Effect of endurance exercise training on muscle glycogen supercompensation in rats

AKIRA NAKATANI, DONG-HO HAN, POLLY A. HANSEN, LORRAINE A. NOLTE, HELEN H. HOST, ROBERT C. HICKNER, AND JOHN O. HOLLOSZY
Department of Internal Medicine, Washington University School of Medicine, St. Louis, Missouri 63110

Nakatani, Akira, Dong-Ho Han, Polly A. Hansen, Lorraine A. Nolte, Helen H. Host, Robert C. Hickner, and John O. Holloszy. Effect of endurance exercise training on muscle glycogen supercompensation in rats. J. Appl. Physiol. 82(2): 711–715, 1997.—The purpose of this study was to test the hypothesis that the rate and extent of glycogen supercompensation in skeletal muscle are increased by endurance exercise training. Rats were trained by using a 5-wk-long swimming program in which the duration of swimming was gradually increased to 6 h/day over 3 wk and then maintained at 6 h/day for an additional 2 wk. Glycogen repletion was measured in trained and untrained rats after a glycogen-depleting bout of exercise. The rats were given a rodent chow diet plus 5% sucrose in their drinking water ad libitum during the recovery period. There were remarkable differences in both the rates of glycogen accumulation and the glycogen concentrations attained in the two groups. The concentration of glycogen in epitrochlearis muscle averaged 13.1 ± 0.9 mg/g wet wt in the untrained group and 31.7 ± 2.7 mg/g in the trained group (P < 0.001) 24 h after the exercise. This difference could not be explained by a training effect on glycogen synthase. The training induced ~50% increases in muscle GLUT-4 glucose transporter protein and in hexokinase activity in epitrochlearis muscles. We conclude that endurance exercise training results in increases in both the rate and magnitude of muscle glycogen supercompensation in rats.

glucose transporter isoform 4; glucose transporter; glycogen synthase; hexokinase

AS CLEARLY SHOWN in the classic study by Bergstrom and Hultman (3), and confirmed in numerous subsequent investigations (2, 9, 16, 17), a glycogen-depleting bout of exercise followed by a high carbohydrate diet results in an increase in muscle glycogen to levels well above those normally seen in the fed state. This “glycogen supercompensation” phenomenon has generally been attributed to the activation of glycogen synthase (GS) after exercise and to the increase in muscle insulin sensitivity that persists for a variable period of time after exercise (6, 15, 16). It has also been proposed that GS activity plays a key role in determining the rates of insulin-stimulated glucose uptake and glycogen synthesis in muscle (5, 18, 29). However, the activation of GS that is present in the glycogen-depleted state after exercise reverses before glycogen supercompensation occurs, approximately at the point when glycogen concentration increases to levels seen in the resting, fed state (4, 8).

Recent studies on transgenic mice overexpressing the GLUT-1 isoform of the glucose transporter (22) and on rats in which the GLUT-4 glucose transporter content was raised by means of exercise training (23) have provided additional evidence that the extent and rate of glycogen accumulation are limited by the rate of glucose uptake, not by GS activity, in muscle. In this context, the goal of the present study was to test the hypothesis that the rate and extent of glycogen super-compensation are higher in endurance exercise-trained than in untrained muscle. This hypothesis was based on the findings that 1) exercise training induces an increase in muscle GLUT-4 (see Ref. 14 for review), 2) insulin-stimulated glucose transport activity is increased in proportion to the increase in GLUT-4 (14), and 3) the rate of glycogen accumulation is greater in trained than in untrained muscle incubated with glucose and insulin in vitro (23). The rat epitrochlearis muscle was used for this research because it is extensively used during swimming, the form of exercise employed in the present study. This is evidenced by glycogen depletion, stimulation of glucose transport and increased insulin sensitivity in response to a bout of exercise, and adaptive increases in GLUT-4 and hexokinase (6, 23, 30).

METHODS

Animal care and exercise program. Seven-wk-old female specific pathogen-free Wistar rats were housed in individual cages and fed a diet of Purina rodent laboratory chow 5001 (which contains 481 g of carbohydrate/kg) and water ad libitum. They were randomized to either an exercise group or a sedentary control group. The exercise group was trained by means of an ~5-wk-long swimming program that has been described previously (10). Animals swam in groups of six, and the duration of swimming was increased 30 min/day until they were swimming a total of 6 h/day 5 days/wk. They were maintained at this level of training for an additional 3 wk. At the time of the glycogen repletion experiment, the average body weight of the exercise group was 187 ± 4 g, whereas the body weight of the sedentary group averaged 180 ± 6 g.

Glycogen depletion exercise bout and tissue collection. After an overnight fast, both sedentary and trained animals were exercised by using a swimming protocol, described previously.
(6, 28), to deplete muscle glycogen. The protocol consisted of four 30-min bouts of swimming separated by 5-min rest periods. During the last three 30-min intervals, the rats swam with a weight equal to 2% of their body weight tied to the base of their tails. Immediately after exercise, subgroups of animals were anesthetized with pentobarbital sodium (5 mg/100 g body weight). The epitrochlearis muscles were dissected out and clamp frozen by using tongs precooled in liquid nitrogen. Blood (~1 ml) was drawn from the abdominal aorta, placed in heparinized microfuge tubes, and centrifuged. Plasma samples were frozen for subsequent determination of insulin concentration.

