Impaired muscle glycogen storage after muscle biopsy

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Costill, D. L., D. R. Pearson, and W. J. Fink. Impaired muscle glycogen storage after muscle biopsy. J. Appl. Physiol. 64(5): 2245-2248, 1988.—To assess the effects of repeated needle biopsies on the rate of muscle glycogen repletion, eight male subjects were studied immediately after and 2 days after an exhaustive exercise bout. A single biopsy was obtained from the right vastus lateralis muscle immediately after an exhaustive cycling bout. Two days later, a sample was taken 1 cm lateral or medial to sample A. In four of these subjects, additional biopsies were taken 3 cm distal and proximal. A control specimen was also taken from the left leg 2 days after the exercise. Ten days after the exercise, muscle was again sampled from each leg of these four subjects. Analysis of these samples revealed that the initial biopsy impaired glycogen storage in the muscle taken 1 cm medial or lateral to the previous site. This reduction in glycogen storage was most pronounced in the first 2 days after the exercise. Samples taken distal and proximal to the initial biopsy contained, on the average, less glycogen than the contralateral leg, but these differences were only significantly different in the distal muscle sample. Alteration in muscle glycogen storage was seen to persist for 10 days after the first biopsy, suggesting that care must be taken in selecting the site for repeated biopsies from the same muscle.

Methods and Procedures

Eight physically active, healthy men participated in this investigation after being fully informed of the risks and stresses associated with this study. The mean (±SE) characteristics of these subjects are presented in Table 1. In an effort to deplete the glycogen content of the vastus lateralis muscle, each subject performed 1 h of exercise on a cycle ergometer (Monarch) at a work load that required the men to use ~70% of their VO$_{2\text{max}}$. After this exercise, the subjects performed six 1-min sprints on the ergometer at an exercise intensity of 125% VO$_{2\text{max}}$ with 1-min rest between sprints. Within 5 min after the final sprint bout, a biopsy was taken from the right vastus lateralis muscle (sample A) ~10-15 cm above the patella. This and subsequent biopsies were taken with the aid of suction, using a 5-mm (OD) Duchenne needle (1, 8, 9). Approximately 1 ml of lidocaine (xylocaine 1%) with epinephrine (1:100,000) was injected into the skin and superficial tissue before each biopsy. Approximately 0.2 ml of this injection was introduced below the fascia in the area of the incision, which was made with a scalpel blade (no. 11). Sufficient tissue (i.e., 28.5-55.4 mg) was obtained for all analyses with a single entry into the muscle. Each specimen was cleaned of all connective tissue and blood, frozen within 30 s, and stored in liquid nitrogen.

In the following 2 days, the men were fed a diet containing 500 g of carbohydrates (CHO) per day and refrained from any strenuous exercise. At that point, a second biopsy (sample B) was then taken 1 cm (medial or lateral) from the site of the first biopsy. In four of these subjects, additional biopsies were taken 3 cm distal (sample C) and 3 cm proximal (sample D) to the first biopsy incision. The distribution of these incisions are shown in Fig. 1. For the purpose of comparison, a single biopsy was also obtained from the left vastus lateralis muscle (sample E) of all the subjects 2 days after the exhaustive exercise. All of these specimens were divided...
TABLE 1. Characteristics of subjects

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Weight, cm</th>
<th>VO₂ max, ml·kg⁻¹·min⁻¹</th>
<th>%Type I*</th>
</tr>
</thead>
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<td>28.3</td>
<td>180.6</td>
<td>75.5</td>
<td>60.1</td>
<td>56.8</td>
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<td>±2.4</td>
<td>±2.2</td>
<td>±2.6</td>
<td>±5.4</td>
</tr>
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VO₂ max, maximal aerobic capacity. * Muscle fibers. Values are means ± SE.

into two pieces. One part of the sample was mounted and frozen in isopentane that was cooled to its freezing point over liquid nitrogen. This specimen was subsequently sectioned and stained for fiber composition [myosin adenosinetriphosphatase (ATPase)], glycogen (periodic acid-Shiff's reaction), and leukocytic infiltration (hematoxylin eosin stain; 12). The second portion of the sample was frozen within 3 min after transection and stored in liquid nitrogen. At a later time, this sample was divided into three or four pieces, while frozen, and analyzed for glycogen (14).

