Effects of high-intensity intermittent swimming on glucose transport in rat epitrochlearis muscle

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J. Appl. Physiol. 84(6): 1852–1857, 1998.—Recently (K.Kawanaka, I. Tabata, and M. Higuchi, J. Appl. Physiol. 83: 429–433, 1997), we demonstrated that glucose transport activity after repeated 10-s-long in vitro tetani in rat epitrochlearis (Epi) muscle was negatively correlated with the postcontraction muscle glycogen concentration. Therefore, we examined whether high-intensity intermittent swimming, which depletes muscle glycogen to a lower level than that observed after ten 10-s-long in vitro tetani, elicits higher glucose transport than that observed after ten 10-s-long in vitro tetani, which has been regarded as the exercise-induced maximal stimulus for glucose transport. In male rats, 2-deoxy-D-glucose transport rate in Epi muscle after eight bouts of high-intensity intermittent swimming with a weight equal to 18% of body mass (exercise duration: 20 s, rest duration between exercise bouts: 40 s) was higher than that observed after the ten 10-s-long tetani (2.25 ± 0.08 vs. 1.02 ± 0.16 μmol·ml intracellular water−1·20 min−1). Muscle glycogen concentration in Epi after eight bouts of high-intensity intermittent swimming was significantly lower than that observed after ten 10-s-long in vitro tetani (7.6 ± 0.5 vs. 14.8 ± 1.4 μmol glucose/g muscle). These observations show that the high-intensity intermittent swimming increases glucose transport in rat Epi to a much higher level than that induced by ten 10-s-long in vitro tetani, which has been regarded as the exercise-related maximal stimulus for glucose transport. Furthermore, this finding suggests that the lower muscle glycogen level after high-intensity intermittent swimming than after in vitro tetani may play a role, because there was a significant negative correlation between glucose transport and muscle glycogen concentration in Epi after high-intensity swimming and in vitro tetani.

Insulin increases the permeability of skeletal muscle to glucose. Furthermore, it is well known that low- or moderate-intensity swimming or running has an insulin-like effect on glucose transport in skeletal muscle (14, 15). In the absence of insulin, in vitro twitch or tetanic contractions induced by electrical stimulation produce an increase in muscle glucose transport (12, 22, 23). Therefore, it is suggested that there are two separate pathways for stimulation of glucose transport in skeletal muscle: one pathway is activated by insulin, the other by muscle contraction.

Several previous studies reported enhanced insulin-stimulated glucose uptake after exercise when the muscle glycogen content was low (5, 7, 28). These results suggest that muscle glycogen content affects insulin-stimulated glucose transport in skeletal muscle.

METHODS

Male Sprague-Dawley rats (Crea Japan, Tokyo) with body weights of 90–110 g were used for this study. Ethical approval for this work was obtained from the Committees on Animal Care at the National Institute of Health and Nutrition, Japan.

Treatment of animals and swimming program. All animals were housed in rooms lighted from 7 AM to 7 PM and were maintained with ad libitum feeding on standard chow and water. Room temperature was maintained at 20–22°C. Food was restricted to 8 g on the evening of the last day before the experiment.

Some of the rats swam in a barrel (4 rats/barrel) filled to a depth of 50 cm, with water maintained at 35°C. A weight equal to 3% of body mass was tied to the body of the rat (low-intensity continuous swimming). These rats swam for 10, 30, or 120 min. At the end of 120 min of low-intensity continuous swimming, the blood lactate concentration was...
Table 1. Effects of high-intensity intermittent swimming on glycogen concentration and glucose transport activity in rat epitrochlearis muscle

<table>
<thead>
<tr>
<th>Bouts of 20-s Swimming With Weight – 18% body wt</th>
<th>Basal (n = 14)</th>
<th>1 (n = 9)</th>
<th>3 (n = 11)</th>
<th>8 (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle glycogen, μmol glucose units/g wet wt</td>
<td>26.2 ± 1.4</td>
<td>22.3 ± 2.0*</td>
<td>12.9 ± 1.3†</td>
<td>7.6 ± 0.5†‡</td>
</tr>
<tr>
<td>2-Deoxy-o-glucose transport, μmol·min⁻¹·g⁻¹</td>
<td>0.34 ± 0.02</td>
<td>0.55 ± 0.05</td>
<td>1.36 ± 0.11†</td>
<td>2.25 ± 0.08†‡</td>
</tr>
</tbody>
</table>

