Glucose uptake and metabolic stress in rat muscles stimulated electrically with different protocols

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Aslesen, Rune, Ellen M. L. Engebretsen, Jesper Franch, and Jørgen Jensen. Glucose uptake and metabolic stress in rat muscles stimulated electrically with different protocols. J Appl Physiol 91: 1237–1244, 2001.—In the present study, the relationship between the pattern of electrical stimulation and glucose uptake was investigated in slow-twitch muscles (soleus) and fast-twitch muscles (epitrochlearis) from Wistar rats. Muscles were stimulated electrically for 30 min in vitro with either single pulses (frequencies varied between 0.8 and 15 Hz) or with 200-ms trains (0.1–2 Hz). Glucose uptake (measured with tracer amount of 2-[3H]deoxyglucose) increased with increasing number of pulses whether delivered as single pulses or as short trains. The highest glucose uptake achieved with short tetanic contractions was similar in soleus and epitrochlearis (10.9 ± 0.7 and 12.0 ± 0.8 mmol·kg dry wt−1·30 min−1, respectively). Single pulses, on the other hand, increased contraction-stimulated glucose uptake less in soleus than in epitrochlearis (7.5 ± 1.1 and 11.7 ± 0.5 mmol·kg dry wt−1·30 min−1, respectively; P < 0.02). Glucose uptake correlated with glycogen breakdown in soleus (r = 0.84, P < 0.0001) and (epitrochlearis: r = 0.91, P < 0.0001). Contraction-stimulated glucose uptake also correlated with breakdown of ATP and PCr and with reduction in force. Our data suggest that metabolic stress mediates contraction-stimulated glucose uptake.

glycogen; adenosine 5′-triphosphate; force; fiber type; fatigue

CONTRACTILE ACTIVITY AND INSULIN are the two main stimuli of glucose uptake in skeletal muscle (34). Although it has been more than 100 yr since Chauveau and Kaufmann (3) reported that contractile activity increases glucose uptake in skeletal muscle, the signaling pathway for contraction-stimulated glucose uptake is still unknown (34). Contraction and insulin, however, regulate glucose uptake via different signaling pathways. Insulin stimulates glucose uptake via phosphatidylinositol 3-kinase, but this kinase is not involved in contraction stimulated glucose uptake (27, 39).

Glucose transport in skeletal muscle is regulated by translocation of the glucose transporter GLUT-4 from intracellular vesicles to the plasma membrane. Both insulin and contractile activity stimulate translocation of GLUT-4, but it is believed that insulin and contraction translocate different pools of GLUT-4 (6, 32). The total amount of GLUT-4 and glucose uptake during insulin stimulation is higher in type I than in type II fibers of rats (2, 11, 15, 21, 34). There is, however, controversy about the ability of contractile activity to stimulate glucose uptake in different fiber types. Most studies using treadmill running report higher glucose uptake in type I fibers (21, 35), whereas most studies using electrical stimulation report higher glucose uptake in type II fibers (11, 23, 31, 36). The low level of glucose uptake in type II fibers during treadmill running may be due to lack of recruitment of these fibers rather than the inability of contractile activity to stimulate glucose uptake in vivo.

Only a few detailed studies exist concerning the relationship between the intensity of electrical stimulation and glucose uptake (23, 27, 30). In general, these studies show that the glucose uptake increases with increasing stimulus intensity (23, 27, 30, 31). Besides, more intense electrical stimulation is required in the slow-twitch soleus muscles than in fast-twitch muscles for activation of glucose uptake (8, 23, 31). The signaling pathway for contraction-stimulated glucose uptake has received much interest because contractile activity stimulates glucose uptake in insulin-resistant muscles (11). Knowledge about the relationship between the intensity of the electrical stimulation and glucose uptake is important to explore the signaling pathway for contraction-stimulated glucose uptake. The present study was designed to investigate thoroughly the relationship between stimulation pattern and glucose uptake in the fast-twitch epitrochlearis and the slow-twitch soleus muscle.

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Several lines of evidence have suggested that metabolic stress may play a role in contraction-stimulation glucose uptake. Recently, AMP-activated protein kinase has been suggested to mediate the effect of contraction (12, 13, 19). Glycogen, however, is also important for regulation of glucose uptake (16, 17, 21, 22), but so far the relationship between glycogen breakdown and glucose uptake has not been studied when muscles with normal glycogen concentration are stimulated with different intensities for the same period of time. The second purpose of this study was, therefore, to investigate the relationship between contraction-stimulated glucose uptake and breakdown of glycogen. Furthermore, relationships between contraction-stimulated glucose uptake and reduction in concentration of ATP and phosphocreatine (PCr) and reduction in force were investigated.

