

# Rapid reversal of adaptive increases in muscle GLUT-4 and glucose transport capacity after training cessation

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**Host, Helen H., Polly A. Hansen, Lorraine A. Nolte, May M. Chen, and John O. Holloszy.** Rapid reversal of adaptive increases in muscle GLUT-4 and glucose transport capacity after training cessation. *J. Appl. Physiol.* 84(3): 798–802, 1998.—Previous studies have shown that when exercise is stopped there is a rapid reversal of the training-induced adaptive increase in muscle glucose transport capacity. Endurance exercise training brings about an increase in GLUT-4 in skeletal muscle. The primary purpose of this study was to determine whether the rapid reversal of the increase in maximally insulin-stimulated glucose transport after cessation of training can be explained by a similarly rapid decrease in GLUT-4. A second purpose was to evaluate the possibility, suggested by previous studies, that the magnitude of the adaptive increase in muscle GLUT-4 decreases when exercise training is extended beyond a few days. We found that both GLUT-4 and maximally insulin-stimulated glucose transport were increased approximately twofold in epitrochlearis muscles of rats trained by swimming for 6 h/day for 5 days or 5 wk. GLUT-4 was 90% higher, citrate synthase activity was 23% higher, and hexokinase activity was 28% higher in triceps muscle of the 5-day trained animals compared with the controls. The increases in GLUT-4 protein and in insulin-stimulated glucose transport were completely reversed within 40 h after the last exercise bout, after both 5 days and 5 wk of training. In contrast, the increases in citrate synthase and hexokinase activities were unchanged 40 h after 5 days of exercise. These results support the conclusion that the rapid reversal of the increase in the insulin responsiveness of muscle glucose transport after cessation of training is explained by the short half-life of the GLUT-4 protein.

2-deoxy-D-glucose; exercise; insulin

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EXERCISE HAS A NUMBER OF EFFECTS on glucose transport in skeletal muscle. These have generally been classified as acute or short-term responses to a single bout of exercise and as the longer term effects of exercise training. Muscle contractions stimulate glucose transport directly, independent of insulin action (13, 22, 33), by inducing translocation of the GLUT-4 isoform of the glucose transporter to the cell surface (5, 12, 17). This phenomenon is responsible for the acute effect of exercise on glucose transport. The acute exercise-induced activation of glucose transport wears off rapidly after exercise is stopped and is replaced by a large increase in insulin sensitivity (4, 25). This increase in insulin sensitivity persists for varying time periods depending on carbohydrate intake, and its reversal appears to coincide with the development of muscle glycogen supercompensation (4).

Exercise training also induces increases in insulin-stimulated and contraction-stimulated glucose trans-

port. This increase in glucose transport capacity is mediated by an adaptive increase in GLUT-4 (7, 23, 27). Although this adaptation was generally thought to be an effect of long-term training, it has become evident that the increase in GLUT-4 protein and the associated increase in maximally stimulated glucose transport occur very rapidly. For example, Ren et al. (24) found that 6 h of swimming per day induced an ~50% increase in GLUT-4 protein that was evident ~16 h after the first period of exercise and a twofold increase in GLUT-4 protein after 2 days of exercise. Additional days of exercise had no further effect on muscle GLUT-4 content or maximally stimulated glucose transport.

It is now well documented that exercise training induces an increase in muscle GLUT-4 that is associated with an increase in the maximal capacity for glucose transport. It is, therefore, puzzling that a number of studies have found no increase in maximally insulin-stimulated glucose uptake by perfused hind-limb muscles of trained rats 48 h after the last exercise bout (6, 14, 15). The finding that 2 days of exercise can induce a twofold increase in GLUT-4 (24) indicates that this protein has a short half-life. The time course of the decline in the concentration of a protein after removal of an adaptive stimulus is also determined by its half-life (29). It, therefore, seemed possible that a rapid decrease in glucose transport capacity could be mediated by reversal of the adaptive increase of GLUT-4 protein. One purpose of the present study was to investigate this possibility. It appeared from the results of some previous studies (3, 7, 8, 11, 19, 23, 26) that prolonged training results in a smaller increase in GLUT-4 than does brief training (24); i.e., that the magnitude of the adaptive increase declines when exercise training is extended beyond a few days. The second purpose of this study was to investigate this possibility.