Values are means ± SE, n = no. of muscles. Rats swam for 20 s at a rate of 1 bout in 1 min with a weight equal to 18% body wt for 1, 3, and 8 bouts. Immediately after swimming, muscles were excised and analyzed. *Significantly different from basal, P < 0.05. †Significantly different from 1 bout of swimming, P < 0.05. ‡Significantly different from 3 bouts of swimming, P < 0.05.
five tetani, muscle glycogen concentration was decreased, compared with the basal level (P < 0.01), but more tetani did not significantly induce any further decrease in muscle glycogen (Table 2). Twenty tetani reduced muscle glycogen concentration to a level of 13.7 ± 1.0 μmol glucose/g muscle (Table 2). 2-DG transport also increased with increasing numbers of in vitro tetani (Table 2). Although 2-DG transport was significantly increased after five tetani compared with the basal level (P < 0.01), more tetani did not result in further increase in 2-DG transport (Table 2).

Effects of low-intensity continuous swimming on muscle glucose transport and glycogen in rat Epi muscle. When rats swam with a weight equal to 3% of body mass, muscle glycogen concentration in Epi decreased as the duration of swimming was increased (Table 3). 2-DG transport also increased as the duration of swimming was prolonged to 120 min (Table 3).

Effects of high-intensity intermittent swimming, in vitro tetani, and combined stimuli on muscle glucose transport and glycogen in rat Epi muscle. Fifteen tetanic contractions, which provide a maximal effect of in vitro tetani on 2-DG transport, resulted in a 3.2-fold increase in 2-DG transport rate above the basal level (Table 4). Eight bouts of high-intensity swimming induced a 7.3-fold increase in the rate of 2-DG transport above the basal level, which was significantly greater than that induced by 15 tetani alone (P < 0.01; Table 4). We also examined the effects of swimming followed by 15 tetani in vitro on 2-DG transport. The combined effects of swimming and subsequent in vitro tetani on 2-DG transport were not significantly greater than the effect of swimming alone (Table 4).

Figure 1 shows the relationship between the mean 2-DG transport rate and the mean muscle glycogen level at the end of swimming and in vitro tetani. When all the data after in vitro tetani, high-intensity intermittent swimming, and low-intensity continuous swimming were plotted, 2-DG transport rate was negatively correlated with muscle glycogen concentration (y = 2.83 – 0.11x, r = −0.95, P < 0.01). When the data after high-intensity intermittent swimming alone were plotted, 2-DG transport rate was negatively correlated with postexercise muscle glycogen level (y = 3.06 – 0.12x, r = −0.99, P < 0.01).

Effects of high-intensity intermittent swimming, insulin, and combined stimuli on muscle glucose transport and glycogen in rat Epi muscle. Incubation of Epi muscle with 2 mU/ml of insulin increased 2-DG transport from a basal value of 0.33 ± 0.04 μmol·ml intracellular water⁻¹·20 min⁻¹ (n = 8) to 1.34 ± 0.07 μmol·ml intracellular water⁻¹·20 min⁻¹ (n = 5). High-intensity intermittent swimming followed by 15 tetani in vitro increased 2-DG transport to 2.32 ± 0.13 μmol·ml intracellular water⁻¹·20 min⁻¹ (n = 5). Furthermore, swimming followed by in vitro tetani and subsequent insulin (2 mU/ml) incubation increased 2-DG transport to 3.41 ± 0.29 μmol·ml intracellular water⁻¹·20 min⁻¹ (n = 7). Thus the combined effects of insulin and swimming followed by in vitro tetani were additive.

DISCUSSION

In the present study, the high-intensity intermittent swimming increased glucose transport in rat Epi to a much higher level than that induced by repeated 10-s-long in vitro tetani alone (Table 4). A previous study (3) reported that ten 10-s-long in vitro tetanic contractions increased glucose transport to a level as high as that achieved after four bouts of 30-min swimming with a weight equal to 2% of body mass. Therefore, glucose transport after ten 10-s-long in vitro tetani has been considered as the exercise-induced maximal activation of glucose transport in Epi. However, the present study showed that high-intensity intermittent swimming raises glucose transport in rat Epi to a higher level than do ten 10-s-long in vitro tetani.

We do not know whether other in vitro contraction- or exercise-related protocols increase glucose transport to a higher level than that observed after ten 10-s-long in vitro tetani.

