Regulation of skeletal muscle glycogen phosphorylase and PDH during maximal intermittent exercise

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Parolin, Michelle L., Alan Chesley, Mark P. Matsos, Lawrence L. Spriet, Norman L. Jones, and George J. F. Heigenhauser. Regulation of skeletal muscle glycogen phosphorylase and PDH during maximal intermittent exercise. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E890–E900, 1999.—The time course for the activation of glycogen phosphorylase (Phos) and pyruvate dehydrogenase (PDH) and their allosteric regulators was determined in human skeletal muscle during repeated bouts of maximal exercise. Six subjects completed three 30-s bouts of maximal isokinetic cycling separated by 4-min recovery periods. Muscle biopsies were taken at rest and at 6, 15, and 30 s of exercise during bouts 1 and 3. Phos was rapidly activated within the first 6 s of bout 1 from 12% at rest to 47% at 6 s. The activation of PDH increased from 14% at rest to 48% at 6 s and 95% at 15 s of bout 1. Phos reverted back to basal values at the end of the first bout, whereas PDH remained fully activated. In contrast, in the third bout, PDH was 42% at rest and was activated more rapidly and was nearly completely activated by 6 s, whereas Phos remained at basal levels (range 14–20%). Lactate accumulation was marked in the first bout and increased progressively from 2.7 to 76.1 mmol/kg dry wt with no further increase in bout 3. Glycogen utilization was also marked in the first bout and was negligible in bout 3. The rapid activation of Phos and slower activation of PDH in bout 1 was probably due to Ca2+ release from the sarcoplasmic reticulum. Lactate accumulation appeared to be due to an imbalance of the relative activities of Phos and PDH. The increase in H+ concentration may have served to reduce pyruvate production by inhibiting Phos transformation and may have simultaneously activated PDH in the third bout such that there was a better matching between pyruvate production and oxidation and minimal lactate accumulation. As each bout progressed and with successive bouts, there was a decreasing ability to stimulate substrate phosphorylation through phosphocreatine hydrolysis and glycolysis and a shift toward greater reliance on oxidative phosphorylation.

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and third bouts of exercise to construct a time course of Phos and PDH activation and to ascertain the regulatory factors responsible for Phos and PDH activity. This protocol also allowed us to calculate the relative contributions to ATP regeneration from substrate level phosphorylation by PCr hydrolysis and glycolysis and from oxidative phosphorylation within the two bouts of exercise.

**METHODS**

**Subjects**

Six male subjects (mean ± SE, age 28.5 ± 1.83 yr, height 178.8 ± 2.1 cm, and weight 85.8 ± 3.1 kg) participated in this study. Informed consent was obtained from each subject after a verbal and written explanation of the experimental protocol and its attendant risks. The study was approved by the McMaster University Ethics Committee.

**Experimental Protocol**

The exercise protocol for this study consisted of three 30-s bouts of maximal isokinetic cycling at 100 rpm separated by 4 min of rest. The isokinetic cycle ergometer used in this study has been described previously (34). Briefly, the cycle was fitted with a 3-hp direct current motor that set the upper limit of crank velocity at 100 rpm. The forces exerted by the subjects were recorded by strain gauges attached to the pedal cranks. Electrical signals were transferred to a computer, and work was calculated for each pedal stroke as the product of impulse and angular velocity. The total work for each 30-s period was calculated as the sum of 50 pedal strokes for two legs. The total work and power output accomplished during each time interval (i.e., 0–6, 6–15, and 15–30 s) were calculated from the sum of the pedal strokes for two legs over each interval.