Ten days after the initial biopsies (sample A), additional muscle samples were taken from four of the subjects 1 cm medial or lateral (sample B) to the site of the first biopsy (sample A) and 3 cm proximal (sample G) to the incision of the biopsy in the control leg (sample E). These specimens were analyzed for glycogen as previously described. During this period, the subjects ate ad libitum and performed only normal daily activity.

A one-way analysis of variance (ANOVA) for repeated measures was used to compare the means for samples A, B, and E, in which all eight subjects were sampled. A separate ANOVA was used to determine the significance of the difference between the mean glycogen values for sites A, B, C, D, E, F, and G, in which only four of the subjects were used. In light of the small number of subjects used for these latter comparisons, we present these results with some reservation. When the F ratio indicated significance (P < 0.05), specific mean differences were identified with the Neuman-Keuls post hoc test.

RESULTS

After the exhaustive exercise, muscle glycogen averaged 12.8 mmol/kg wet weight (Fig. 2). Histochemical analysis of this muscle specimen confirmed this low-glycogen content, with both type I and II fibers equally depleted. On the first 2 days after the exercise and first biopsy (sample A), all of the men experienced some muscle soreness at the site of the incision. There was no soreness, however, in any other areas of that muscle, or in other leg muscles, as a consequence of the exercise routine.

As shown in Fig. 2, 2 days of rest and a rich CHO diet resulted in a significant rise (P < 0.05) in muscle glycogen of the contralateral, control muscle (sample E). Muscle specimens taken 1 cm lateral or medial (sample B) to the site of sample A were found to have significantly less glycogen (P < 0.05) than the control leg. Although the mean glycogen content of this specimen was 30 mmol/kg wet weight greater than the immediate postexercise sample (sample A), three of the subjects showed no glycogen storage in the immediate area of sample A after the 2 days of rest.

Muscle samples taken proximal (sample D) to the site of the first biopsy showed a smaller, but significant, decrement (P < 0.05) in glycogen storage (Fig. 3). Of the four subjects biopsied proximal (3 cm) to the initial biopsy, all had markedly lower muscle glycogens (mean 85.3 mmol/kg wet wt) compared with the control specimen (mean 138.9 mmol/kg wet wt). Samples taken distal to the initial biopsy were not significantly different (126.7 mmol/kg wet wt) from the control specimen (138.9 mmol/kg wet wt). Because only four subjects were biopsied proximal and distal to the location of the first biopsy, it may be misleading to treat these means statistically. Nevertheless, these data show a significantly (P < 0.05) lower rate of glycogen storage in the immediate area (1 cm) or proximal to the initial biopsy (sample A). On the other hand, there appears to be little or no impairment in muscle glycogen storage in the area distal to the original biopsy.

Ten days after the first biopsy (sample A), four of these subjects were again biopsied 3 cm from the site of the control incision (sample E) and 1 cm from the location of the first biopsy (sample A). Although no differences were found between the glycogen contents of the control

![Diagram of muscle biopsies](image-url)
IMPAIRED MUSCLE GLYCOGEN STORAGE

FIG. 2. Mean (±SE) muscle glycogen content immediately after (sample A) and 2 days after exhaustive cycling in control (sample E) and test (sample B) legs. Note that both samples A and B are significantly lower (P < 0.05) than the control muscle (sample E). **Glycogen content in sample B was significantly greater than sample taken immediately after exercise (Sample A). * Sample A significantly different from Samples E and B.