Table 2. Effects of in vitro tetanic contractions on glycogen concentration and glucose transport activity in rat epitrochlearis muscle

<table>
<thead>
<tr>
<th>No. of Tetani</th>
<th>Basal (n = 6)</th>
<th>1 (n = 5)</th>
<th>5 (n = 5)</th>
<th>10 (n = 6)</th>
<th>15 (n = 7)</th>
<th>20 (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle glycogen, μmol glucose units/g wet wt</td>
<td>25.9 ± 2.3</td>
<td>22.8 ± 1.6</td>
<td>18.1 ± 3.6†</td>
<td>14.8 ± 1.4†</td>
<td>14.9 ± 1.0†</td>
<td>13.7 ± 1.0†</td>
</tr>
<tr>
<td>2-Deoxy-D-glucose transport, μmol·ml⁻¹·20 min⁻¹</td>
<td>0.33 ± 0.05</td>
<td>0.58 ± 0.10</td>
<td>0.93 ± 0.06†</td>
<td>1.02 ± 0.16†</td>
<td>1.05 ± 0.12†</td>
<td>1.14 ± 0.10†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of muscles. Muscles were excised and stimulated at 100 HZ for 10 s at a rate of 1 contraction/min for 1, 5, 10, 15, and 20 min. *Significantly different from basal, P < 0.05. †Significantly different from 1 tetani, P < 0.05.

Table 3. Effects of low-intensity continuous swimming on glycogen concentration and glucose transport activity in rat epitrochlearis muscle

<table>
<thead>
<tr>
<th>Duration of Swimming With Weight ~ 3% Body Wt</th>
<th>Basal (n = 5)</th>
<th>10 min (n = 4)</th>
<th>30 min (n = 6)</th>
<th>120 min (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle glycogen, μmol glucose units/g wet wt</td>
<td>26.8 ± 2.8</td>
<td>17.0 ± 1.4*</td>
<td>13.5 ± 1.2*</td>
<td>9.9 ± 0.8†</td>
</tr>
<tr>
<td>2-Deoxy-D-glucose transport, μmol·ml⁻¹·20 min⁻¹</td>
<td>0.38 ± 0.02</td>
<td>0.78 ± 0.09*</td>
<td>1.23 ± 0.14†</td>
<td>1.67 ± 0.15††</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of muscles. Immediately after swimming, muscles were excised and analyzed. * Significantly different from basal, P < 0.05. †Significantly different from 10-min swimming, P < 0.05. ††Significantly different from 30-min swimming, P < 0.05.
GLUCOSE TRANSPORT AFTER HIGH-INTENSITY INTERMITTENT SWIMMING

Concentration and mean rate of 2-deoxy-D-glucose (2-DG) transport in vitro tetani (Table 1). Therefore, the higher glucose transport induced by high-intensity intermittent swimming seems to be related to a lower muscle glycogen content after swimming than that observed after repeated 10-s-long in vitro tetani. However, a causal mechanism explaining the negative correlation between muscle glycogen and glucose transport after exercise is not known. Some novel experimental approach seems necessary to explain the high glucose transport activity after high-intensity intermittent swimming.

In the present study, 120-min-long, low-intensity swimming reduced muscle glycogen concentration to a lower level and activated glucose transport to a greater extent than did ten 10-s-long in vitro tetani (Tables 2 and 3), whereas a previous study (3) reported that low-intensity swimming with the same duration activated glucose transport to the same extent as did ten 10-s-long in vitro tetani. Apparently, the results obtained from the two studies are in conflict. Because the weight attached to the swimming rat (3% of body weight) in the present study was heavier than that used in the previous study (2% of body weight), the total muscle work (probably substrate consumption) of the Epi during swimming in the present study is thought to be greater than that in the previous study. Therefore, it is possible that muscle glycogen content in the present study was lower than that in the previous study. Consequently, we believe that this apparent difference can also be explained by our hypothesis that the lower the glycogen content in muscle, the higher the glucose transport activity.

