Decreased insulin action on muscle glucose transport after eccentric contractions in rats

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Asp, Sven, and Erik A. Richter. Decreased insulin action on muscle glucose transport after eccentric contractions in rats. J. Appl. Physiol. 81(5): 1924–1928, 1996.—We have recently shown that eccentric contractions (Ecc) of rat calf muscles cause muscle damage and decreased glycogen and glucose transporter GLUT-4 protein content in the white (WG) and red gastrocnemius (RG) but not in the soleus (S) (S. Asp, S. Kristiansen, and E. A. Richter. J. Appl. Physiol. 79: 1338–1345, 1995). To study whether these changes affect insulin action, hindlimbs were perfused at three different insulin concentrations (0, 200, and 20,000 µU/ml) 2 days after one-legged eccentric contractions of the calf muscles. Compared with control, basal glucose transport was slightly lower (P < 0.05) in Ecc-WG and -RG, whereas it was lower (P < 0.05) at both submaximal and maximal insulin concentrations in the Ecc-WG and at maximal concentrations in the Ecc-RG. In the Ecc-S, the glucose transport was unchanged in hindquarters perfused in the absence or presence of a submaximal stimulating concentration of insulin, whereas it was slightly (P < 0.05) higher during maximal insulin stimulation compared with control S. At the end of perfusion the glycogen concentrations were lower in both Ecc-gastrocnemius muscles compared with control muscles at all insulin concentrations. Fractional velocity of glycogen synthase increased similarly with increasing insulin concentrations in Ecc- and control WG and RG. We conclude that insulin action on glucose transport but not glycogen synthase activity is impaired in perfused muscle exposed to prior eccentric contractions.

skeletal muscle; insulin resistance

A single bout of concentric exercise (which involves shortening of active muscle) is a recognized enhancer of insulin action systemically and in muscle in rats and humans (7, 19, 21, 22), whereas it has been reported that a bout of eccentric exercise (which involves forced lengthening of active muscle) transiently impairs whole body insulin action 2 days after the bout (16). The underlying mechanism(s) for this apparent insulin-resistant state remains obscure, but both local and systemic changes are present. In recent studies in humans and rats, we showed that eccentric contractions induce a transient decrease in the skeletal muscle glucose transporter isoform (GLUT-4) protein content (4, 5). GLUT-4 is the predominant glucose transporter in skeletal muscle fibers (17), and translocation of GLUT-4 from an intracellular pool to the sarcolemma and t-tubules occurs by insulin stimulation (12, 13, 29). Because insulin-induced glucose transport (15) and uptake (1, 10) have been found to correlate with muscle GLUT-4 content, decreased GLUT-4 content could be part of the explanation for the insulin resistance found systemically. Also, this could be part of the reason for the sustained low muscle glucose concentration after eccentric contractions (6, 9, 11, 20, 28). Thus, in the present study, we used the perfused rat hindquarter technique to investigate whether eccentric damage decreases insulin’s ability to stimulate muscle glucose transport and glycogen synthase activity. Because we previously showed that muscle GLUT-4 protein content was decreased maximally 2 days after eccentric contractions (5), insulin action on glucose transport and glycogen synthase activity was studied in muscle perfused 2 days after eccentric muscle contractions.

MATERIALS AND METHODS

Animals and diets. All experiments were approved by the Danish Animal Experiments Inspectorate and complied with the “European Convention for the Protection of Vertebrate Animals Used for Experiments and Other Scientific Purposes” (Council of Europe no. 123, Strasbourg, France, 1985). Male Wistar rats weighing ~200–240 g were housed three per cage on a 12:12-h light-dark cycle and were fed on an ad libitum standard chow diet (Altromin no. 1324, Petersen, Ringsted, Denmark; 62% energy intake as carbohydrate), which was maintained until the end of the experiment.

Eccentric contraction model. Rats were anesthetized by an intraperitoneal injection of Dormicum (midazolam, 0.5 µg/kg body wt; Roche, Switzerland) and Hypnorm (fentanyl, 20 µg/kg body wt, and fluoanison, 1 mg/kg body wt; J. ansseen), and the calf muscles on one side were stimulated for eccentric contractions ( Ecc-muscles) as described previously (5), whereas the contralateral muscles were unstimulated controls. In brief, muscles were subjected to 4 × 10 eccentric contractions with 1 min of rest between the 4 series. Stimulation sessions were carried out between 0800 and 1200, and after recovery from anesthesia the gait of the rats appeared completely normal.

