Effects of endurance exercise training on muscle glycogen accumulation in humans

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Grewie, Jeffrey S., Robert C. Hickner, Polly A. Hansen, Susan B. Racette, May M. Chen, and John O. Holloszy. Effects of endurance exercise training on muscle glycogen accumulation in humans. J. Appl. Physiol. 87(1): 222–226, 1999.—The purpose of this investigation was to determine whether endurance exercise training increases the ability of human skeletal muscle to accumulate glycogen after exercise. Subjects (4 women and 2 men, 21 ± 8 yr old) performed high-intensity stationary cycling 3 days/wk and continuous running 3 days/wk for 10 wk. Muscle glycogen concentration was measured after a glycogen-depleting exercise bout before and after endurance training. Muscle glycogen accumulation rate from 15 min to 6 h after exercise was twofold higher (P < 0.05) in the trained than in the untrained state: 0.5 ± 0.2 and 4.5 ± 1.3 mmol·kg wet wt · h⁻¹, respectively. Muscle glycogen concentration was higher (P < 0.05) in the trained than in the untrained state at 15 min, 6 h, and 48 h after exercise. Muscle GLUT-4 content after exercise was twofold higher (P < 0.05) in the trained than in the untrained state (10.7 ± 1.2 and 4.7 ± 0.7 optical density units, respectively) and was correlated with muscle glycogen concentration 6 h after exercise (r = 0.64, P < 0.05). Total glycogen synthase activity and the percentage of glycogen synthase I were not significantly different before and after training at 15 min, 6 h, and 48 h after exercise. We conclude that endurance exercise training enhances the capacity of human skeletal muscle to accumulate glycogen after glycogen-depleting exercise.

GLUT-4 glucose transporter; glycogen supercompensation; glycogen synthase activity

Individuals who exercise on a regular basis generally have higher muscle glycogen levels than their sedentary counterparts (26, 30, 32). This is to be expected, because exercise sessions that result in muscle glycogen depletion are followed by glycogen supercompensation when an adequate amount of carbohydrate (CHO) is ingested (1). Skeletal muscle adapts to endurance exercise with an increase in the GLUT-4 isoform of the glucose transporter protein (5–8, 15, 25, 28, 29). In the absence of insulin resistance, stimulated glucose transport is proportional to muscle GLUT-4 content, and the exercise-induced increase in muscle GLUT-4 is associated with proportional increases in insulin- and contraction-stimulated glucose transport (25, 28, 29).

In this context, it seemed possible that exercise training might also result in an enhanced capacity for muscle glycogen supercompensation. Therefore, studies on rats were conducted to evaluate the possibility that training enhances muscle glycogen supercompensation. It was found that muscles of rats that have adapted to exercise with an increase in GLUT-4 accumulate glycogen more rapidly, and to much higher levels, than untrained muscles if glucose is made available after glycogen-depleting exercise (22, 28). It appears that, in rats and mice, glucose transport is the rate-limiting step in muscle glycogen accumulation under physiological conditions (14, 27, 28). Whether glucose transport or glycogen synthase activity limits glycogen synthesis in human muscle is a matter of controversy (2, 3). Therefore, it could not be assumed that an exercise-induced increase in muscle GLUT-4 is also associated with enhanced glycogen supercompensation after glycogen-depleting exercise in humans.

As a first approach to this question, we performed a study comparing highly trained cyclists with untrained subjects (11). It was found that the rate of glycogen accumulation in response to CHO feeding was twofold faster in the cyclists than in the untrained subjects during the first 6 h after glycogen-depleting exercise. Furthermore, the muscle glycogen level attained by 48 or 72 h after exercise was ~66% higher in the cyclists. Muscle GLUT-4 concentration was threefold higher in the cyclists than in the untrained subjects. This is a remarkably large difference in muscle GLUT-4 content compared with the increases that have been found to occur with training in human subjects (5, 8, 15). The possibility that genetic factors, in addition to training status, could have contributed to the differences in muscle glycogen accumulation between the cyclists and untrained subjects must therefore be considered. Possible genetic differences are, of course, always a potential confounding factor in this type of study. Therefore, comparisons of athletes with nonathletes are usually useful only as an initial screening test to determine whether a longitudinal training study is warranted.

In this context, the purpose of this investigation was to determine whether a period of endurance exercise training results in enhanced glycogen supercompensation in response to CHO feeding after glycogen-depleting exercise.