**MATERIALS AND METHODS**

**Animals.** Male Wistar rats were obtained from Mollegård Breeding Center (Lille Skensved, Denmark) and housed for at least 1 wk at 21°C and a 12:12-h light-dark cycle (light from 7:00 AM to 7:00 PM). The rats had free access to rat chow and water. The experiments were performed during the light cycle (10:00 AM to 2:00 PM), and the weight of the rats on the day of the experiment was 120–150 g. The experiments were conducted in conformity with the laws and regulations controlling experiments on live animals in Norway and the European Convention for the Protection of Vertebrate Animals used in Experimental and Other Scientific Purposes.

**Muscle preparation and incubation.** The rats were anesthetized with an intraperitoneal injection of ~7.5 mg pento-barbital (50 mg/ml), and the epitrochlearis and soleus muscles were dissected out. The epitrochlearis muscles were studied intact, whereas the soleus muscles were split in two strips. Some muscles were used for measurements of force development, and other muscles were used for measurements of glucose uptake. All muscles were preincubated for 30–40 min in 3.5 ml Krebs Henseleit buffer containing 5.5 mM glucose, 2 mM sodium acetate, 5.5 mM HEPES, and 0.1% bovine serum albumin (Fraction V, Sigma Chemical) before stimulation and measurements of force or glucose uptake as described previously (2). The preincubations and all other incubations were performed at 30°C while 95% O₂ and 5% CO₂ were continuously gassed through the buffer.

**Force measurements.** The apparatus for measurements of force development was built at our institute (The Institute of Basic Medical Sciences, University of Oslo) according to the description by Neshet et al. (29). The force transducer (FORT 100, Precision Instruments, Sarasota, FL) was connected to an amplifier (built at The Institute of Basic Medical Sciences, University of Oslo), and the force was printed directly (Omni recorder, Houston Instrument, Austin, TX). The equipment was calibrated by hanging a 20-g weight (~0.2 N) on the force transducer. Muscles were mounted at the resting tension where the highest twitch force was obtained (~0.5 g) and rested for at least 30 min. Force development was measured during 30 min when muscles were stimulated with trains 200 ms long at a frequency of 100 Hz (square-wave pulses of 0.2-ms duration and 10-V amplitude) delivered every 0.5, 1, 2, 5, or 10 s (2, 1, 0.5, 0.2, and 0.1 Hz). After the stimulation, the muscles were removed from the contraction apparatus and frozen in liquid nitrogen. Tension-time product was calculated by summation of force multiplied with stimulation time (200 ms).

**Glucose uptake.** Glucose uptake was measured after the preincubation as described previously (2). In brief, the muscles were transferred to fresh buffer (containing 5.5 mM glucose) where 0.25 μCi/ml of 2-[1,2-3H(N)]-deoxy-o-glucose (30.6 Ci/mmol; DuPont, NEN) and 0.1 μCi/ml of [1-14C]-d-mannitol (54.5 mCi/mmol; DuPont, NEN) were added. The muscle from the one leg was stimulated to contract isometrically for 30 min, whereas the other served as resting control. Some groups of muscles were stimulated with single pulses (square-wave pulses of 0.2-ms duration and 10-V amplitude) delivered at frequencies of 0.83, 1.33, 2, 3.33, 6.66, 10, and 15 Hz. Other groups of muscles were stimulated with 200-ms trains at a frequency of 100 Hz (square-wave pulses of 0.2-ms duration and 10-V amplitude) delivered at rates of 2, 1, 0.5, 0.2, and 0.1 Hz. After incubations, the muscles were removed from the contraction apparatus, blotted on filter paper, frozen in liquid nitrogen, freeze-dried, and weighed. For measurements of glucose uptake, muscles were digested in 600 μl of 1 M KOH for 20 min at 70°C. Of the digest, 400 μl were added to 3 ml of scintillation solution (High-Ionic Flour, Packard) mixed, and counted for radioactivity (TRI-CARB 460C, Packard). Glucose uptake was determined as the intracellular accumulation of 2-[3H]deoxy-d-glucose during 30 min of incubation. Glucose uptake is presented as millimoles glucose per kilogram dry weight (dw), assuming similar uptake kinetics for glucose and 2-deoxyglucose. Contraction-stimulated glucose uptake is calculated as the difference between the rested and stimulated muscle.