Hindquarter perfusion. Two days after the stimulation the rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body wt) and prepared for hindquarter perfusion as described by Ruderman et al. (25) modified for male rats. Before insertion of the perfusion catheters, the rat was heparinized with 500 IU heparin in the inferior vena cava. The rat was killed by an intracardial injection of pentobarbital sodium just before being placed in the perfusion cabinet. The perfusion apparatus was similar to that described previously (24). The perfusate consisted of Krebs-Henseleit solution, 1- to 2-day-old bovine erythrocytes, 5% bovine serum albumin, 10 mM glucose, and 0.5–1.0 mM lactate originating from the erythrocytes. Human insulin was present in the perfusate at 0, 200, or 20,000 µU/ml cell-free perfusate from the beginning of the perfusion. The perfusate was continuously gassed with a mixture of 3% CO2-35% O2-62% N2. To ensure a perfusate temperature of 37°C, the perfusate was warmed in a water-perfused heating coil (37°C) immediately before entering the hindquarter. The first 25 ml of perfusate that passed through the hindquarter were discarded, and then the perfusate was recirculated. The initial volume of perfusate was 300 ml. To allow equilibration, the hindquarter was preperfused and then the actual experi-
tal period began. When insulin was omitted from the perfusate (basal), the hindquarters were preperfused for 10 min and the experimental period, where tracer was added to the perfusate, was 30 min. In the experiments with insulin at 200 µU/ml (submaximal), the hindquarters were preperfused for 15 min followed by a 15-min experimental period and finally the experiments with insulin at 20,000 µU/ml (maximal); the preperfusion period was 15 min followed by a 10-min experimental period. The different exposure times for the isotopes were constructed to obtain intramuscular concentrations of 3-O-methyl-D-glucose of <30% of the cell-free perfusate concentration to avoid nonlinearity of uptake. At the end of perfusion, the pump was stopped and muscle samples were cut out, trimmed of connective tissue and visible blood, and blotted. The superficial part of the gastrocnemius (WG) muscle, which consists mainly of fast-twitch white fibers (2), the soleus (S) muscle, which consists mainly of slow-twitch fibers (2), and, finally, a portion of the deep part of the medial head of the gastrocnemius (RG) muscle, consisting mainly of fast-twitch red fibers (2), were cut out. The muscle samples were freeze-dried with tongs cooled in liquid nitrogen and stored at −80°C until analyzed.

For measurement of muscle membrane glucose transport, 10–20 µCi of 3-O-[methyl-14C]-methyl-D-glucose (specific activity 315 mCi/mmol; New England Nuclear, Boston, MA) along with 20 µCi [3H]mannitol (specific activity 22.5 Ci/mmol; New England Nuclear) were added at the start of the actual experimental period. Also, cold mannitol yielding a perfusate concentration of 1 mM was added simultaneously. Because transit time from the reservoir to the hindquarter was 2.75 min, the hindquarters were exposed to isotopes for 27.25, 12.25, or 7.25 min, at basal, submaximal, and maximal insulin concentrations, respectively. Muscle glycogen was measured by a hexokinase method after acid hydrolysis (18). Glycogen synthase activity was measured with a modification of the filter paper method of Thomas et al. (27), where maximal activity was measured at saturating (8 mM) glucose 6-phosphate concentration and the percent fractional velocity was calculated as activity at a submaximal glucose 6-phosphate concentration (0.17 mM) in percentage of maximal activity. Total water content of muscle was determined by weighing before and after lyophilization for 48 h.

