glucose transport rates in the fasted sedentary and exercised animals. Because there was not sufficient epitrochlearis muscle, we measured GLUT-1 in the triceps muscle. This seems a reasonable approach because the rat epitrochlearis (27) and triceps (7) muscles are similar in their fiber-type composition and show a similar adaptive response to the swimming program used in this study (20). As shown in Fig. 3, there were no differences in GLUT-1 content between the muscles of the fed or fasted rats or between the muscles of the exercised and sedentary groups.

Hexokinase. As in previous studies, the swimming program induced a significant increase in hexokinase activity in epitrochlearis muscle (Fig. 4). Feeding the rats after the last exercise bout had no effect on hexokinase activity.

Muscle glycogen. Epitrochlearis muscle glycogen concentration in the swimmers that were fed was ~7.5-fold higher than that in the fasted swimmers and ~3-fold higher than that in the fed sedentary animals (Fig. 5).

Glycogen synthase. The 2 days of swim training had no significant effect on glycogen synthase activity in the epitrochlearis muscle (Fig. 6). The percentage of glycogen synthase in the I form was approximately twofold higher in the fasted than in the fed groups.
DISCUSSION

The present results show that feeding a high carbohydrate diet that causes glycogen supercompensation after exercise largely prevents the increase in maximally insulin-stimulated glucose transport induced by exercise training. Normally, stimulated glucose transport in muscle roughly parallels GLUT-4 content (17, 24, 33). The reduction in glucose transport activity with glycogen supercompensation is mediated by a masking of the effect of the twofold increase in GLUT-4 protein induced by exercise training rather than by prevention or reversal of the increase in GLUT-4. A number of previous studies have provided evidence that there is a relationship between muscle glycogen and glucose transport (4, 9, 18, 21, 29, 45). Three approaches have been used to change muscle glycogen concentration: exercise, fasting, and β-adrenergic stimulation.

The effects of exercise on muscle glucose transport are complex and are made more so by the interactions between exercise and nutritional state/muscle glycogen. Exercise has three separate effects on muscle glucose transport. The first is a direct stimulation of glucose transport that is independent of (39, 40) and additive to the effects of insulin (17, 28, 38, 46). Indirect evidence suggests that the decrease in muscle glycogen induced by contractions is not responsible for the stimulation of glucose transport. This evidence consists of the finding that the increase in glucose transport reverses after exercise even when glycogen resynthesis is prevented by the absence of glucose (14, 44). Second, as the acute stimulation of glucose transport wears off after exercise, it is replaced by an increase in insulin sensitivity (11, 38) (insulin sensitivity is defined as the concentration of insulin required to induce a half-maximal increase in glucose transport). This increase in insulin sensitivity is markedly affected by carbohydrate feeding and muscle glycogen level. If glycogen is kept low, i.e., in the fasting range, either by fasting or feeding a carbohydrate-free diet (4), the increase in insulin sensitivity persists for at least 48 h and probably longer. On the other hand, if muscle glycogen is raised by feeding carbohydrate, the increase in insulin sensitivity rapidly reverses as muscle glycogen concentration rises above the usual fed level, i.e., when glycogen supercompensation occurs (4).

The third effect of exercise is the training-induced increase in muscle GLUT-4, which occurs very rapidly (33) and is associated with an increase in insulin responsiveness of glucose transport (32, 33, 35) (insulin responsiveness of glucose transport is defined as the glucose transport rate that results from a maximally effective insulin stimulus). As shown in the present study, the effect of the training-induced increase in muscle GLUT-4 on the insulin responsiveness of glucose transport is masked by glycogen supercompensation.

The effects of short-term fasting on muscle glycogen and glucose transport are less dramatic than the effects of exercise. In the present study the fasted rats were not fed for ~24 h before their muscles were harvested; however, the period in which they were actually in the fasted state was considerably shorter, because the upper one-half of the rat's stomach serves as a food storage pouch that empties only gradually. As in previous studies on the effects of fasting (2, 12) or food restriction (3), basal glucose transport was higher in the fasted than in the fed rats' muscles in both the sedentary and the exercise-trained groups. It has been shown that prolonged fasting, for 3–4 days, induces a two- to threefold increase in muscle GLUT-4 in rats (1, 5). The finding that muscle GLUT-4 concentration was not increased in the present study is probably due to the relatively short duration of the fast. Muscle GLUT-1 concentration was also unchanged in the fasting rats in the present study, confirming the finding of Woloschak et al. (41). Thus the increase in basal glucose transport activity is not due to an increase in GLUT-1.

In the present study there was no significant difference in maximally insulin-stimulated glucose transport between the muscles of the fed and the fasted sedentary rats. This finding suggests that relatively small differences in muscle glycogen such as that found in our fed and fasted control animals (28.11 ± 1.99 compared with 14.02 ± 0.77 μmol/g wet wt) do not have a major effect on insulin responsiveness. However, in a study that also compared epitrochlearis muscles of fed and 24-h-fasted rats, Jensen et al. (21) found a large (~2-fold) effect of fasting on maximally insulin-stimulated glucose transport. The reason for this difference between our results and those of Jensen et al. is not obvious because muscle glycogen levels and the methodology used were similar in the two studies. Other studies on the effect of fasting on maximally insulin-stimulated glucose uptake in the perfused rat hindquarter or using the euglycemic clamp technique also provide evidence that short-term fasting does not increase insulin responsiveness (2, 42). On the other hand, fasting or food restriction that results in a decrease in muscle glycogen does appear to increase muscle insulin sensitivity (2, 12). Another approach that has been used to lower...
Muscle glycogen is treatment of rats with epinephrine. Muscles of rats injected with epinephrine had an ~40% reduction in glycogen content and showed significant increases in both basal glucose transport and in the insulin sensitivity of glucose transport but no increase in insulin responsiveness (29).

Taken together these results show that 1) reduction of muscle glycogen concentration into the range seen with fasting is associated with increases in basal glucose transport and in the insulin sensitivity of the glucose transport process in muscle and 2) muscle glycogen supercompensation is associated with reversal of increased insulin sensitivity after a bout of exercise, and, as found in the present study, masking of the increase in maximally insulin-stimulated glucose transport associated with an exercise training-induced increase in GLUT-4. At this time, the mechanism(s) underlying the relationship between muscle glycogen concentration and glucose transport is still a mystery. One possibility that we are pursuing is that some of the GLUT-4 molecules are, like glycogen synthase and phosphorylase, incorporated into the glycogen-protein complex (6, 8) so that they are not available for translocation to the cell surface. It is, of course, also possible that muscle glycogen is not directly involved in the inhibition of glucose transport and that a process that occurs concomitantly with glycogen supercompensation is responsible. One possibility that we are currently trying to evaluate is that a sufficient fraction of the glucose flooding into the muscle cells after exercise enters the hexosamine, rather than the glycogen, synthetic pathway, resulting in inhibition of GLUT-4 translocation (23). Another is that the GLUT-4 protein is modified (for example, by glycation) so as to result in a decrease in intrinsic glucose transport activity.

In conclusion, the results of this study show that muscle glycogen supercompensation, or some closely associated process for which muscle glycogen supercompensation serves as a marker, prevents the training-induced increase in GLUT-4 from manifesting itself as an increase in maximally insulin-stimulated glucose transport. This phenomenon may represent a feedback mechanism by which high muscle glycogen levels reduce glucose transport activity so as to prevent muscle glycogen from increasing to levels that cause muscle stiffness and impair performance.

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REFERENCES


