Effect of Time of Carbohydrate Ingestion on Muscle Glycogen Resynthesis after Exhaustive Exercise in Rats

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We studied the optimal time of carbohydrate ingestion required to restore muscle glycogen storage after exhaustive exercise in rats. The animals were divided into 4 groups (IN0, IN30, IN60 and IN120), each receiving 30% glucose solution (30 g/kg body weight) through a stomach tube 0, 30, 60 or 120 minutes after exercise. Six hours after administration of glucose, the glycogen concentration in the m. extensor digitorum longus returned to the baseline in the IN0 group, while reaching only 77%, 80% and 73% of the baseline in the IN30, IN60 and IN120 groups, respectively. There was a significant difference in this variable between the IN0 group and any of the three other groups. On the other hand, the glycogen concentration in the m. soleus returned to the baseline in all groups with no difference. These findings suggest that muscle glycogen may be most efficiently resynthesized if carbohydrate is given immediately after exercise, and that muscle glycogen resynthesis may vary with the type of muscle.

(Key Words: glycogen synthesis, carbohydrate ingestion, muscle type, exhaustive exercise)

INTRODUCTION
Glycogen is the primary energy substrate at a high intensity of exercise. The duration of exercise can be limited by glycogen supply. Particularly muscle glycogen store serves as an important energy fuel during exercise (7). The rate of muscle glycogen synthesis is thought to depend on the extent of glycogen depletion and the ratio activity of glycogen synthase after exercise (7, 10). Recent studies have focused on how to maximize the rate of muscle glycogen resynthesis during the early hours following exhaustive exercise (9). Ivy et al. (6) reported that delaying the ingestion of a carbohydrate supplement post-exercise would result in a reduced rate of muscle glycogen storage. But there is little information on the effect of time of carbohydrate ingestion on the restoration of muscle glycogen after exercise. In the present study we investigated muscle glycogen resynthesis following carbohydrate ingestion at different times after prolonged exhaustive exercise.

MATERIALS AND METHODS

Animals
Twenty-eight male Wistar rats, aged 7 weeks, were used in this study. The rats were given standard chow (51.6% carbohydrate, 24.8% protein, 4.4% fat, 3.5% fiber, 7.0% ash, and 8.7% water; CE-2, Clea, Tokyo) and water ad libitum. The animal room was maintained at a temperature of about 25 °C. The room was illuminated between 19:00 and 07:00, and darkened between 07:00 and 19:00.

Experiment protocols
All rats were familiarized for one week with a treadmill (KN73, Natume, Tokyo) set at a 6% gradient and a speed of about 15 m/min.

Eight of the twenty-eight rats were used for a preliminary experiment. Four of them were exercised to exhaustion on the treadmill. Its speed was initially set at 20 m/min and increased by 5 meters every 5 minutes up to 40 m/min. Exhaustion was defined as the stage in which the animals refused to run any more and did not try to escape when placed on a table (10). Four other rats were not exercised and used as controls. The two groups of rats were...
immediately anesthetized with sodium pentobarbital (50 mg/kg body weight, i.p.) and killed without being given carbohydrate. Blood samples were taken from the left ventricle of each rat to measure blood glucose, serum free fatty acid (FFA) and serum glycerol concentrations. The m. soleus (SOL) and m. extensor digitorum longus (EDL) were quickly excised to measure muscle glycogen concentration. The values obtained from the rested controls were used as the baseline of each variable.

The remaining twenty rats were exercised to exhaustion in the same manner. They were equally divided into four groups and given 30% glucose solution (30 g/kg body weight) through a stomach tube immediately (IN0), 30 minutes (IN30), 60 minutes (IN60) and 120 minutes (IN120) after exercise. The rats were dealt with in the above mentioned manner six hours after carbohydrate ingestion.

Measurements

Blood glucose was determined using an auto glucose/lactate analyzer (YSI 2300 STAT, American). The serum concentrations of FFA and glycerol were determined using NEFA kit-U (Nihon Shoji, Tokyo) and F-kit glycerol (Boehringer Mannheim), respectively. The glycogen content in muscle tissue was determined according to the method of Carroll (4).

Data analysis

Each mean was calculated and the one way analysis of variance (ANOVA) test was used for group comparison. Significance was determined at the P<0.05 level.

RESULTS

The body weight neither differed among the six groups before exercise, nor changed after exercise.

Glycogen concentration

The glycogen concentration in the EDL dropped from 5.01±0.52 mg/g wet wt at rest to 0.33±0.26 mg/g wet wt after exercise (Fig. 1). Six hours after administration of glucose, it rose to 5.58±0.83 mg/g wet wt and completely returned to the baseline in the IN0 group, while reaching only 77% (3.84±0.60 mg/g wet wt), 80% (4.01±1.09 mg/g wet wt) and 73% (3.64±0.64 mg/g wet wt) of the baseline in the IN30, IN60 and IN120 groups, respectively (Fig. 2). The recovery rate of glycogen concentration in the EDL was significantly higher in the IN0 group than in the three other groups.

The glycogen concentration in the SOL decreased from 3.72±0.26 mg/g wet wt at rest of 0.44±0.21 mg/g wet wt after exercise (Fig. 1). Six hours after administration of glucose, it almost or completely returned to the baseline in all groups, increasing to 3.95±0.88,
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3.62±1.24, 4.20±0.84 and 4.44±0.29 mg/g wet wt in the IN0, IN30, IN60 and IN120 groups, respectively (Fig. 2). There was no significant difference in the recovery rate of glycogen concentration in the SOL among the four groups.

Blood variables

The blood glucose concentration was significantly lower after exercise than at rest (Table 1), but did not differ among the four carbohydrate-ingested groups (Table 2).