Glucose transport. Uptake of 3-O-methyl-D-glucose in the individual muscles was determined in perchloric acid extracts and corrected for label in the extracellular space determined by the 3H counts for mannitol. From the uptake of labeled 3-O-methyl-D-glucose, rates of glucose transport were calculated by using a specific activity of glucose determined by the glucose concentration in the cell-free arterial perfusate and the 3-O-methyl-D-glucose counts. To compare transport rates into muscles despite differences in mannitol space in control and Ecc-muscles, transport was expressed as micromoles of glucose per gram of non-mannitol-accessible muscle per hour, and for this calculation we used an equation derived from Richter et al. (23), assuming the mass density of the mannitol space being 1 g/ml.

\[ C_{\text{mna}} = (C_m - C_p) \cdot E \cdot (W - E)^{-1} \cdot T^{-1} \]

where \( C_{\text{mna}} \) is glucose transport rate (in µmol glucose·g of non-mannitol-accessible muscle·h⁻¹), \( C_m \) is glucose concentration in muscle calculated from 3-O-[methyl-14C]-methyl-D-glucose counts in muscle, with the assumption of the same specific activity in muscle and plasma (µmol glucose/g wet wt muscle), \( C_p \) is glucose concentration in perfusate plasma water (µmol glucose/ml perfusate plasma water), \( E \) is extracellular (mannitol) space (ml/g wet wt muscle), \( W \) is muscle weight (g wet wt), and \( T \) is time that muscles are exposed to tracers (h).

Statistics. Mean values from control and Ecc-muscles were compared by Student's paired t-test. To compare mean glycogen values at the different insulin concentrations, a one-way analysis of variance was used. Student's unpaired t-test was used as post hoc test.

RESULTS

Compared with the control muscle, basal glucose transport rate was slightly higher in the Ecc-WG and -RG but not in the Ecc-S (Fig. 1). Insulin-stimulated glucose transport was impaired in the Ecc-WG both at submaximal and maximal insulin concentrations (Fig. 1A). In the Ecc-RG, the maximal transport rate was lower compared with control muscle, whereas at submaximal insulin concentrations there was no significant difference (Fig. 1B). In the Ecc-S, maximal insulin-stimulated glucose transport surprisingly was slightly but significantly higher compared with control muscle (Fig. 1C).

The muscle glycogen concentration at the end of the perfusions was lower in the Ecc-WG and -RG, whereas in the Ecc-S the glycogen concentration was similar to the corresponding control leg at all insulin concentrations (Table 1). In control muscle, glycogen concentrations at the end of perfusion were significantly higher when insulin was present in the perfusate than when it was absent. This was also the case in the Ecc-RG and -S but not in the Ecc-WG, in which no net insulin-stimulated glycogen synthesis was apparent (Table 1).

The fractional velocity of glycogen synthase increased with increasing insulin concentrations in WG and not significantly in RG, and there were no differences between Ecc- and control muscles (Table 2). The maximal activity of glycogen synthase was on average 16% lower in the Ecc-WG compared with control, whereas it was unaffected in the Ecc-RG (Table 2).

The mannitol space was larger in all Ecc-muscles compared with control (Table 3). The water content was 4.2 ± 0.5% higher in the Ecc-WG and 2.5 ± 0.5% higher in the Ecc-RG compared with control. No change was found in the Ecc-S (Table 3).

DISCUSSION

The principal finding in this study is that insulin action on skeletal muscle glucose transport was impaired in fast-twitch muscle by prior eccentric contractions. The decrease was most pronounced in the WG, whereas it was less marked in the RG and no suppression was found in the S.

It has been reported that a bout of eccentric exercise transiently impairs the stimulating action of a submaximal insulin concentration on whole body glucose disposal 2 days after the bout (16), but the underlying mechanism(s) for this apparent insulin-resistant state remains obscure, involving local and/or systemic changes. The in vitro hindquarter technique allows us to measure changes of insulin action in muscle caused by prior eccentric contractions, and the results indicate that local muscle effects might at least partly be
responsible for the previously observed whole body insulin resistance (16). We found the largest effect on glucose transport in the WG, in which we recently showed that muscle GLUT-4 protein content is decreased by 65% 2 days after eccentric contractions (5). In comparison, in the RG in which the decrease in GLUT-4 protein is only 30% (5), prior eccentric contractions impaired insulin-stimulated glucose transport less (Fig. 1). The results from these muscle types are in agreement with the view that the insulin-induced increase in muscle glucose uptake is dependent on the GLUT-4 protein content (1, 10, 15). Finally, in the S muscle, eccentric contractions had no effect on the GLUT-4 protein content (5), and the maximal insulin stimulated glucose transport was actually slightly but