The remaining rats were given Purina chow and 5% sucrose in their drinking water ad libitum until 4, 24, or 48 h after the exercise, when tissues were collected as described above. We have observed that rats generally start drinking soon after completion of swimming. The rationale for sucrose in the drinking water was to ensure an adequate carbohydrate intake. This research was approved by the Animal Studies Committee of the Washington University School of Medicine.

Analytic methods. Muscle glycogen was measured by using the amyloglucosidase method as described previously (20, 30). GS activity was determined fluorometrically by using the method of Passonneau and Lowry (21). Muscle hexokinase was determined as described by Uyeda and Racker (27) on the 700-g supernatant fraction of muscle homogenates prepared in HES buffer [in mM] 10 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, 1 EDTA, 250 sucrose, pH 7.4]. All of the enzyme assays were conducted at 30°C.

Muscle GLUT-4 glucose transporter content was determined by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis/Western blotting as described previously (11). Glucose transporter protein was detected with a rabbit polyclonal antibody directed against the COOH-terminus of GLUT-4 (F349; the generous gift of Dr. Mike Mueckler, Washington University School of Medicine), followed by horse-radish peroxidase-conjugated donkey anti-rabbit immunoglobulin G (Jackson ImmunoResearch Laboratories, West Grove, PA). Antibody-bound transporter protein was visualized by using enhanced chemiluminescence (Amersham) according to manufacturer’s specifications. Films were scanned with an imaging densitometer (Bio-Rad GS-670, Hercules, CA).

Plasma insulin concentration was determined by Washington University’s Diabetes Research and Training Center Radioimmunoassay Core Laboratory by using a double antibody radioimmunoassay (19). Plasma glucose was analyzed by using the glucose oxidase method (Beckman Instruments, Fullerton, CA).

Statistics. The results are expressed as means ± SE. The significance of differences between the trained and untrained groups was evaluated by using Student’s unpaired t-test.

RESULTS

Muscle glycogen concentration. As shown in Fig. 1, there were remarkable differences in the rate of glycogen accumulation, and in the magnitude of the final glycogen concentration attained, between epitrochlearis muscles of the exercise-trained and the untrained rats after the glycogen-depleting exercise. The glycogen concentration in epitrochlearis muscle 24 h after exercise averaged 73 ± 5 µmol/g wet weight in the untrained animals compared with 176 ± 15 µmol/g in the trained group 24 h after the exercise. There was no further increase in muscle glycogen level between 24 and 48 h after exercise (Fig. 1).

GS. The training program induced a small (18%) but statistically significant increase in total GS activity in the epitrochlearis muscle. Total GS activity [i.e., GS in I form (GS I) + GS in D form (GS D)] averaged 1.52 ± 0.10 µmol·min⁻¹·g muscle⁻¹ in the untrained group and 1.80 ± 0.10 µmol·min⁻¹·g muscle⁻¹ in the trained group (P = 0.05). However, the faster rate of glycogen supercompensation in the trained group cannot be explained on the basis of a higher level of GS I activity. This is because, by 4 h after the glycogen-depleting exercise, the percentage of GS I was sufficiently lower in the trained than in the untrained group to more than counter the effect of the small increase in total GS activity (Fig. 2). The greater %GS I in the untrained group after 4 h probably is due to the muscle glycogen
level attained at this time not being sufficiently high to exert a maximal inhibitory effect on GS activation. It is interesting in this context that the massive glycogen supercompensation that occurred between 4 and 24 h after exercise in the muscles of the trained group took place despite the suppression of GS I activity to a very low level.

GLUT-4 and hexokinase. As shown in Table 1, both total GLUT-4 concentration and hexokinase activity were increased ~50% in the epitrochlearis muscles of the swim-trained group. Thus the swimming program resulted in significant increases in the capacities of muscle both to take up and to phosphorylate glucose.

Plasma glucose and insulin. As shown in Fig. 3, plasma insulin levels were extremely low immediately after exercise and increased progressively over the 48-h postexercise period. Plasma insulin levels were not significantly different in the two groups. It, therefore, seems clear that the differences in the rate and extent of glycogen accumulation between the trained and untrained groups cannot be explained on the basis of differences in the insulin levels. The plasma glucose values are probably raised as the result of the stress of anesthesia but do at least provide some evidence that the trained animals did not have higher plasma glucose concentrations.

DISCUSSION

The results of this study confirm our hypothesis that both the rate and extent of muscle glycogen accumulation after glycogen-depleting exercise are increased in rats that have adapted to endurance exercise training. To our knowledge, only two previous studies have provided evidence suggesting that exercise training might enhance glycogen supercompensation. One was an early study by Lamb et al. (17), in which trained and untrained guinea pigs were exercised by means of running for 30 min and muscle glycogen was measured either immediately or 48 h after exercise. Muscle glycogen concentrations were higher in the trained than in the untrained group at 48 h. However, the exercise resulted in much less glycogen depletion in the trained than in the untrained group. As a result, the total increase in muscle glycogen over the 48 h was actually smaller in the trained group. In the other study, Tan et al. (26) examined muscle glycogen repletion in trained and untrained rats 1 h after a glycogen-depleting exercise bout. They found that the increase in muscle glycogen was about twofold greater in the trained than in the untrained rats during the 60-min recovery period.