## MATERIALS AND METHODS

### Materials

2-[1,2-<sup>3</sup>H]-deoxy-D-glucose (2-DG) was obtained from American Radiolabeled Chemicals (St. Louis, MO), and D-[1-<sup>14</sup>C]mannitol was obtained from NEN Life Science Products (Boston, MA). Purified porcine insulin (Iletin II) was purchased from Lilly. Polyclonal antiserum specific for the GLUT-4 glucose transporter (F349) was the generous gift of Dr. Mike Mueckler (Washington University, St. Louis, MO). Horseradish peroxidase-conjugated donkey anti-rabbit immunoglobulin G 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.

**Short-term training.** Six-week-old (body weight 128–148 g) female, specific-pathogen-free Wistar rats were housed in individual cages and fed a diet of Purina rodent laboratory chow and water ad libitum. They were randomized to one of three groups: sedentary control, 5-day trained, and 5-day trained/40-h detrained. The rats were trained by using a swimming protocol that has been described previously (24). Briefly, rats swam in groups of five to six in steel barrels filled with water maintained at 34–35°C. The animals were accustomed to swimming for 10 min/day on the 2 days before the start of the training protocol. They swam for two 3-h-long bouts separated by a 45-min-long rest period. Approximately 16 h after the last exercise bout, the 5-day-trained rats and one-half of the control rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body wt), and the epitrochlearis and triceps muscles were dissected out. Sixteen hours is long enough for the acute effect of exercise on insulin responsiveness to wear off (4). The animals were not fed during the 16-h period between the last exercise bout and time the muscles were taken.

Forty hours after the last exercise bout, the remaining control and 5-day-trained animals were anesthetized in the same manner. They were fasted for the same length of time (~22 h) as were the animals that were studied 16 h after the swimming. All anesthetized rats were killed by exsanguination.

**Long-term training.** A separate group of 6-wk-old female specific-pathogen-free Wistar rats was housed in individual cages and fed a diet of Purina rodent laboratory chow and water ad libitum. They were randomly assigned to one of three groups: sedentary controls, 5-wk trained, and 5-wk trained/40-h detrained. Animal care and exercise training were performed in exactly the same manner as with the 5-day trained animals, except these animals were trained for 5 wk (6 days/wk). The animals weighed ~210 g at the end of the 5-wk training program.

#### Muscle Incubations: Effects of Insulin

The epitrochlearis is a small, thin muscle of the forelimb that is suitable for measurement of glucose transport activity in vitro (20, 34). ATP, creatine phosphate, and glycogen levels are stable for at least 9 h in epitrochlearis muscles incubated with substrate in oxygenated medium (9). Epitrochlearis muscles were incubated for 60 min in 2 ml of oxygenated Krebs-Henseleit buffer (KHB) supplemented with 8 mM glucose, 32 mM mannitol, and 0.1% radioimmunoassay-grade bovine serum albumin (BSA), in the presence or absence of a maximally effective concentration of purified porcine insulin (2 mU/ml). Muscles were then incubated for 10 min in KHB containing 40 mM mannitol, 0.1% BSA, and insulin, if present in previous incubations, to wash glucose out of the extracellular space. All incubations were performed in stoppered Erlenmeyer flasks with a gas phase of 95% O<sub>2</sub>-5% CO<sub>2</sub>. The flasks were shaken in a Dubnoff incubator at 30°C.

#### Measurement of Glucose Transport Activity

Glucose transport activity was measured as described previously (10). After the rinse, muscles were incubated at 30°C for 20 min in 1.0 ml KHB containing 4 mM 2-DG (1.5  $\mu$ Ci/ml), 36 mM [<sup>14</sup>C]mannitol (0.2  $\mu$ Ci/ml), 0.1% BSA, and

insulin if it was present in the previous incubation. Extracellular space and intracellular 2-DG concentration ( $\mu$ mol·ml intracellular water<sup>-1</sup>·20 min<sup>-1</sup>) were determined as previously described (34).

#### Measurement of Immunoreactive GLUT-4 Protein

Epitrochlearis and triceps GLUT-4 glucose transporter content were determined by Western blotting as described previously (11), by using a rabbit polyclonal antibody directed against the COOH terminus of GLUT-4 (F349) followed by horseradish peroxidase-conjugated anti-rabbit immunoglobulin G. Antibody-bound transporter protein was visualized by using enhanced chemiluminescence according to the manufacturer's specifications. Films were scanned by using an imaging densitometer.