Table 1. Glycogen in calf muscles 2 days after one-legged eccentric contractions

<table>
<thead>
<tr>
<th>Insulin, µU/ml</th>
<th>0</th>
<th>200</th>
<th>20,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>WG-C</td>
<td>197.7 ± 14.4</td>
<td>238.6 ± 9.1†</td>
<td>238.0 ± 8.4†</td>
</tr>
<tr>
<td>Ecc-WG</td>
<td>132.0 ± 6.1*</td>
<td>134.1 ± 16.6*</td>
<td>141.0 ± 6.7*</td>
</tr>
<tr>
<td>RG-C</td>
<td>213.8 ± 16.3</td>
<td>255.6 ± 11.4†</td>
<td>275.7 ± 9.7†</td>
</tr>
<tr>
<td>Ecc-RG</td>
<td>137.8 ± 17.7*</td>
<td>180.8 ± 11.1†</td>
<td>191.1 ± 7.1†*</td>
</tr>
<tr>
<td>S-C</td>
<td>151.0 ± 10.3</td>
<td>188.2 ± 8.7†</td>
<td>215.6 ± 10.2†</td>
</tr>
<tr>
<td>Ecc-S</td>
<td>152.9 ± 10.1</td>
<td>187.2 ± 10.8†</td>
<td>209.5 ± 9.0†</td>
</tr>
</tbody>
</table>

Values are means ± SE of 7–9 observations in each group given in mmol/kg dry wt. WG-C, control white gastrocnemius; Ecc-WG, eccentrically stimulated white gastrocnemius; RG-C, control red gastrocnemius; Ecc-RG, eccentrically stimulated red gastrocnemius; S-C, control soleus; Ecc-S, eccentrically stimulated soleus. *Significantly different from control value, P < 0.05. †Significantly different from basal (0 µU/ml insulin) value, P < 0.05.

Table 2. Glycogen synthase fractional velocity and maximal activity in calf muscles 2 days after one-legged eccentric contractions

<table>
<thead>
<tr>
<th>Insulin, µU/ml</th>
<th>0</th>
<th>200</th>
<th>20,000</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS fractional velocity, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WG-C</td>
<td>30.4 ± 2.5</td>
<td>38.7 ± 3.8</td>
<td>42.7 ± 2.1†</td>
<td></td>
</tr>
<tr>
<td>Ecc-WG</td>
<td>30.1 ± 3.9*</td>
<td>34.2 ± 3.3</td>
<td>40.4 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>RG-C</td>
<td>30.8 ± 3.6</td>
<td>32.7 ± 4.6</td>
<td>37.5 ± 3.6</td>
<td></td>
</tr>
<tr>
<td>Ecc-RG</td>
<td>30.1 ± 3.8</td>
<td>32.7 ± 4.6</td>
<td>37.5 ± 3.6</td>
<td></td>
</tr>
<tr>
<td>GS maximal activity, nmol·min⁻¹·mg dry wt⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ecc-WG</td>
<td>11.1 ± 1.1</td>
<td>12.7 ± 0.8</td>
<td>12.0 ± 0.7</td>
<td>11.9 ± 0.5</td>
</tr>
<tr>
<td>WG-Ecc</td>
<td>8.7 ± 1.0*</td>
<td>12.1 ± 1.3</td>
<td>9.7 ± 0.9*</td>
<td>10.0 ± 0.6*</td>
</tr>
<tr>
<td>RG-C</td>
<td>13.0 ± 1.2</td>
<td>10.8 ± 1.8</td>
<td>11.9 ± 1.4</td>
<td>11.0 ± 0.8</td>
</tr>
<tr>
<td>Ecc-RG</td>
<td>10.1 ± 0.8</td>
<td>9.4 ± 1.2</td>
<td>10.0 ± 0.9</td>
<td>9.7 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE of 7–9 observations in each group. Glycogen synthase (GS) fractional velocity was calculated as activity at submaximal glucose 6-phosphate concentration (0.17 mM) in percentage of maximal activity. Maximal activity was measured at saturating (8.0 mM) glucose 6-phosphate concentration. Average is average maximal activity for 22–26 observations in each group. *Significantly different from control value, P < 0.05. †Significantly different from basal value, P < 0.05.