In a previous study by Gulve et al. (9), when the Epi was allowed to recover for 3 h after prolonged low-intensity swimming in incubation medium without glucose, glucose transport returned to basal levels. In this study, during the recovery period, muscle glycogen in Epi was not resynthesized, because the incubation medium did not contain glucose. Furthermore, when Epi was allowed to recover in incubation medium without glucose for 2 h after high-intensity intermittent swimming, we observed that glucose transport returned to near-basal levels while muscle glycogen content was as low as that observed immediately after the swim (unpublished observations). Therefore, this suggests that a reduction of muscle glycogen itself does not initiate the biochemical reactions that lead to activation of glucose transport. In addition to reduced muscle glycogen content, other factor(s) may be necessary for activating glucose transport. The transient increase in the cytoplasmic Ca\textsuperscript{2+} during muscle contraction may be a possible factor. Previous studies (1, 13, 14).
24, 26) suggest that an increase in cytoplasmic Ca\(^{2+}\), that activates phosphorylase kinase and induces a burst of glycogenolysis activates the process that results in increased glucose transport. An increase in cytoplasmic Ca\(^{2+}\) may increase glucose transport through activation of a Ca\(^{2+}\)-activated enzyme, in cooperation with a reduction in muscle glycogen.

Muscle contraction stimulates glucose transport by translocating glucose transporter GLUT-4-containing vesicles from an intracellular pool to the plasma membrane (6, 8, 19). An increase in GLUT-4 at the plasma membrane is considered to be due both to stimulation of translocation from an intracellular pool to the plasma membrane and to a decrease in the rate of endocytosis (16, 25). In terms of explaining the data obtained in the present investigation, we raise the hypothesis that translocation of GLUT-4 vesicles is interfered with by muscle glycogen. Coderre et al. (2) reported that ~30% of the GLUT-4 in rat skeletal muscle coprecipitated with glycogen and that transporters could be released from the glycogen particles by amylase digestion. Therefore, it may be possible that the more muscle glycogen is broken down, the more GLUT-4 is freed from glycogen, resulting in an increased intracellular pool of free GLUT-4 available in response to muscle contraction. Thus less muscle glycogen binding to intracellular GLUT-4 vesicles may facilitate GLUT-4 vesicles to undergo translocation in response to muscle contraction. We speculate that another factor (for example, Ca\(^{2+}\)), other than a reduction in muscle glycogen, may trigger the translocation of GLUT-4 vesicles, and that the amount of muscle glycogen binding to GLUT-4 vesicles may control the degree of GLUT-4 vesicle translocation.

Previous studies in which rat Epi muscle was used indicated that the maximal effects of insulin and in vitro tetani on glucose transport in Epi were additive (11, 22). Therefore, these previous studies suggest the hypothesis that muscle contractile activity and insulin increase glucose transporter through two different mechanisms. In the present study, although the effect of high-intensity intermittent swimming on glucose transport was much greater than the effect of in vitro tetani, the combined effects of swimming and insulin on glucose transport were also additive (see RESULTS). Therefore, the present study supports the hypothesis that there are two separate mechanisms of glucose transport activation in skeletal muscle.

During exercise, muscle glycogen is catabolized to lactate or CO\(_2\) for the resynthesis of ATP. When rats swim with a weight equal to 18% of body mass, the blood lactate concentrations were significantly elevated from resting levels of 1.3 ± 0.2 mM (n = 7) to 10.4 ± 0.3 mM (n = 7), and the mean exhaustion time was 63 s (see METHODS). Because the increase in blood lactate concentration roughly reflects production of lactate in active skeletal muscle, large amounts of glycogen in active skeletal muscle are considered to be catabolized to lactate during the high-intensity swimming. Furthermore, Medbo and Tabata (20) demonstrated that most of the glycogen broken down during 60 s of exhaustive exercise was catabolized to lactate. On the other hand, rats with a weight equal to 3% of body mass could swim for >120 min. During such a low-intensity exercise, the blood lactate concentration did not increase above resting levels. Therefore, consumed glycogen during low-intensity swimming was assumed to be metabolized not to lactate but to CO\(_2\). However, the data of muscle glycogen and glucose transport after low-intensity continuous swimming fell on the same regression line obtained from the data after high-intensity swimming (Fig. 1). Therefore, we suggest that the effect of exercise on glucose transport is mediated by a process associated with a decrease in muscle glycogen content, regardless of the end product from glycogen breakdown.

In summary, high-intensity intermittent swimming increases glucose transport in rat Epi to a higher level than that induced by ten 10-s-long in vitro tetani, which has been regarded as the maximal exercise-related stimulus for glucose transport. This result may be explained by the lower muscle glycogen level after high-intensity intermittent swimming than after in vitro tetani, because there was a significant negative correlation between glucose transport and muscle glycogen concentration in Epi after the high-intensity swimming and the in vitro tetani.

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