Biopsies of the vastus lateralis were taken at rest and at 6, 15, and 30 s of bouts 1 and 3. Because it was not possible to obtain all of the biopsies on the same day, the sampling was distributed over three trials each separated by 1 wk (Fig. 1). The study was designed such that 1) the predetermined exercise intervals (i.e., 6, 15, or 30 s) were completed uninterrupted from rest before each biopsy was taken and 2) the resting and 30-s biopsies were obtained on the same day for bouts 1 and 3, respectively, so that muscle glycogenolysis could be determined within each bout from the difference between resting glycogen content and glycogen content at 30 s of exercise. During trial 1, two biopsies were taken at rest, one at 30 s of bout 1, and another at 6 s of bout 3. During trial 2, one biopsy was taken at 15 s of bout 1, two biopsies were taken at rest before bout 3, and one biopsy was taken at 30 s of bout 3. During trial 3, biopsies were taken at 6 s of bout 1 and 15 s of bout 3. A total of 10 biopsies were taken. The order of the trials was randomized for each subject and took place at the same time each day. Subjects were asked to consume a high-carbohydrate diet 48 h before trial 1 and to replicate this diet before the remaining two trials. The subjects were instructed to abstain from the consumption of caffeine and to refrain from intense exercise for 48 h before each trial.

**Muscle Sampling**

Before each trial, one thigh was prepared for needle biopsies of the vastus lateralis. Two or four incisions were made through the skin to the deep fascia under local anesthesia (2% lidocaine without epinephrine). Alternate legs were used on each visit so that one leg received four biopsies and the contralateral leg received six biopsies in total. Biopsies of the vastus lateralis were obtained as described by Bergström (5). All muscle biopsies were taken while the subject was on the bike and were immediately plunged in liquid N2. At the appropriate time, the exercise was interrupted and the clock was stopped. After the biopsy, cycling was resumed for the time remaining in the bout so that subjects completed a total of 30 s of maximal cycling for each bout. The time delay between interrupting and resuming cycling was ~9 s. The time elapsed between the start of the biopsy to complete freezing was ~10 s. One of the resting biopsies before bouts 1 and 3 was frozen in liquid N2 after a 30-s delay to obtain resting measurements of the active form of Phos (Phos; see Ref. 41). The biopsies were removed from the needle while frozen and were stored in liquid N2.

**Analyses**

A 5- to 15-mg piece of muscle was chipped from each biopsy under liquid N2 and was dissected free of blood and connective tissue for the determination of the active form of PDH (PDHa). On two larger biopsies, another piece of wet muscle was used for the determination of total PDH activity (PDHt).
PDHa and PDHt were measured as described by Constantin-Teodosiu et al. (13) and modified by Putman et al. (39). The remaining muscle was freeze-dried, dissected free of blood, connective tissue, and fat, and stored dry at −70°C for subsequent analysis.

One aliquot of powdered muscle was used for the determination of Phos activity, as described by Young et al. (52). Briefly, 3–4 mg of powdered muscle were homogenized at −25°C in 200 μl of buffer containing 100 mM Tris, 60% glycerol, 50 mM KF, and 10 mM EDTA (pH 7.5). Homogenates were then diluted with 800 μl of the above buffer without glycerol and were homogenized further at 0°C. Total (a + b) Phos activity (in the presence of 3 mM AMP) and Phos activity (in the absence of AMP) were measured by following the production of glucose 1-phosphate spectrophotometrically at 30°C. Maximal velocity ($V_{\text{max}}$) and the mole fraction of Phos a and a + b was calculated from the measured activities as described by Chasiotis et al. (11).

A second aliquot of powdered muscle was assayed enzymatically for glycogen, as described by Harris et al. (21). The remaining dry powdered muscle was extracted in a solution of 0.5 M perchloric acid (PCA), and 1 mM EDTA and was neutralized with 2.2 M KHCO3. The neutralized PCA extracts were assayed for ATP, PCr, creatine, pyruvate, lactate, glucose, glucose-6-phosphate (G-6-P), fructose-6-phosphate (F-6-P), and glyceral 3-phosphate (Gly-3-P) as described by Bergmeyer (4) and acetyl-CoA, the reduced form of coenzyme A (CoASH), and acetyl carnitine as described by Cederblad et al. (8). Muscle metabolites and enzyme activities were corrected for nonmuscle contamination.