Fig. 1. Glucose transport in calf muscles 2 days after one-legged eccentric contractions. Values are means ± SE of 7–9 observations in each group. When no SE is shown, it is contained within the symbols. A: white gastrocnemius. B: red gastrocnemius. C: soleus. Open symbols, control muscles; solid symbols, eccentrically stimulated muscles. *Significantly different from control, P < 0.05.
muscles 2 days after one-legged eccentric contractions. In the Ecc-WG compared with control muscle, it was increased approximately sixfold compared with basal by 200 µU/ml insulin and yet absolutely no tendency to an increase in muscle glycogen from 0 to 200 µU/ml insulin was found (Table 1). This might suggest that not only is glycogen synthesis impaired by decreased glucose transport but also glycogen breakdown may be accelerated. The latter may also be part of the explanation behind the lower glycogen values in the Ecc-RG compared with control because the decrease in insulin-stimulated glucose transport, especially at low insulin concentrations, was quite small in this muscle (Fig. 1B). These findings could suggest that the subnormal muscle glycogen concentrations found for several days after eccentric contractions (5, 6, 9, 11, 20, 28) are the result of both decreased insulin action on glucose transport secondary to decreased muscle GLUT-4 protein content and increased glycogen degradation.

The extracellular space, measured by the use of [3H]mannitol, was higher in all Ecc-muscles compared with control, and also the water content was higher in the Ecc-WG and -RG compared with control. The changes in the extracellular space were pronounced with a 12 and 7 ml/g increase in the Ecc-WG and -RG compared with control muscle, whereas the total water content only was 3 and 2 ml/100 g higher in the Ecc-WG and -RG compared with control muscle, respectively. The disparate changes in mannitol space and water content indicate that the major fraction of the increase in the former after the stimulation originates from the intracellular space probably secondarily to membrane damage. Because mannitol and 3-O-methyl-D-glucose have approximately the same molecular weight (182 and 194, respectively) and are both nonpolar compounds, they likely diffuse at nearly the same rate and any increase in distribution space for mannitol should be equally large for 3-O-methyl-D-glucose. Therefore, the increased apparent distribution space for mannitol should not be a problem in the calculation of specific glucose transport. We chose to express glucose transport as specific uptake of 3-O-methyl-D-glucose into the space that is not accessible to mannitol rather than per gram of muscle because in Ecc-muscle the larger mannitol space leaves less tissue for specific glucose transport per gram of muscle. Thus expression per gram muscle would tend to decrease specific glucose transport in eccentric muscle simply because of division by a larger mass of tissue that does not participate in specific glucose transport.

The maximal activity of the enzyme glycogen synthase, which presumably reflects enzyme concentration, was on average 16% lower in the Ecc-WG, whereas it was largely unchanged in the Ecc-RG. In previous studies we found no significant decrease in the maximal activity of glycogen synthase (4, 5), which was in accordance with the results from Doyle et al. (11). However, although we found a small decrease in the maximal activity, this was much smaller than the previously reported decrease in GLUT-4 (~65%) (5) in this fiber type, suggesting that the insulin- and/or exercise-regulatable glucose transporter (GLUT-4) is especially susceptible to this type of muscle damage.

We conclude that the decrease in the muscle content of the insulin- and/or exercise-regulatable glucose transporter (GLUT-4) 2 days after eccentric contractions is accompanied by impaired insulin-stimulated muscle glucose transport. This could at least partly explain the previously described systemic insulin resistance found 2 days after eccentric exercise (16) and the sustained decreased muscle glycogen concentration after eccentric contractions. However, increased glycogen degrada-

| Table 3. Mannitol space and water content in calf muscles 2 days after one-legged eccentric contractions |
|--------------------------------------------------|-----------------|
|                                 | Ecc             |              |
| Mannitol space, ml/100 g         | 14.0 ± 0.6      | 26 ± 1.5*    |
|                                 | 13.6 ± 0.8      | 20.9 ± 1.4*  |
|                                 | 17.7 ± 0.7      | 20.8 ± 0.7*  |
| Water content, ml/100 g         | 76.9 ± 0.2      | 80.0 ± 0.3*  |
|                                 | 76.1 ± 0.2      | 78.1 ± 0.4*  |
|                                 | 78.1 ± 0.2      | 78.0 ± 0.2   |

Values are means ± SE of 22–26 observations in each group. *Significantly different from control value, P < 0.05.
tion may also play a significant role in muscle after eccentric contractions.

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