**Glycogen.** For glycogen determination in muscles where glucose uptake was measured, 100 μl of the 1 M KOH muscle digest was neutralized with 17 μl glacial acetic acid, and 500 μl 0.3 M acetate buffer containing 0.5% amyloglucosidase (Boehringer-Mannheim) were added to hydrolyze the glycogen. In the muscles where force was measured, muscle samples and glycogen were hydrolyzed in 500 μl 1 M HCl for 2 h at 100°C. Glucose was measured fluorometrically according to Lowry and Passonneau (25). Glycogen is presented as millimoles glucose per kilogram dry weight. Contraction-stimulated glycogen breakdown is calculated as the difference between the rested and stimulated muscle.

**ATP, PCr, and lactate.** Metabolites were extracted from freeze-dried muscle samples in 3 M HClO₄ for 20 min on ice and neutralized with KHCO₃. The neutralized extracts were centrifuged at 4°C and frozen for later analysis. ATP, PCr, and lactate concentrations were measured fluorometrically according to Lowry and Passonneau (26).

**Statistics.** The data are presented as means ± SE. Glucose uptake, force reduction, and time-tension product in soleus and epitrochlearis during different stimulation protocols were compared by two-way analysis of variance with muscle and stimulation rate as factors. Analysis of variance with Fisher’s least significant difference as post hoc test were used to compare effects of 200-ms trains delivered at different frequencies. Maximal contraction-stimulated glucose uptake in epitrochlearis and soleus was compared with Students t-test. Correlations were assessed by linear regression analysis.

**RESULTS**

Stimulation of epitrochlearis and split soleus muscles with 200-ms trains produced similar force initially [0.16 ± 0.01 N (n = 32) and 0.16 ± 0.01 N (n = 29) respectively]. Mean weights of split soleus and epitrochlearis muscles used for force measurements were also similar (5.38 ± 0.28 and 5.20 ± 0.21 mg dw,
with identical protocol. In contrast to force, tension-time product tended to increase with increasing rate of stimulation (Tables 1 and 2). In epitrochlearis, however, there was a decrease in time-tension product at the highest rate of stimulation (Table 1).

Basal glucose uptake was 5.5 ± 0.2 (n = 104) and 4.1 ± 0.1 (n = 100) mmol·kg dw⁻¹·30 min⁻¹ in soleus and epitrochlearis (P < 0.00001), respectively. Glucose uptake increased with increasing rate of stimulation both when stimulated with short trains and with single pulses. The contraction-stimulated glucose uptake correlated with the total number of pulses in both soleus (r = 0.89; P < 0.0001) and epitrochlearis (r = 0.64; P < 0.03). In muscles stimulated with 200-ms trains, two-way analysis of variance showed differences between stimulation protocol and muscles (P < 0.0001; Tables 1 and 2) and interaction (P < 0.01). At a lower rate of stimulation, glucose uptake was increased more in epitrochlearis than in soleus (Tables 1 and 2). The highest glucose uptakes achieved when the muscles were stimulated with 200-ms trains were, however, similar in soleus and epitrochlearis (10.9 ± 0.7 and 12.0 ± 0.8 mmol·kg dw⁻¹·30 min⁻¹, respectively). In muscles stimulated with single pulses, two-way analysis of variance showed differences between muscles and stimulation (Fig. 2; P < 0.0001) but no interaction (P = 0.25). Half-maximal glucose uptake was obtained at a stimulation frequency of ~1.5 and 3 Hz in epitrochlearis and soleus muscles, respectively. This is in agreement with previous in vitro studies (27, 30). In soleus, the highest glucose uptake achieved when muscles were stimulated with single pulses was lower than when muscles were stimulated with 200-ms trains [10.9 ± 0.7 (n = 18) vs. 7.5 ± 1.1 mmol·kg dw⁻¹·30 min⁻¹ (n = 9); P < 0.02].