Calculations

Intracellular H+ concentration ($[H^+]$) was calculated from muscle lactate and pyruvate concentrations according to Sahlin et al. (44) and was validated by Spriet et al. (45) for the type of exercise used in the present study. The concentrations of free ADP and free AMP were calculated from the near-equilibrium reactions of creatine kinase and adenylate kinase, respectively (15); however, these calculations do not provide information as to the exact location and local concentrations of these metabolites within the cell.

The concentration of free P1 was calculated as the difference between the concentrations of resting and exercise PCr, less the accumulation of G-6-P, F-6-P, and Gly-3-P plus the assumed resting concentration of 10.8 mM/kg dry wt (15). The concentrations of the monoprotonated (HPO4$^{2-}$) and diprotonated (H2PO4$^{-}$) forms of P1 were calculated from the total concentration of P1, and the calculated pH1 using a pK1 of 6.78.

The rate of glycolysis, expressed as millimole glycosyl units per kilogram dry weight per second, was estimated according to Spriet et al. (47) from the accumulation of glycolytic intermediates plus the flux of pyruvate through PDHa during each time interval. Because previous studies have shown that flux through PDH is equivalent to its level of activity (19, 22, 38), PDHa was converted to units of millimole per kilogram dry weight per second, assuming a wet-to-dry muscle ratio of 4:1 at rest and 4.5:1 during exercise (37)

glycolysis

= $\frac{\Delta([G-6-P] + [F-6-P]) + \Delta([Gly-3-P] + [lactate] + [pyruvate])/2}{\text{time} + \text{PDHa}_a/2}$

where $\Delta$ indicates change and brackets indicate concentration.

The rate of pyruvate production, expressed as millimole pyruvate units per kilogram dry weight per second, was calculated from the accumulation of pyruvate and lactate plus the flux of pyruvate through PDHa during each time interval. From a previous study using the same protocol (31), lactate efflux from the muscle was found to be very small and was thus not included in the calculation of pyruvate production.

pyruvate production = $\frac{\Delta([\text{lactate}] + [\text{pyruvate}])}{\text{time} + \text{PDHa}_a}$

The rate of pyruvate oxidation was estimated from the flux through PDHa, which was converted to units of millimole per kilogram dry weight per second. The rate of lactate accumulation was calculated from the differences in concentration between two time points, divided by the elapsed time.

The rate of ATP turnover from PCr was calculated from the breakdown of PCr, whereas the rate of ATP turnover from glycolysis was calculated from the accumulation of lactate and the flux of pyruvate through PDHa. The rate of ATP turnover from oxidative phosphorylation was calculated from total acetyl-CoA production as the area under the PDHa curves for each bout; 1 mmol of acetyl-CoA from glycolysis was equal to 15 mmol of ATP. The contribution of fat fuels (25) and exogenous glucose (29) was assumed to be negligible.

Statistical Analysis

Data were analyzed by a two-way ANOVA with repeated measures over time. When a significant F ratio was found, the Newman-Keuls post hoc test was used to compare the means. Net changes of metabolites during bouts 1 and 3 were compared using a paired dependent-samples t-test. Results were considered significant at P < 0.05.

RESULTS

Total Work and Average Power Output

Total work performed during the 30-s bouts of isokinetic cycling was 18.7 ± 0.8 and 13.8 ± 1.0 kJ in bouts 1 and 3, respectively, and was significantly greater in bout 1. The total power output generated during each bout decreased significantly from 622 ± 27 W during

![Fig. 2. Average power generated during each time interval of maximal isokinetic cycling at 100 rpm in the first bout (open bars) and third bout (filled bars). Data are means ± SE. *Significantly different from first 6 s of same bout. †Significant difference between bouts 1 and 3. ‡Significantly different from 7 to 15 s of same bout.](http://apendocophysiology.org/download/article/336x93/342x540/20171014_122033.5)
the first bout to 459 ± 32 W in the third bout. The average power declined progressively during each bout, and this decline was more pronounced in bout 1 (Fig. 2).