#### Analytic Methods

Muscle glycogen was measured by using the amyloglucosidase method (21). Citrate synthase activity was measured as described by Srere (30). Muscle hexokinase activity was determined as described by Uyeda and Racker (31).

#### Statistics

The results are expressed as means  $\pm$  SE. The significance of differences between the trained, 40-h detrained, and sedentary control groups was evaluated with one-way analysis of variance. When significant differences were found, a Tukey's honestly significant difference test was performed with the significance level set at  $P < 0.05$ .

## RESULTS

#### Effects of 5 Days of Swimming on Muscle Glucose Transport and GLUT-4

GLUT-4 protein content in the epitrochlearis was increased by 100% in the 5-day-trained animals compared with their untrained counterparts (Fig. 1). The adaptive increase in GLUT-4 was completely lost within 40 h. The 5 days of exercise also resulted in a parallel approximately twofold increase in maximally insulin-

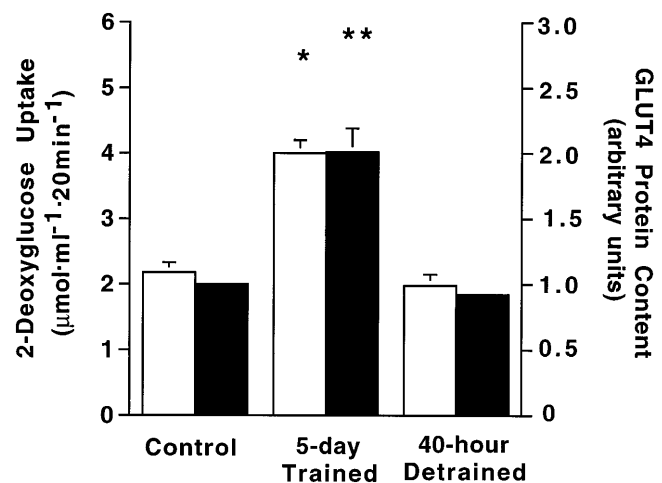


Fig. 1. Effect of 5 days of swim training and 40 h of rest, posttraining, on maximally insulin-stimulated 2-deoxy-D-glucose (2-DG) uptake and GLUT-4 protein content in epitrochlearis muscle. Values are means  $\pm$  SE for 6–12 rats per group. Open bars, insulin-stimulated 2-DG uptake; filled bars, GLUT-4 protein content. \* $P < 0.001$  vs. control and 40-h detrained. \*\* $P < 0.01$  vs. control and 40-h detrained.

stimulated glucose transport that was completely lost within 40 h after cessation of exercise (Fig. 1). There was a similar increase in GLUT-4 in the triceps muscles (1.9-fold increase in the 5-day-trained animals) (Table 1). Like the epitrochlearis, the triceps GLUT-4 protein returned to sedentary control levels within 40 h postexercise.

### Muscle Glycogen

Muscle glycogen concentration in the three groups was determined on epitrochlearis muscle. The muscle glycogen concentration was higher in the 5-day swimmers studied 40 h after the exercise than in those studied after 16 h but was still in the range normally seen in the fasted state (Fig. 2).

### Muscle Enzyme Activities

The 5 days of swimming induced a 28% increase in hexokinase activity in the triceps muscle. This increase was still present 40 h after cessation of exercise (Table 1). There was also an ~23% increase in citrate synthase activity; this increase was not statistically significant by using analysis of variance. However, because there was no difference between the 16- and 40-h postexercise values, we combined these results and compared them with the control value by using a *t*-test, which gave a significance value of  $P < 0.05$ .

### Effects of 5 Wk of Swimming on Muscle Glucose Transport and GLUT-4

To evaluate the effect of longer term training, another group of animals was trained with the same swimming protocol for 5 wk. After 5 wk of swim training, there was an ~2.5-fold increase in GLUT-4 protein content in the epitrochlearis; 40 h after the last exercise session, GLUT-4 protein content was back to control levels (Fig. 3). Maximally insulin-stimulated glucose transport in the 5-wk-trained group was approximately two times higher than that in the sedentary controls (Fig. 3). This increase in glucose transport activity was lost within 40 h after exercise was stopped.