In resting soleus and epitrochlearis muscles, the glycogen concentration was 85.3 ± 2.3 (n = 104) and 153.9 ± 3.4 mmol/kg dw (n = 100), respectively. In muscles stimulated electrically, the glycogen concentration decreased with increasing rate of stimulation (Tables 1 and 2, Fig. 3). There was a high correlation

![Figure 1](http://jap.physiology.org/content/91/9/1239/F1)

**Fig. 1.** Force development in soleus (A) and epitrochlearis (B) muscles stimulated with 200-ms trains delivered at different rates for 30 min. Trains were delivered at rates of 0.1 Hz (●), 0.2 Hz (○), 0.5 Hz (▪), 1 Hz (▲), or 2 Hz (◆). Values are means ± SE; n = 6–8 for epitrochlearis and n = 5–7 for soleus.

### Table 1. Tension-time products, contraction-stimulated glucose uptake, and concentration of glycogen, ATP, PCr, and lactate in soleus muscles stimulated 30 min with 200-ms trains delivered at different frequencies

<table>
<thead>
<tr>
<th>Stimulation Protocol</th>
<th>Tension-Time Product, N’s</th>
<th>Contraction-Stimulated Glucose Uptake, mmol·kg⁻¹·30 min⁻¹</th>
<th>Glycogen, mmol/kg</th>
<th>ATP, mmol/kg</th>
<th>PCr, mmol/kg</th>
<th>Lactate, mmol/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of 200-ms trains, Hz</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0.1</td>
<td>401 ± 56* (5)</td>
<td>1.6 ± 0.6* (13)</td>
<td>85.2 ± 9.6* (13)</td>
<td>15.8 ± 1.4* (5)</td>
<td>51.7 ± 5.6* (5)</td>
<td>13.4 ± 1.8* (5)</td>
</tr>
<tr>
<td>0.2</td>
<td>583 ± 59* (6)</td>
<td>2.9 ± 0.8* (13)</td>
<td>63.8 ± 6.4* (13)</td>
<td>14.0 ± 1.5* (6)</td>
<td>42.8 ± 8.7* (6)</td>
<td>16.2 ± 1.9* (6)</td>
</tr>
<tr>
<td>0.5</td>
<td>1,129 ± 142* (5)</td>
<td>6.2 ± 1.0* (8)</td>
<td>67.4 ± 6.5* (8)</td>
<td>12.0 ± 1.0* (4)</td>
<td>36.3 ± 3.5* (4)</td>
<td>20.4 ± 3.6* (4)</td>
</tr>
<tr>
<td>1.0</td>
<td>1,136 ± 105* (6)</td>
<td>8.9 ± 0.7* (5)</td>
<td>46.6 ± 8.0* (5)</td>
<td>12.5 ± 0.9* (6)</td>
<td>40.9 ± 3.2* (6)</td>
<td>25.0 ± 4.6* (6)</td>
</tr>
<tr>
<td>2.0</td>
<td>945 ± 84* (7)</td>
<td>10.9 ± 0.7* (18)</td>
<td>46.9 ± 3.7* (18)</td>
<td>10.8 ± 0.4* (5)</td>
<td>33.3 ± 3.0* (5)</td>
<td>37.3 ± 6.9* (7)</td>
</tr>
</tbody>
</table>

Values are means ± SE; no. of muscles is given in parentheses. Contraction-stimulated glucose uptake was calculated as the difference between rested and stimulated muscles. Basal glucose uptake was 5.1 ± 0.2 mmol·kg⁻¹·dry wt⁻¹·30 min⁻¹ (57). Concentrations of metabolites in control soleus muscles: glycogen 86.1 ± 3.5 mmol/kg dry wt (57), ATP 15.3 ± 0.6 mmol/kg dry wt (7), PCr 54.3 ± 2.9 mmol/kg dry wt (7), and lactate 2.2 ± 1.0 mmol/kg dry wt (8). PCr, phosphocreatine. *Significantly different from †, P < 0.05. ‡Significantly different from §, P < 0.05.
between the glycogen content after the contraction and glucose uptake both in soleus ($r = 0.93$, $P < 0.00001$) and epitrochlearis ($r = 0.87$, $P < 0.00001$). For all stimulation protocols except 2 Hz, muscles from one leg were stimulated electrically, whereas contralateral muscles served as resting controls. Assuming that the initial glycogen concentration and basal glucose uptake were the same on both sides, contraction-stimulated glucose uptake and glycogen breakdown were calculated for each rat. There was a high correlation between contraction-stimulated glucose uptake and glycogen breakdown for both soleus ($r = 0.84$, $P < 0.003$) and with mean PCr breakdown ($r = 0.70$, $P < 0.03$).