Phos

The maximal total Phos activities were similar between bouts and across time (Table 1). In contrast, the calculated maximal Phos activity increased from rest during bout 1, returned to basal levels at the end of bout 1, and remained unchanged throughout bout 3 (Table 1). Consequently, during bout 1, the percentage of Phos in the a form increased rapidly from a resting value of 11.8 ± 2.7 to 46.8 ± 5.3% at 6 s, remained elevated at 15 s, and reverted back to basal levels at 30 s (Fig. 3). In bout 3, the resting Phos mole fraction was similar to the resting value before bout 1 and remained unchanged throughout exercise (Fig. 3).

PDH

Before bout 1, resting PDH was 0.53 ± 0.02 mmol·min⁻¹·kg wet wt⁻¹ and increased in a linear fashion to 1.81 ± 0.16 mmol·min⁻¹·kg wet wt⁻¹ at 6 s and to 3.56 ± 0.50 mmol·min⁻¹·kg wet wt⁻¹ at 15 s (Table 1). Because PDH was 3.74 ± 0.59 mmol·min⁻¹·kg wet wt⁻¹, this represented complete activation of PDH (95.2 ± 13.2%), which was maintained for the remainder of bout 1 (Fig. 3). Resting PDH was approximately threefold higher before bout 3 vs. bout 1. In bout 3, PDH was nearly completely activated to PDH by 6 s and was fully activated for the remainder of exercise (Table 1 and Fig. 3).

Muscle Metabolites

Muscle glycogen utilization was 89.2 ± 31.3 mmol/kg dry wt during bout 1 but was negligible in bout 3 (4.2 ± 28.5 mmol/kg dry wt; Fig. 4). The rate of muscle glycogenolysis during the initial 6 s of bout 1 was 4.04 mmol glucosyl units·kg dry wt⁻¹·s⁻¹, remained elevated from 6 to 15 s, and dropped to 0.58 mmol glucosyl units·kg dry wt⁻¹·s⁻¹ in the final 15 s (Fig. 5). Glycogenolytic rates were depressed throughout bout 3 to levels similar to those in the last 15 s of bout 1. The concentrations of G-6-P, F-6-P, and Gly-3-P increased significantly from rest to 15 s of exercise in bout 1 and remained elevated (Table 2). In contrast, the concentrations of G-6-P, F-6-P, and Gly-3-P did not increase above resting levels during bout 3. The concentration of free glucose did not change within either bout but was significantly greater in bout 3 (Table 2).

During bout 1, the concentrations of lactate, pyruvate, and H⁺ increased progressively from rest until 15 s and remained elevated at 30 s (Fig. 4 and Table 2). Before bout 3, the concentrations of lactate, pyruvate, and H⁺ were similar to those found at the end of bout 1 and did not increase further during bout 3.

The rates of total pyruvate production and oxidation and lactate accumulation are shown in Fig. 6. The rate of pyruvate production was highest in the first 15 s of bout 1 but was reduced to <1/3 to 1/6 in the last 15 s of bout 1 and throughout bout 3. In contrast, the rate of pyruvate oxidation increased more than threefold dur-

Table 1. Muscle Phos activities and PDH at rest and during maximal intermittent isokinetic cycling

<table>
<thead>
<tr>
<th></th>
<th>( V_{\text{max}} ) for Phos a + b</th>
<th>( V_{\text{max}} ) for Phos a</th>
<th>PDH a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>1.97 ± 0.22</td>
<td>0.24 ± 0.08</td>
<td>0.53 ± 0.02</td>
</tr>
<tr>
<td>Bout 1</td>
<td>2.56 ± 0.43</td>
<td>0.35 ± 0.13</td>
<td>1.56 ± 0.25†</td>
</tr>
<tr>
<td>6 s</td>
<td>2.73 ± 0.30</td>
<td>1.28 ± 0.22*</td>
<td>1.81 ± 0.16*</td>
</tr>
<tr>
<td>Bout 3</td>
<td>3.08 ± 0.55</td>
<td>0.39 ± 0.05†</td>
<td>3.08 ± 0.55†</td>
</tr>
<tr>
<td>15 s</td>
<td>2.38 ± 0.25</td>
<td>1.09 ± 0.17*</td>
<td>3.56 ± 0.50††</td>
</tr>
<tr>
<td>Bout 3</td>
<td>2.60 ± 0.33</td>
<td>0.54 ± 0.10†</td>
<td>3.31 ± 0.40*</td>
</tr>
<tr>
<td>30 s</td>
<td>2.30 ± 0.29</td>
<td>0.52 ± 0.19†</td>
<td>3.71 ± 0.38*</td>
</tr>
<tr>
<td>Bout 3</td>
<td>2.21 ± 0.35</td>
<td>0.27 ± 0.14</td>
<td>3.46 ± 0.42*</td>
</tr>
</tbody>
</table>