It has been proposed, on the basis of studies in humans in which glucose and insulin infusions were combined with muscle biopsies, that GS activity limits both glucose uptake and glycogen accumulation in muscle (5, 18, 29). One line of evidence arguing against this interpretation was provided by studies on transgenic mice overexpressing the GLUT-1 glucose transporter in their skeletal muscles (22). In these animals, the basal glucose transport rate in muscle was sevenfold higher than normal and greater than the maximally insulin-stimulated glucose transport rate in the nontransgenic controls. These high glucose uptake rates resulted in muscle glycogen concentrations that were 10-fold greater than those seen in the nontransgenic controls (22). This enormous increase in muscle glycogen concentration occurred despite significantly lower than normal total GS and %GS I activities in muscles of the transgenic mice. Further evidence against a primary role of GS activity in regulating the rate of muscle glycogen accumulation came from a study of rat epitrochlearis muscles, in which GLUT-4 content had been increased by exercise training (23). When muscles were incubated in vitro with glucose and insulin, the trained muscles accumulated glycogen more rapidly than control muscles, despite lower levels of %GS I activity in the trained group. In the present

Table 1. Effects of training on epitrochlearis muscle GLUT-4 content and hexokinase activity

<table>
<thead>
<tr>
<th></th>
<th>Untrained</th>
<th>Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUT-4 content</td>
<td>1.18 ± 0.06</td>
<td>1.75 ± 0.13**</td>
</tr>
<tr>
<td>Hexokinase activity</td>
<td>2.17 ± 0.10</td>
<td>3.06 ± 0.22*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 18 for untrained control and 19 from swim-trained animals. Values for GLUT-4 glucose transporter protein are in arbitrary densitometric units. Values for hexokinase are in µmol·min⁻¹·g muscle wet wt⁻¹. Significantly different compared with untrained control: *P < 0.01, **P < 0.001.
study also, the more than twofold higher rates of glycogen accumulation and final glycogen concentrations in the trained group were attained despite %GS I activities that were similar to, or lower than, those in the untrained muscles. As in previous studies (4, 8), the muscle glycogen supercompensation in both the trained and control groups occurred in the face of muscle %GS I activities that were far lower than those found in the resting, fasting state or immediately postexercise. Taken together the results of these studies indicate that glucose uptake, i.e., glucose-6-phosphate availability, limits the rate of glycogen synthesis in skeletal muscle.

There is considerable evidence that depletion of muscle glycogen results in development of fatigue and that raising muscle glycogen concentration delays onset of exhaustion during prolonged, strenuous exercise (1, 2, 7). It is well documented that the adaptations induced by endurance exercise training, primarily the increase in muscle mitochondria, have a potent glycogen-sparing effect during exercise that plays a major role in improving endurance (13, 15). The results of the present study show that endurance exercise training induces additional adaptations that result in a remarkable enhancement of postexercise glycogen supercompensation. This adaptation could improve endurance exercise performance in two ways: 1) by making possible more rapid recovery in response to carbohydrate ingestion between glycogen-depleting bouts of exercise and 2) by elevating muscle glycogen concentration and thus prolonging the time before glycogen depletion results in exhaustion during strenuous exercise. On the basis of the finding in previous studies that increases in muscle GLUT-4 content are associated with increases in glucose transport activity (12, 23–25), and the findings in the present study that %GS I activity and plasma insulin concentrations were similar in the trained and control groups, it seems likely that the exercise training-induced increases in muscle GLUT-4 and hexokinase play an important role in the enhancement of glycogen supercompensation.

In conclusion, the results of this study show that endurance exercise training results in marked increases in both the rate and magnitude of muscle glycogen supercompensation after exercise in rats. This greater rate of glycogen accumulation appears to be mediated by an enhanced skeletal muscle glucose uptake because %GS I was not higher in the trained group during the postexercise period. Among the factors that are probably involved are an adaptive increase in muscle GLUT-4 and a parallel increase in hexokinase activity.

We are grateful to May Chen and Tim Meyer for excellent technical assistance and to Victoria Reckamp for expert assistance in preparation of the manuscript.

This research was supported by National Institutes of Health Grants AG-00425 and DK-18986. L. A. Nolte and D. H. Han were supported by Mentor-Based Fellowships from the American Diabetes Association. R. C. Hickner was supported by National Institute on Aging Postdoctoral Research Training Grant AG-00078.

Address for reprints: J. O. Holloszy, Washington Univ. School of Medicine, Section of Applied Physiology, Campus Box 8113, 4566 Scott Ave., St. Louis, MO 63110 (E-mail: J HOLLOSZ@IMGATE.WUSTL.EDU.). Received 1 October 1996; accepted in final form 18 November 1996.

REFERENCES