Table 1. Effect of 5 days of swim training on GLUT-4, hexokinase, and citrate synthase activity in the triceps

	Control	5-day Trained	40-h Detrained
GLUT-4 protein content, arbitrary units	1.00 ± 0.00 (11)	1.90 ± 0.33* (5)	0.82 ± 0.25 (5)
Hexokinase activity, $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g protein}^{-1}$	18.25 ± 1.08 (12)	23.39 ± 0.93* (6)	24.35 ± 1.70† (6)
Citrate synthase activity, $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g muscle wet wt}^{-1}$	18.65 ± 1.78 (12)	22.31 ± 2.69* (6)	23.47 ± 3.85* (6)

Values are means ± SE for each group; no. of animals in each group is given in parentheses. Significantly different compared with sedentary control: \*  $P < 0.05$ ; †  $P < 0.01$ .

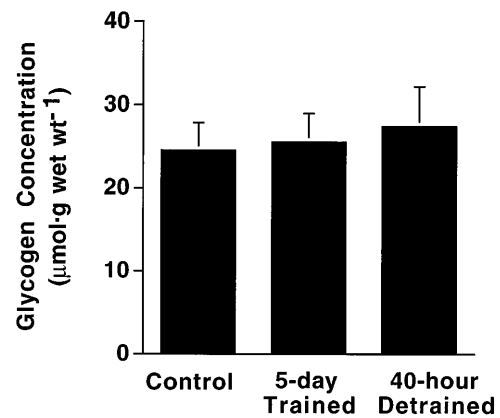


Fig. 2. Effect of 5 days of swim training followed by 40 h of rest on glycogen concentration in epitrochlearis muscle. Values are means ± SE for 5–9 rats per group.

### DISCUSSION

One purpose of this study was to determine whether the adaptive increase in muscle GLUT-4 in response to exercise reverses as rapidly as it develops. It was previously found that there was a twofold increase in epitrochlearis muscle GLUT-4 16 h after the second bout of the same 6-h swimming protocol that was used in the present study (24). A similar increase was seen after 5 days of the swimming program in the present study, indicating that the entire adaptive response occurs within 2 days. Our finding that the adaptive increase in muscle GLUT-4 had completely reversed within 40 h after the last exercise bout shows that the time course of the decrease in GLUT-4 is similar to the time course of the increase. This finding is not surprising because the time required to attain a new steady state in response to an adaptive increase in protein synthesis and the time required to return to baseline level after the adaptive stimulus is removed are both determined by the half-life of the protein (29). It seems clear from these findings that the GLUT-4 protein has a

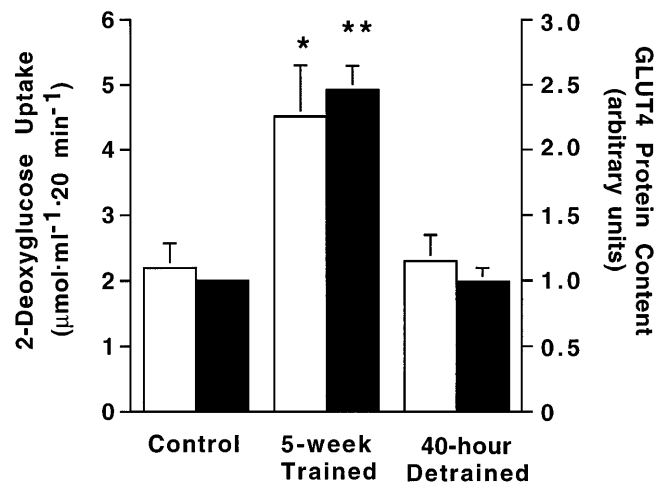


Fig. 3. Effect of 5 wk of swim training and 40 h of rest, posttraining, on maximally insulin-stimulated 2-DG uptake and GLUT-4 protein content in epitrochlearis muscle. Values are means ± SE for 5 rats per group. Open bars, 2-DG uptake; filled bars, GLUT-4 protein content. \*  $P < 0.05$  vs. control and 40-h detrained. \*\*  $P < 0.01$  vs. control and 40-h detrained.

short half-life, probably in the range of 8–10 h. This is in contrast to citrate synthase, which has a half-life of ~7 days (1). An adaptive increase of a protein can, theoretically, also result from a decrease in the rate of degradation, i.e., an increase in half-life; in this case the rate of reversal of the adaptation would occur more rapidly than its development. However, it seems likely that in the case of GLUT-4 the adaptive increase in response to exercise results from an increase in protein synthesis, because there is a large increase in GLUT-4 mRNA that occurs more rapidly than the increase in protein (24).