Data are means ± SE. \( V_{\text{max}} \) for phosphorylase (Phos) a + b, calculated maximal total Phos activity; \( V_{\text{max}} \) for Phos a, calculated maximal Phos a activity in mmol·kg dry wt⁻¹·s⁻¹. Active form of pyruvate dehydrogenase (PDH a) is expressed in mmol·kg wet wt⁻¹·min⁻¹. *Different from rest of same bout. †Difference between bouts 1 and 3. ‡Different from previous time point in same bout.
ing the last 15 s of bout 1. Lactate accumulated at a rate that mirrored the rate of pyruvate production.

High energy phosphates. The concentration of PCr decreased from a resting value of 88.2 ± 4.7 to 7.6 ± 3.2 mmol/kg dry wt at 30 s of exercise in bout 1 (Table 3). PCr resynthesis was incomplete before bout 3 and was 79% of the amount available before bout 1. Total PCr hydrolysis was greater in bout 1 compared with bout 3 (80.7 vs. 59.9 mmol/kg dry wt).

The concentration of ATP was unchanged during exercise and between bouts (Table 3). The calculated concentrations of free ADP and AMP increased in a time-dependent fashion and were significantly greater at 30 s of exercise in bout 1 compared with bout 3 (Table 3).

The concentration of total free Pi increased rapidly during the first 6 s of exercise and was significantly elevated throughout bout 3 compared with bout 1, due to increased binding to accumulated hexose monophosphates in bout 1 (Table 3). Due to a greater change in [H+] in the first bout compared with the third, differences were shown between the two bouts when total free Pi was partitioned into its two molecular species.

The diprotonated form (H₂PO₄⁻) followed a similar pattern of change as total Pi, whereas the monoprotonated form (HPO₄²⁻) increased as a function of time only with no differences between bouts 1 and 3 (Table 3).

Acetyl group accumulation. The concentrations of total and free CoA remained constant throughout both bouts of cycling (Table 2). The concentration of acetyl-CoA increased from resting values during each bout and was significantly elevated throughout bout 3 compared with bout 1 (Table 2). The concentration of acetylcarnitine remained constant throughout each exercise bout but was greater in bout 3 compared with bout 1 (Table 2).