Methods

Subjects. Six healthy untrained subjects (4 women and 2 men) participated in this investigation. Informed consent was obtained from each subject. This research was approved by the Human Studies Committee at Washington University School of Medicine.

O₂ uptake. Peak O₂ uptake (V̇O₂peak) was measured during a continuous cycle ergometer test to exhaustion 3 wk before the glycogen depletion trial and again at the end of a 10-wk
exercise-training program. The VO2peak protocol consisted of cycling at 50, 100, and 150 W for 3 min per exercise intensity, followed by 25-W increments every minute until exhaustion. Expired air was collected and analyzed immediately throughout the exercise test with an automated on-line system (Max-1, Physio-Dyne Instrument, Farmingdale, NY).

Glycogen depletion trial. Subjects reported to the laboratory after an overnight fast. They then performed a glycogen-depleting exercise bout, which consisted of cycling at ~75% of VO2peak for four 30-min periods with 4-min rest periods between bouts. Subjects then performed five 1-min exercise bouts at ~100% of VO2peak with 3-min rest periods between bouts. O2 uptake was measured for ~10 min during each of the 30-min exercise bouts to ensure the subjects were exercising at ~75% of VO2peak. This protocol was performed before and after a 10-wk exercise-training program.

Muscle biopsies. A biopsy was taken from the vastus lateralis of the quadriceps femoris muscle 15 min after the glycogen-depleting exercise bout. Biopsies were also taken at 6 and 48 h after the subject began eating the first meal (~15 min after initial biopsy) from the vastus lateralis of the contralateral leg and ~3 cm distal to the initial biopsy site, respectively. Muscle samples were immediately frozen in liquid nitrogen and stored at -80°C for subsequent analysis. Muscle samples from all time points were analyzed for glycogen (23) and glycogen synthase (24). The 15-min postexercise muscle samples were analyzed for GLUT-4 and citrate synthase protein content and hexokinase activity. Muscle hexokinase activity was determined at 30°C, as described by Uyeda and Racker (33). Muscle GLUT-4 content and citrate synthase content were determined by Western blotting, as described previously (9). Briefly, protein content was detected with rabbit polyclonal antibodies directed against GLUT-4 or citrate synthase, followed by horseradish peroxidase-conjugated donkey anti-rabbit IgG. Antibody-bound protein was visualized using enhanced chemiluminescence (Amersham). The intensity of the bands was determined by densitometry (model GS-670, Bio-Rad).

Diet. Subjects were provided food for 48 h before and after the glycogen depletion trial. The composition of the diet before the glycogen depletion trial consisted of 50% CHO, 30% fat, and 20% protein and was prepared to be 37 kcal·kg body wt²·day⁻¹. After the glycogen-depleting exercise bout, meals were eaten at 15 min, 2 h, and 4 h after the initial biopsy and provided 1.4 g CHO·kg body wt⁻¹·h⁻¹ for 6 h. During the next 42 h the diet provided 10 g CHO·kg body wt⁻¹·day⁻¹, and the composition of the diet was 80% CHO, 7% fat, and 13% protein. Diet composition and content were the same before and after exercise training.

Blood sampling and analysis. A polyethylene catheter was inserted into an antecubital vein and kept patent with saline throughout the glycogen depletion trial. Blood samples were obtained at the following times: immediately before exercise, at the end of the last 30-min exercise bout, after the last sprint, and every 0.5 h for 6 h after the subject started eating the first meal after the initial biopsy. Blood samples were collected in tubes containing heparin for determination of plasma glucose (glucose oxidase method; Beckman Instruments, Fullerton, CA), Trasylol for determination of insulin (20) and C peptide (18), and reduced glutathione and EGTA for catecholamine determination (31). Samples were subjected to centrifugation (15 min at 2,000 g), and the supernatant was collected and stored at -80°C until subsequent analyses.

Exercise-training program. Subjects began a 10-wk training program 72 h after the glycogen depletion trial. The program consisted of high-intensity cycle ergometry exercise sessions. The intensity of the bands was determined by densitometry (model GS-670, Bio-Rad). The average increase in VO2peak in response to 10 wk of exercise training was ~22% (P < 0.05).

Muscle glycogen. Muscle glycogen concentrations were very low immediately after exercise in the untrained and trained states 15 min, 6 h, and 48 h after a glycogen-depleting exercise bout. Values are means ± SE. *Significantly different from untrained (P < 0.05).