A number of studies using the perfused rat hindquarter preparation have shown that the increase in insulin-stimulated glucose uptake induced by exercise training is lost within 48 h after exercise is stopped (6, 14, 15). The second purpose of this study was to test the hypothesis that this finding is explained by a rapid decrease in GLUT-4 with an accompanying rapid reversal of the increase in maximally insulin-stimulated glucose transport. The present results support this hypothesis because the increase in GLUT-4 in response to the exercise and the decrease in GLUT-4 after cessation of exercise were paralleled by proportional changes in maximally insulin-stimulated glucose transport. These findings are somewhat different from those of Etgen et al. (6), who found that a training-induced increase in glucose uptake in perfused rat hindlimb muscles was lost 48 h after the last exercise bout despite a persistent increase in muscle GLUT-4 content. We have no explanation for the difference between our results and those of Etgen et al. It is of interest, however, that the running program used by Etgen et al. resulted in a much smaller increase in GLUT-4 protein in hindlimb muscles, ~30%, than that induced in the epitrochlearis muscle by our swimming program, ~100%.

In previous studies, involving longer periods of training with various types of exercise (3, 7, 8, 11, 19, 23, 26), the increases in muscle GLUT-4 protein concentration were considerably smaller than those induced by 2 or 5 days of our swimming program. Thus it seemed possible that, as a result of some of the other adaptations induced by training, the magnitude of the adaptive increase in GLUT-4 might be greatest at the onset of training and then decrease as the training progressed. To determine whether the initial adaptive increase in GLUT-4 partially reverses with more prolonged training, some of the rats in the study were trained by means of a 5-wk-long swimming program. Our results provide evidence that the full adaptive response is maintained for at least 5 wk of training. The finding that the increases in GLUT-4 and insulin-stimulated glucose transport were similar in the 5-day-trained and 5-wk-trained animals was unexpected, because in two previous studies in which rats were exercised by swimming for 6 h a day for 3–5 wk the increase in epitrochlearis GLUT-4 averaged ~50% (11, 19). In retrospect, this apparent discrepancy can be explained by differences in the design of the three studies. In the study by Hansen et al. (11), the rats were much fatter and, therefore,

more buoyant than the rats in the present study; the smaller increase in GLUT-4 in their muscles is, therefore, probably explained by a smaller exercise stimulus. In the study by Nakatani et al. (19), the final exercise session involved only 2 h of swimming and groups of rats were killed over a 48-h period after the exercise. Because it was not realized at that time that the adaptive increase in GLUT-4 reverses so quickly, the GLUT-4 data on all of the rats were pooled.

Numerous studies in which glucose tolerance or insulin action was evaluated in humans after cessation of exercise training have provided evidence that the effect of training on insulin-stimulated glucose disposal is lost rapidly (16, 18, 28, 32). In some of these studies, the period of detraining was 7–10 days (16, 18, 28); however, judging from the present results, the loss of this training effect would have been evident in a much shorter time period. In a study by King et al. (16) in which the effects of training and detraining were evaluated by using an euglycemic hyperinsulinemic clamp procedure, it was concluded that the effects of training and detraining were on insulin sensitivity rather than on insulin responsiveness. It now seems clear from studies on isolated muscles that the effect of training is actually on insulin responsiveness and is mediated by an increase of GLUT-4. It seems probable that the increase in insulin responsiveness was missed in the study by King et al., because glucose delivery, i.e., blood flow, rather than muscle glucose transport becomes rate limiting at the unphysiologically high insulin concentration required to study insulin responsiveness (2).

In conclusion, the results of this study provide evidence that, because of the short half-life of the GLUT-4 protein, the adaptive increases in GLUT-4 and in insulin-stimulated glucose transport induced by exercise training reverse within 2 days after the exercise is stopped. Therefore, to maintain this adaptation, it is necessary to exercise at least every other day.

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