Force reduction correlated with glycogen breakdown in soleus ($r = 0.70$; $P < 0.0001$) and epitrochlearis ($r = 0.71$; $P < 0.0001$). Reduction in ATP concentration also correlated with the reduction in force in epitrochlearis ($r = 0.67$; $P < 0.0001$) and soleus ($r = 0.57$; $P < 0.002$).

**DISCUSSION**

In the present study, we have made a thorough investigation of how different stimulation protocols stimulate glucose uptake in a slow-twitch oxidative (soleus) and fast-twitch glycolytic (epitrochlearis) muscle. Several conclusions can be made: 1) the highest glucose uptake achieved with tetanic contractions was similar in soleus and epitrochlearis, 2) higher glucose uptake could be obtained in soleus when the muscle was stimulated with short tetanic contractions compared with single pulses, 3) much higher glucose uptake was obtained in epitrochlearis than in soleus.

**Table 2. Tension-time products, contraction-stimulated glucose uptake, and concentration of glycogen, ATP, PCr, and lactate in epitrochlearis muscles stimulated 30 min with 200-ms trains delivered at different frequencies**

<table>
<thead>
<tr>
<th>Stimulation Protocol</th>
<th>Rate of 200-ms trains, Hz</th>
<th>Tension-Time Product, N·s</th>
<th>Glycogen, mmol/kg</th>
<th>ATP, mmol/kg</th>
<th>PCr, mmol/kg</th>
<th>Lactate, mmol/kg</th>
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<tr>
<td>0.1</td>
<td>254 ± 22 (6)</td>
<td>3.8 ± 0.4 (11)</td>
<td>108.6 ± 13.2 (11)</td>
<td>16.4 ± 0.8 (6)</td>
<td>47.8 ± 3.8 (6)</td>
<td>33.7 ± 6.9 (6)</td>
</tr>
<tr>
<td>0.2</td>
<td>475 ± 46 (7)</td>
<td>8.5 ± 0.6 (13)</td>
<td>81.1 ± 6.7 (13)</td>
<td>14.6 ± 0.9 (7)</td>
<td>49.2 ± 7.4 (7)</td>
<td>37.9 ± 4.9 (7)</td>
</tr>
<tr>
<td>0.5</td>
<td>549 ± 55 (6)</td>
<td>9.1 ± 1.2 (7)</td>
<td>70.8 ± 4.2 (7)</td>
<td>10.3 ± 1.2 (6)</td>
<td>33.8 ± 5.9 (6)</td>
<td>42.0 ± 8.8 (6)</td>
</tr>
<tr>
<td>1.0</td>
<td>527 ± 39 (5)</td>
<td>9.2 ± 0.8 (6)</td>
<td>52.7 ± 3.9 (6)</td>
<td>10.6 ± 1.1 (7)</td>
<td>43.1 ± 6.6 (7)</td>
<td>58.6 ± 8.5 (7)</td>
</tr>
<tr>
<td>2.0</td>
<td>394 ± 52 (6)</td>
<td>12.0 ± 0.8 (15)</td>
<td>63.3 ± 6.2 (15)</td>
<td>11.1 ± 0.9 (6)</td>
<td>44.1 ± 5.6 (6)</td>
<td>41.5 ± 5.2 (6)</td>
</tr>
</tbody>
</table>

Values are means ± SE; no. of muscles is given in parentheses. Contraction-stimulated glucose uptake was calculated as the difference between rested and stimulated muscles. Basal glucose uptake was $3.9 ± 0.2$ mmol·kg dry wt$−1$·30 min$−1$ (52). Concentrations of metabolites in control epitrochlearis: glycogen $148.1 ± 5.1$ mmol/kg dry wt (52), ATP $17.7 ± 0.6$ mmol/kg dry wt (9), PCr $66.8 ± 3.8$ mmol/kg dry wt (9), and lactate $5.9 ± 1.9$ mmol/kg dry wt (10). *Significantly different from †, $P < 0.05$. ‡Significantly different from §, $P < 0.05$.

![Fig. 2. Glucose uptake epitrochlearis (a) and soleus (b) muscles stimulated for 30 min with single pulses delivered at frequencies varying between 0.83 and 15 Hz. Contraction-stimulated glucose uptake was calculated as the difference between rested and stimulated muscles. dw, Dry weight. Values are means ± SE; n = 5–18 for soleus and n = 6–15 for epitrochlearis.](image)

![Fig. 3. Glycogen concentration in epitrochlearis (a) and soleus (b) muscles after 30 min with single pulses delivered at frequencies varying between 0.83 and 15 Hz. Values are means ± SE; n = 5–18 for soleus and n = 6–15 for epitrochlearis.](image)
when muscles performed short tetanic contractions at low rates, and 4) contraction-stimulated glucose uptake correlated with glycogen breakdown as well as with other markers of fatigue (decrease in force, reduction ATP and PCr).