FIG. 3. Mean (±SE) glycogen content in muscle samples taken immediately after (sample A) and 2 days after exhaustive cycling in test and control legs. Samples were taken medial/lateral (test), distal, and proximal to sample A after 2 days of recovery. * Samples A, B, and D are significantly lower than Samples C and E (P < 0.05). samples (E and G), all of the subjects had lower muscle glycogen in the area of the first biopsy specimen (Fig. 4). The mean values for the control samples (E and G) averaged 127.6 ± 4.5 (SE) mmol/kg wet weight on day 2 and 129.0 ± 0.5 on day 10. The mean (±SE) value for sample F, on the other hand, was 112.6 ± 3.9 mmol/kg wet weight, significantly below (P < 0.05) the mean for the control leg (129.0 ± 0.5 mmol/kg wet wt).

DISCUSSION

The design of the present study assumes that the muscle glycogen content was similar in both legs after the exercise. To confirm this assumption, we examined the glycogen content in both legs of seven subjects after exhaustive cycling and have noted no significant difference between the mean values [right leg, 33.2 ± 2.4 (SE)mmol/kg; left leg, 31.8 ± 2.8 (SE) mmol/kg]. It should also be noted that there was no preferential depletion of glycogen from the subjects dominant leg.

Thus we feel it is valid to assume that in the present study, muscle glycogen content was similar in both legs after the exhaustive cycling.

This research illustrates that the trauma associated with the muscle biopsy procedure impairs the storage of muscle glycogen. The effect of tissue damage on glycogen storage is most dramatic in the immediate area (1 cm) of the original biopsy. Although this effect is most noticeable in the first 2 days after an initial biopsy, this influence is measurable 10 days after the initial surgery. These findings suggest that previous studies that have measured muscle glycogen resynthesis after exhaustive exercise and taken repeated biopsy samples from a small area of the same muscle may have reported falsely low mean values (2, 4, 6, 7, 15, 17). This is not to say that the present data invalidates all previous research on muscle glycogen storage, but they do emphasize the importance of site selection during such studies. Unless the previous studies obtained repeated samples from a site distal to the preceding biopsy, their data may have underestimated glycogen synthesis in the whole muscle and/or in the contralateral muscle.

Bonen et al. (5), for example, obtained repeated biopsies from the same incision in a study of muscle glycogen repletion during 4 h of recovery from cycling. During the recovery period, the subjects consumed a CHO-rich solution (1.5 g/kg wt; 20% solution) and either performed light exercise with one leg or were bed rested. They observed no glycogen repletion in either leg when one leg was exercised. In the bed-rested condition, there was no significant rise in muscle glycogen between 2 and 4 h of recovery. In light of the present study, there is some question concerning the validity of these measurements. On the other hand, the degree of glycogen storage during the first 2 h of bed rest is similar to that observed in the present study (samples A vs. B), suggesting that glycogen repletion slows or stops after 4 h in the area of the biopsy. We are led to speculate that the effect of previous biopsies may be a delayed reaction. Although the results by Bonen et al. (5) serve as a caution to universalizing our data, it is difficult to accept their speculation of a central catecholamine drive that inhibits glycogen repletion in the nonexercising leg during mild cycling with...
the other leg. Histochemical examination (i.e., hematoxylin eosin stain) of the muscle specimens taken from the test leg 2 and 10 days after the initial exercise-biopsy regimen revealed frequent sites of leukocyte infiltration, suggestive of an inflammatory reaction. On the other hand, there were no signs of leukocyte infiltration in the muscle samples taken from the control leg on either days 2 or 10. Such inflammatory reactions in muscle have been reported after marathon running and eccentric exercising (10, 11). The relationship between this inflammatory response and the observed delay in muscle glycogen storage remains to be determined.

In summary, this investigation demonstrates the influence of muscle trauma associated with the needle biopsy procedure on glycogen storage. This research may also raise some questions concerning the use of repeated muscle biopsies to describe the traumatic effects of exhaustive exercise (9, 13). During studies that require repeated muscle biopsies over several days, care must be taken to minimize the influence of previous biopsies on the analysis and interpretation of the results. This problem appears to be minimized by sampling muscle distal (~3 cm) to the site of the previous biopsy, although even these specimens may experience some reduction in glycogen storage.

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