The serum glycerol concentrations differed neither between the baseline and the post-exercise level (Table 1), nor among the four carbohydrate-ingested groups (Table 2).

The serum FFA concentration did not differ between the baseline and the post-exercise level (Table 1). It was 29% to 52% higher in the IN30, IN60 and IN120 groups than in the IN0 group. However, there was no significant difference in this variable among the four carbohydrate-ingested groups (Table 2).

DISCUSSION

Among the local factors affecting muscle glycogen synthesis during post-exercise recovery are muscle blood flow and glucose transport through the muscle plasma membrane (3, 7, 10). The muscle blood flow is quite low at rest, but can increase 20 times during moderately intense exercise (3). After exercise, it declines rapidly to a level slightly higher than that at rest and then continues to decrease slowly over an hour. Glucose uptake by muscle seems to take a similar course. Ivy et al. measured glucose transport in the plasma membrane vesicles of rat gastrocnemius before and after exercise, and reported that the variable after two hours of recovery showed a four-fold drop compared with the level at the end of exercise (6). These findings suggest that the two physiological variables may be at peak immediately after exercise.

The biochemical aspect of glycogen synthesis also deserves due consideration. The rate of glycogen synthesis is limited by glycogen synthase and shows a strong negative correlation with glycogen concentration (3, 5). This means that the lower muscle glycogen concentration favors the higher ratio of glycogen synthase activity (5, 9). Our study showed a sharp decrease in muscle glycogen concentration after exhaustive exercise. This suggests that the glycogen synthase activity immediately after exercise might have become high enough to promote transamination of ingested glucose.

![Graph](image)

**Fig. 2** Glycogen content in muscles of rats 6 hours after carbohydrate ingestion. IN0: carbohydrate ingested immediately after exercise, IN30: carbohydrate ingested 30 minutes after exercise, IN60: carbohydrate ingested 60 minutes after exercise, IN120: carbohydrate ingested 120 minutes after exercise. SOL: m. soleus, EDL: m. extensor digitorum longus. §: P<0.05 vs IN0, †: P<0.01 vs IN0.
Table 1 Blood glucose, serum FFA and serum glycerol concentration at rest and after exercise in rats.

<table>
<thead>
<tr>
<th></th>
<th>At rest</th>
<th>After exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFA (μEq/l)</td>
<td>476±176</td>
<td>561±175</td>
</tr>
<tr>
<td>glycerol (mmol/l)</td>
<td>10.15±2.05</td>
<td>13.36±3.76</td>
</tr>
<tr>
<td>glucose (mg/dl)</td>
<td>134±12</td>
<td>65±14*</td>
</tr>
</tbody>
</table>

All data expressed as Mean±SD. FFA: free fatty acid. *P<0.05 vs at rest.

Table 2 Blood glucose, serum FFA and serum glycerol concentrations 6 hours carbohydrate ingestion in rats.

<table>
<thead>
<tr>
<th></th>
<th>IN0</th>
<th>IN30</th>
<th>IN60</th>
<th>IN120</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFA (μEq/l)</td>
<td>368±114</td>
<td>476±176</td>
<td>561±175</td>
<td>504±122</td>
</tr>
<tr>
<td>glycerol (mmol/l)</td>
<td>6.51±0.88</td>
<td>5.35±1.29</td>
<td>5.86±1.83</td>
<td>5.10±1.14</td>
</tr>
<tr>
<td>glucose (mg/dl)</td>
<td>122±7</td>
<td>116±9</td>
<td>112±9</td>
<td>111±13</td>
</tr>
</tbody>
</table>

All data expressed as Mean±SD. FFA: free fatty acid. IN0: carbohydrate ingested immediately after exercise, IN30: carbohydrate ingested 30 min after exercise, IN60: carbohydrate ingested 60 min after exercise and IN120: carbohydrate ingested 120 min after exercise.

into glycogen.

The role of FFA and glycerol in muscle glycogen resynthesis should also be considered. These two biochemical variables, particularly the latter, indicate the status of lipolysis in adipose tissue (2). The relatively high serum concentrations of the two variables immediately after exhaustive exercise may reflect increased lipolysis in adipose tissue. Two things are furthermore known about FFA. FFA is suppressed in the presence of a high level of blood glucose. However, elevated blood FFA impairs the glucose tolerance and insulin sensitivity of tissue (8), thereby inhibiting glycogen synthesis. This may at least in part explain our study results concerning the relationship of glycogen concentration in the EDL to serum FFA concentration, i.e., a significantly higher glycogen concentration and a relatively lower serum FFA concentration in the IN0 group than in the IN30, IN60 and IN120 groups. On the other hand, a relatively lower serum glycerol concentration after carbohydrate ingestion than the post-exercise level is likely to reflect the decrease of lipolysis and the replenishment of muscle glycogen store from available glucose.

The above considerations of our study results will allow us to conclude that a carbohydrate supplement will be most efficiently and effectively utilized for muscle glycogen resynthesis, if it is provided immediately after exercise.

Interestingly, the change of glycogen concentration in the SOL showed a different pattern from that in the EDL. This is mainly attributed to the difference in the type of predominant muscular fibers. Ariano et al. reported that slow oxidative fibers occupy 84% in the SOL, while fast oxidative glycolytic fibers and fast glycolytic fibers occupy 59% and 38%, respectively, in the EDL (1). Furthermore, Conlee et al. showed that glycogen synthesis occurs most rapidly in the fast oxidative glycolytic fibers, most slowly in the fast glycolytic fibers and at an intermediate rate in the slow oxidative fibers (5).

REFERENCES


5) Conlee RK, Hickson RC, Winder WW, Hagberg JM, and Holloszy JO: Regulation of glycogen resynthesis in...
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