The highest glucose uptake rates achieved with short tetanic contractions were similar in epitrochlearis and soleus muscles. Although soleus muscles have a higher total concentration of GLUT-4 (11, 15) and higher insulin-stimulated glucose uptake than epitrochlearis, (2, 11, 15), most studies have reported that contraction-stimulated glucose uptake is higher in fast-twitch than in slow-twitch muscles (11, 23, 31).

Our data, however, suggest that the highest glucose uptake achieved with short tetanic contractions is similar in fast- and slow-twitch muscles. Surprisingly, the importance of stimulation intensity has not been focused with major interest previously, and only a few protocols with tetanic contractions have been compared (9, 31). Our data clearly show that much higher glucose uptake was obtained in the glycolytic epitrochlearis muscle than in the soleus when stimulated with tetanic contraction at lower rates, whereas maximal contraction-stimulated glucose uptake was similar. Because metabolic stress has been suggested to mediate contraction-stimulated glucose uptake, the fatigue-re-

![](https://example.com/image1.png)

Fig. 4. Relationship between glycogen breakdown and glucose uptake in soleus (A) and epitrochlearis (B) muscles. Muscles were stimulated for 30 min with different protocols, and the contraction-stimulated glucose uptake and glycogen breakdown were calculated as the difference between control and stimulated muscles. ●, ▲, muscles stimulated with single pulses; ○, △, muscles stimulated with 200-ms impulse trains. Values are means ± SE; n = 5–18 for each point.

![](https://example.com/image2.png)

Fig. 5. Relationship between force reduction and contraction-stimulated glucose uptake. Soleus (●) and epitrochlearis (▲) muscles were stimulated for 30 min with 200-ms trains delivered at different rates. Contraction-stimulated glucose uptake was calculated as the difference between rested and stimulated muscles. Values are means ± SE; (force reduction: n = 4–7 for soleus and n = 6–8 for epitrochlearis; glucose uptake: n = 5–18 for soleus and n = 6–15 for epitrochlearis).

![](https://example.com/image3.png)

Fig. 6. Relationship between reduction in ATP and contraction-stimulated glucose uptake. Soleus (●) and epitrochlearis (▲) muscles were stimulated for 30 min with 200 ms trains delivered at different rates. Contraction-stimulated glucose uptake was calculated as the difference between rested and stimulated muscles. Values are means ± SE (ATP: n = 5–6 for soleus and n = 6–7 for epitrochlearis; glucose uptake: n = 5–18 for soleus and n = 6–15 for epitrochlearis).
sistant soleus muscle may require more intense stimulation than epitrochlearis to achieve the metabolic perturbations that activate contraction-stimulated glucose uptake.

A new finding of the present study is that lower glucose uptake can be achieved in soleus when muscles are stimulated with single pulses compared with short trains delivered at various rates. On the other hand, this is not the case in epitrochlearis muscles. Fast- and slow-twitch muscles, however, differ both in the frequency required for tetanic contraction and in the amount of sarcoplasmic reticulum (5, 14), which makes it difficult to compare intracellular effects of a stimulation protocol in different muscle fiber types. Our data clearly highlight that the stimulation protocol has to be considered carefully when the effect of contractile activity is compared in different muscles.

In vivo, glucose uptake increases with increasing workload, indicating that energy consumption and energy status in the muscles are important for the regulation of glucose uptake (1). Most in vitro studies use isometric contractions where no external work is performed, and a thorough investigation of dynamic contractions should be performed to establish the relationship between work and glucose uptake during electrical stimulation. Holloszy and Narahara (18), however, compared glucose uptake in frog muscles stimulated with similar frequency while lifting different loads but found no relationship between work and glucose uptake. Ihlemann et al. (19) used a different procedure to vary energy turnover. By mounting muscles at different length, force development was varied and glucose uptake was related to force development and energy turnover (19, 20). In the present study, a similar force was developed initially during the repeated tetanic contractions. The calculated tension-time product did not show any clear relationship between tension-time product and glucose uptake. In epitrochlearis, for example, the highest glucose uptake was achieved when muscles were stimulated with 200-ms trains at a frequency of 2 Hz, whereas higher tension-time products were obtained when trains were delivered at frequencies of 1 and 0.5 Hz. Tension-time products were also much higher in soleus than in epitrochlearis during stimulation protocols, whereas higher glucose uptake was obtained in epitrochlearis. Time-tension product, however, does not necessarily reflect energy turnover because a major part of ATP is used for Ca\(^{2+}\) handling, which furthermore differs between muscle fiber types (5, 38).