3 days/wk and continuous running 3 days/wk (12). The cycle ergometer exercise consisted of four 5-min exercise bouts at ~90–100% of VO2peak for the first 2 wk. Two minutes of recovery separated the 5-min exercise bouts, during which the subject cycled at ~50–100 W. After the 2nd wk the cycling protocol was increased to five 5-min exercise bouts for the remaining 8 wk. Power output on the cycle ergometer was adjusted throughout the training protocol to compensate for the subject’s increasing maximal exercise capacity. The running exercise consisted of continuous running for 30 min/day for the 1st wk, 35 min/day for the 2nd wk, and 40 min/day for the remaining 8 wk. Subjects were encouraged to run at as fast a pace as they could maintain during the exercise sessions.

Statistics. Values are means ± SE. Untrained and trained muscle tissue variables as well as the area under the curves for blood variables were analyzed with paired t-tests. Pearson correlation coefficient was determined for glycogen concentration vs. GLUT-4 and the percentage of glycogen synthase I. Statistical significance for all statistical tests was accepted at the P < 0.05 α-level.

RESULTS

Body weight and O2 uptake. The training program had no effect on body weight (Table 1). The average increase in VO2peak in response to 10 wk of exercise training was ~22% (P < 0.05).

Muscle glycogen. Muscle glycogen concentrations were very low immediately after exercise in the untrained and trained states. At all time points after the glycogen-depleting exercise bouts, muscle glycogen concentrations were significantly higher in the trained than in the untrained state (Fig. 1). The rate of muscle glycogen accumulation was significantly higher in the trained than in the untrained state (Table 1). The average increase in VO2peak in response to 10 wk of exercise training was ~22% (P < 0.05).

Table 1. Subject characteristics

<table>
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<th>Untrained</th>
<th>Trained</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>31 ± 8</td>
<td>63 ± 9</td>
</tr>
<tr>
<td>Height, cm</td>
<td>165 ± 8</td>
<td>155 ± 8</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>63 ± 8</td>
<td>63 ± 8</td>
</tr>
<tr>
<td>VO2peak</td>
<td>2.3 ± 0.7</td>
<td>2.8 ± 0.9*</td>
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<tr>
<td>ml·kg⁻¹·min⁻¹</td>
<td>34.9 ± 6.5</td>
<td>43.8 ± 9.2*</td>
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Values are means ± SD. VO2peak, peak O2 uptake. *Significantly different from untrained (P < 0.05).
accumulation from 15 min to 6 h after glycogen-depleting exercise was approximately twofold higher in the trained than in the untrained state (10.5 ± 0.2 and 4.5 ± 1.3 mmol·kg wet wt⁻¹·h⁻¹, respectively, P < 0.05). The total increase in muscle glycogen during the 48-h glycogen supercompensation period was also much greater in the trained state: 170.7 ± 23.8 and 95.3 ± 19.0 mmol/kg wet wt after and before training, respectively (P < 0.05).

Muscle GLUT-4 content. Muscle GLUT-4 content was twofold higher in the trained than in the untrained state (Table 2). Muscle glycogen concentration 6 h after exercise was significantly correlated with muscle GLUT-4 content (Fig. 2).

Muscle glycogen synthase activity. Total muscle glycogen synthase activity was similar in the untrained and trained states: 2.88 ± 0.52 and 2.85 ± 0.74 µmol·g⁻¹·min⁻¹ before and after training, respectively. The percentage of glycogen synthase I at 15 min after exercise was slightly higher (28%) in the trained than in the untrained state, but this difference was not statistically significant (Fig. 3). The percentage of muscle glycogen synthase I was not significantly correlated with muscle glycogen concentration 6 h after exercise (r = 0.29, P = 0.36).

Citrate synthase and hexokinase activity. Muscle citrate synthase protein content was significantly higher after than before exercise training (Table 2). Muscle hexokinase activity was 62% higher in the trained than in the untrained state, but this difference was not statistically significant (Table 2).

Hormones and metabolites. The area under the curve for plasma glucose concentration after the glycogen-depleting exercise was smaller (P < 0.05) in the trained than in the untrained state (Fig. 4). Plasma insulin, C peptide, epinephrine, and norepinephrine concentrations in the trained state were not significantly different from those in the untrained state after the glycogen-depleting exercise (data not shown).