DISCUSSION

In the present study, we examined the regulation of metabolic changes in muscle during the first and last of three bouts of maximal exercise for 30 s separated by 4 min of rest. During bout 1, the challenge of the extreme ATP demand was met through substrate phosphorylation by rapid PCr breakdown and the rapid onset of glycogenolysis. Glycogenolysis and pyruvate production were maximal during the first 15 s and far exceeded the rate of oxidation through PDH, resulting in significant lactate accumulation. However, during the last 15 s, Phos had returned to resting levels with low rates of glycogenolysis and pyruvate production, whereas PDH was fully active by 15 s and pyruvate oxidation was more closely matched to its rate of production, resulting in minimal further lactate accumulation. In bout 3, Phos b-to-a transformation was inhibited such that glycogenolysis and pyruvate production were low. PDH activity was elevated before bout 3, and full activation was rapidly achieved during the third bout such that pyruvate oxidation was more closely matched to pyruvate production with minimal lactate accumulation. As each bout progressed and with
Table 2. Muscle metabolite content in the vastus lateralis at rest and during maximal intermittent isokinetic cycling.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Bout 1</th>
<th></th>
<th></th>
<th></th>
<th>Bout 3</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>6 s</td>
<td>15 s</td>
<td>30 s</td>
<td>Rest</td>
<td>6 s</td>
<td>15 s</td>
<td>30 s</td>
</tr>
<tr>
<td>Glucose, mmol/kg dry wt</td>
<td>1.3±0.2</td>
<td>4.0±2.2</td>
<td>2.0±0.3</td>
<td>4.3±1.6</td>
<td>12.8±3.4†</td>
<td>12.9±2.8†</td>
<td>9.7±0.9†</td>
<td>10.2±1.4†</td>
</tr>
<tr>
<td>G-6-P, mmol/kg dry wt</td>
<td>0.9±0.2</td>
<td>9.0±2.1*</td>
<td>18.4±2.6‡</td>
<td>16.8±1.6*</td>
<td>8.5±2.5†</td>
<td>7.0±1.4</td>
<td>11.8±1.6†</td>
<td>10.9±2.6†</td>
</tr>
<tr>
<td>F-6-P, mmol/kg dry wt</td>
<td>0.2±0.1</td>
<td>1.5±0.4*</td>
<td>3.4±0.5‡</td>
<td>3.9±1.3*</td>
<td>1.6±0.4†</td>
<td>1.8±0.5</td>
<td>2.4±0.4</td>
<td>2.0±0.6†</td>
</tr>
<tr>
<td>Glycogenolytic rate, mmol/kg dry wt</td>
<td>0.5±0.1</td>
<td>4.0±0.7*</td>
<td>7.4±0.3‡</td>
<td>6.5±1.4*</td>
<td>11.1±1.7†</td>
<td>10.8±1.4†</td>
<td>11.7±0.6†</td>
<td>11.6±1.6†</td>
</tr>
<tr>
<td>PCr, mM</td>
<td>62.3±0.3</td>
<td>110.3±5.4</td>
<td>157.4±9.8</td>
<td>182.9±12.4</td>
<td>216.4±48.0</td>
<td>254.5±45.8</td>
<td>231.4±13.0</td>
<td>254.5±36.1</td>
</tr>
<tr>
<td>Acetyl-CoA, µmol/kg dry wt</td>
<td>8.0±1.4</td>
<td>9.6±0.7</td>
<td>11.0±1.1*</td>
<td>14.4±4.3‡</td>
<td>14.3±1.5†</td>
<td>18.9±1.1‡</td>
<td>18.1±1.4†</td>
<td>21.2±1.8‡</td>
</tr>
<tr>
<td>Free CoASH, µmol/kg dry wt</td>
<td>58.4±9.6</td>
<td>67.2±13.2</td>
<td>66.6±7.6</td>
<td>60.9±21.6</td>
<td>64.0±12.0</td>
<td>62.8±8.8</td>
<td>66.1±10.6</td>
<td>58.9±8.4</td>
</tr>
<tr>
<td>Total CoASH, µmol/kg dry wt</td>
<td>66.4±9.9</td>
<td>76.8±13.6</td>
<td>77.7±8.1</td>
<td>75.2±23.4</td>
<td>78.3±13.2</td>
<td>81.7±9.8</td>
<td>84.1±12.0</td>
<td>80.0±9.7</td>
</tr>
<tr>
<td>Acetyl carnitine, mmol/kg dry wt</td>
<td>4.6±1.6</td>
<td>2.0±0.4</td>
<td>3.3±1.0</td>
<td>5.4±18</td>
<td>11.8±0.9†</td>
<td>11.7±2.0†</td>
<td>10.3±0.7†</td>
<td>11.1±1.1†</td>
</tr>
</tbody>
</table>

Data are means ± SE. G-6-P, glucose 6-phosphate; F-6-P, fructose 6-phosphate; Gly-3-P, glycerol 3-phosphate. * Different from rest of same bout. † Different from previous time point in same bout. ‡ Different from previous time point in previous bout.

each progressive bout, there was decreased substrate phosphorylation by PCr hydrolysis and glycolysis and a greater reliance on oxidative phosphorylation.

