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

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Kawanaka, Kentaro, Izumi Tabata, Ayumi Tanaka, and Mitsuru Higuchi. Effects of high-intensity intermittent swimming on glucose transport in rat epitrochlearis muscle. 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.

Furthermore, we found recently that in vitro tetani-stimulated glucose transport in rat epitrochlearis (Epi) muscle was negatively correlated with postcontraction muscle glycogen concentration (17). This result also suggests that the lower the concentration of muscle glycogen, the higher the contraction-stimulated glucose transport rate.

In rat Epi muscle, ten 10-s-long in vitro tetani had a maximal effect on glucose transport, and more tetanic contractions did not induce a further increase in glucose transport (11, 17). Furthermore, glucose transport after this protocol was elevated to a level as high as that observed after 120 min of swimming with a weight equal to 2% of body mass (3). Therefore, activation of 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, our previous study showed that mean muscle glycogen concentration was ~15 µmol/g muscle after ten 10-s-long in vitro tetani, and more tetani did not further decrease muscle glycogen content (17). Therefore, it is not known whether a significant negative relationship between postcontraction muscle glycogen and glucose transport exists in the lower range of muscle glycogen concentration (i.e., much less than 15 µmol/g muscle).

Therefore, we examined glucose transport in Epi after high-intensity intermittent exercise that has been used as a tool to deplete muscle glycogen (4). For this purpose, we adopted intermittent swimming with a weight equivalent to 18% of body mass. After this protocol, muscle glycogen concentration decreased to much less than 15 µmol/g muscle, and glucose transport was further increased to a level that was much higher than that observed after ten 10-s-long in vitro tetani.

**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
Effects of high-intensity intermittent swimming on glycogen concentration and glucose transport activity

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⁻¹·20 min⁻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.

RESULTS

Effects of high-intensity intermittent swimming on muscle glucose transport and glycogen in rat Epi muscle. When rats swam with a weight equal to 18% of body mass, muscle glycogen concentration in Epi decreased as the number of bouts of swimming was increased (Table 1). Eight bouts of swimming reduced muscle glycogen concentration to a level of 7.6 ± 0.5 µmol glucose/g muscle (Table 1). 2-DG transport also increased as the number of bouts of swimming was increased (Table 1).

Effects of in vitro tetanic contractions on muscle glucose transport and glycogen in rat Epi muscle. Muscle glycogen concentration in Epi decreased with increasing numbers of in vitro tetani (Table 2).
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. 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 that observed after 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.
Table 4. Interactions among effects of in vitro tetanic contractions and high-intensity swimming on glycogen concentration and glucose transport activity in rat epitrochlearis muscle

<table>
<thead>
<tr>
<th>Muscle glycogen, µmol glucose units/g wet wt</th>
<th>Basal (n = 8)</th>
<th>15 Tetani (n = 5)</th>
<th>8 Bouts of 18% Swimming (n = 7)</th>
<th>Swimming + Tetani (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Deoxy-D-glucose transport, µmol·mL⁻¹·20 min⁻¹</td>
<td>26.7 ± 1.8</td>
<td>13.7 ± 1.0*</td>
<td>8.3 ± 0.7†</td>
<td>6.9 ± 0.5†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of muscles. Swimming + Tetani, rats swam with weight = 18% body wt for 20 s at a rate of 1 swim/min for 8 min, then muscles were excised and stimulated at 100 Hz to contract for 10 s at a rate of 1 contraction/min for 15 min. *Significantly different from basal, P < 0.05. †Significantly different from 15 tetani, P < 0.05.
tetani, the combined effects of swimming and insulin on 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 glyco-
gen, 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 contrac-
tion. 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 \(1.3 \pm 0.2\) mM (\(n = 7\)) to \(10.4 \pm 0.3\) 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 metabo-
ized 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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GLUCOSE TRANSPORT AFTER HIGH-INTENSITY INTERMITTENT SWIMMING