**DISCUSSION**

The present findings show that endurance exercise training markedly enhances the muscle glycogen supercompensation response to CHO ingestion after glycogen-depleting exercise. It was previously found that exercise training increases the rate and extent of muscle glycogen accumulation in rats fed CHOs after a glycogen-depleting bout of exercise (22, 28). Furthermore, Hickner et al. (11) found glycogen accumulation rates twofold higher in highly trained cyclists than in untrained individuals, providing evidence that endurance

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**Table 2.** Effects of exercise training on skeletal muscle GLUT-4 protein, citrate synthase protein, and hexokinase activity

<table>
<thead>
<tr>
<th></th>
<th>Untrained</th>
<th>Trained</th>
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<tr>
<td>GLUT-4 content</td>
<td>4.7 ± 0.7</td>
<td>10.7 ± 1.2*</td>
</tr>
<tr>
<td>Citrate synthase</td>
<td>7.7 ± 0.9</td>
<td>9.2 ± 0.6*</td>
</tr>
<tr>
<td>Hexokinase activity</td>
<td>18.2 ± 5.0</td>
<td>29.4 ± 4.3</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed in arbitrary densitometric units (for citrate synthase and GLUT-4 proteins) and in µmol·min⁻¹·g protein⁻¹ (for hexokinase). *Significantly different from untrained (P < 0.05).

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**Fig. 2.** Relationship between muscle GLUT-4 content and muscle glycogen concentration 6 h after a glycogen-depleting exercise bout (P < 0.05). OD, optical density.

**Fig. 3.** Percentage of muscle glycogen synthase I in untrained and trained states 15 min, 6 h, and 48 h after a glycogen-depleting exercise bout. Values are means ± SE.

**Fig. 4.** Plasma glucose in untrained and trained states before exercise (−2.4 h), after endurance cycling (−0.35 h), after final sprint (0), and every 0.5 h for 6 h after exercise. Values are means ± SE. Area under curve after glycogen-depleting exercise was smaller (P < 0.05) in trained than in untrained state.
Endurance exercise training induces an increase in skeletal muscle GLUT-4 that, as in this study, is generally larger than the increases in mitochondrial enzymes and hexokinase (5–8, 15, 25, 28, 29). A growing body of evidence supports the interpretation that glucose transport rates are correlated with muscle GLUT-4 content (10, 17, 25, 28, 29). There is also considerable evidence that glucose transport is the rate-limiting step for glucose uptake and glycogen synthesis in skeletal muscle (see Ref. 13 for review). The findings from this investigation demonstrate that the glycogen concentration 6 h after exercise is correlated with muscle GLUT-4 concentration in subjects fed CHO after glycogen-depleting exercise. This relationship is in agreement with the findings of McCoy et al. (19) and Hickner et al. (11). The results of this study are in keeping with those of previous studies on rats showing that a training-induced increase in muscle GLUT-4 is associated with increases in the rate and extent of muscle glycogen supercompensation after glycogen-depleting exercise (14, 22). It is clear from the finding that plasma glucose and insulin levels were not higher in the trained than in the untrained state that changes in these variables did not play a role in the enhanced glycogen supercompensation in the trained state.

It has been suggested that the activation of glycogen synthase is responsible for the glycogen supercompensation after glycogen-depleting exercise (3). The findings from this investigation as well as those from other studies do not support this concept (4, 11, 14, 22, 27). In the present study, total glycogen synthase and the percentage of glycogen synthase I in the trained state were not significantly different from those in the untrained state. In addition, the percentage of glycogen synthase I explained only a small portion of the variance in muscle glycogen accumulation 6 h after exercise. Although the increase in the percentage of glycogen synthase I likely plays an important role in the rapid initial increase in muscle glycogen, it does not appear to play a role in the glycogen supercompensation phenomenon. This is evidenced by the finding that glycogen continues to increase to “supersaturated levels” after the increase in percentage of glycogen synthase I has reversed (2, 4, 22). A number of investigators have reported that endurance exercise training induces an increase in total glycogen synthase activity in skeletal muscle (16, 21, 32). In the present study we were unable to detect an increase in total glycogen synthase activity in skeletal muscle. We have no explanation for this apparent discrepancy.

In conclusion, the results of this study show that the increase in the muscle GLUT-4 isoform of the glucose transporter that occurs in response to exercise training is associated with increases in 1) the rate of muscle glycogen accumulation early during recovery and 2) the extent of muscle glycogen supercompensation, in women and men fed CHO after glycogen-depleting exercise. It appears likely that this adaptation helps prevent and/or enhances reversal of muscle fatigue associated with glycogen depletion.

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