Metabolic stress seems to be involved in contraction-stimulated glucose uptake, and AMP-activated protein kinase has recently been suggested to mediate contraction-stimulated glucose uptake in skeletal muscle (12, 20, 13). The AMP-activated protein kinase is a fuel gauge, and the kinase is activated by reduction in ATP/AMP and PCr/Cr ratios and in pH (12, 33). In the present study, contraction-stimulated glucose uptake correlated with the reduction in force, suggesting that metabolic stress plays a role. Contraction-stimulated glucose also correlated with the reduction in ATP and PCr, which supports the hypothesis that activation of AMP-activated protein kinase could mediate contraction-stimulated glucose uptake. However, the role of AMP-activated protein kinase for contraction-stimulated glucose uptake has recently been questioned by Derave et al. (8), who reported that high glycogen concentration inhibits contraction-stimulated activation of AMP-activated protein kinase without affecting glucose uptake.

In the present study, we show a correlation between glucose uptake and glycogen breakdown when muscles are stimulated with different intensities for a fixed period of time. Surprisingly, this correlation has not been established, although a great number of studies have suggested that glycogen participates in the regulation of glucose uptake in skeletal muscles (16, 17, 21, 22). Our laboratory has previously studied the relationship between muscle glycogen and glucose uptake and the intensity of electrical stimulation in the anaesthetized rat (23). In that study, glucose uptake was positively correlated to glycogen breakdown for all muscles (E. Johannsson, J. Jensen, and H. A. Dahl, unpublished observation). Kawanaka and co-workers (24) have also reported a correlation between glucose uptake and glycogen concentration after activity, both when the period of time for muscle activity was varied during in vitro contractions and during swimming (25). Furthermore, the fact that contraction-stimulated glucose uptake is reduced in muscles where the initial glycogen concentration is increased (9, 17) also supports the hypothesis that glycogen breakdown participates in the regulation of contraction-stimulated glucose uptake.

A correlation between glycogen breakdown and glucose uptake does not prove a causal relationship. Glycogen seems, however, to have actions other than as an energy source for ATP production. Stephenson et al. (37) reported that excitation-contraction coupling and Ca\(^{2+}\) release was reduced in skinned muscle fibers where glycogen concentration was reduced, although the concentrations of ATP and PCr were maintained high. In agreement with previous studies we also find a correlation between glycogen breakdown and decrease in force (4). Because glycogen seems to be so important for excitation-contraction coupling and contractile activity (37), glycogen may also be the link to stimulation of glucose uptake. The glycogen molecule is associated with the enzymes that regulate its own metabolism in a glycogen-protein sarcoplasmic reticulum complex (10), and a major part of GLUT-4-containing vesicles are localized in the same area and translocated to the transverse tubules during contractile activity (32). It has been suggested that the GLUT-4-containing vesicles are physically attached to the glycogen particle and are released as the glycogen molecules diminish (7), but this theory has not been confirmed. An alternative explanation may be that during glycogen breakdown, phosphorylase kinase, phosphorylase, phosphatase, glycogen synthase, and other enzymes are released from the glycogen particle into the cytosol (10, 28) and that one of these enzymes...
in the non-glycogen-bound state starts a signaling pathway for translocation of GLUT-4.

In conclusion, soleus muscles require more intense electrical stimulation than epitrochlearis to achieve high rates of glucose uptake. The highest glucose uptake rates achieved with tetanic contractions are, however, similar in the slow-twitch soleus muscle and the fast-twitch epitrochlearis muscle when stimulated with short tetanic contractions. When muscles are stimulated with different protocols for a fixed period of time, contraction-stimulated glucose uptake correlates with breakdown of ATP, PCr, and glycogen as well as with reduction in force. The present findings suggest that metabolic stress mediates the effect of contraction on glucose uptake. Whether activation of AMP-activated protein kinase or reduced glycogen concentration mediates the effect has yet to be determined.

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