Contribution of Substrate Phosphorylation and Oxidative Phosphorylation to ATP Production

In response to the high demand for ATP turnover during the initial 6 s of maximal exercise, substrate level phosphorylation by rapid PCr hydrolysis provided an immediate source of ATP at a rate of 7.0 mmol·kg dry wt⁻¹·s⁻¹ (Fig. 7). The activation of Phos and glycolysis allowed substrate level phosphorylation by glycolysis at a rate of 6.2 mmol·kg dry wt⁻¹·s⁻¹, whereas the rate of ATP turnover by oxidative phosphorylation was 1.32 mmol·kg dry wt⁻¹·s⁻¹ in the first 6 s of exercise. Thus a total maximal ATP turnover rate of 14.5 mmol·kg dry wt⁻¹·s⁻¹ was obtained in the first 6 s of bout 1 and was in agreement with values obtained in other studies using similarly strenuous protocols (24). In the last 15 s of bout 1, when the PCr stores had been depleted and glycogenolysis was inhibited, substrate level phosphorylation accounted for a decreasing proportion of the total ATP turnover rate. In bout 3, Phos was still an important source of ATP regeneration, but, due to the inhibition of Phos, substrate level phosphorylation by glycolysis contributed very little. As each bout progressed and with each progressive bout, the decreasing ability to maintain substrate phosphorylation by PCr hydrolysis and glycolysis was supplemented by oxidative phosphorylation. As each bout progressed, and the average power output was reduced, the ATP turnover rate was reduced accordingly (Figs. 2 and 7).

Regulation of Phos

Glycogen supplies the majority of the energy required for rapid ATP provision during short-term maximal exercise (17, 18, 26, 35, 46). During the initial 6 s of the first bout, the glycogenolytic rate was estimated to be 4.04 mmol glucosyl units·kg dry wt⁻¹·s⁻¹, which remained elevated during the next 9 s and declined to
The high glycogenolytic rate attained during the first 6 s of bout 1 and maintained during the next 9 s would require a Phos activity of 4.04 mmol glucosyl units·kg dry wt⁻¹·s⁻¹. The calculated Vₐ₅₀ of Phos a at 6 s was not sufficient to accommodate such a high glycogenolytic rate. This suggests that complete activation of Phos a + b would be required to achieve this flux. Due to the rapid reversal of Phos a to b (41), the 10-s delay from muscle sampling to complete freezing in our study may have led to an underestimation of Phos a activity and hence Phos activation.

Previous studies using submaximal exercise intensities ranging from 30 to 90% VO₂max have shown that activation of Phos is in excess of the estimated flux through the enzyme and that glycogenolytic flux through the sarcolemmal reticulum at the onset of contraction.

Posttransformational regulation of Phos activity and glycogenolysis occurs via the availability of substrate (Pi and glycogen) and the presence of positive (free AMP and IMP) and negative (G-6-P) allosteric modulators. The Michaelis constant (Kₘ) values of Phos a and a + b for Pi are 26.2 and 6.8 mM, respectively (11). Furthermore, the presence of 0.01 mM free AMP reduces the Kₘ of Phos a for Pi to 11.8 mM (42). Phos b is also active in the presence of high levels of free AMP (>0.02 mM) and IMP (1). In response to the high demand for ATP turnover during the initial 6 s of maximal exercise, the rapid hydrolysis of PCr resulted in significant accumulation of free Pi, which falls within the range of the Kₘ for Phos.

The breakdown of glycogen is regulated by the rate-limiting enzyme Phos. Phos is regulated by reversible enzymatic phosphorylation and exists in two interconvertible forms, a less active b form and a more active a form. Phos b is transformed to its a form by Phos kinase, whereas dephosphorylation back to the b form is catalyzed by Phos phosphatase. The transformation of Phos is regulated at the hormonal level by cAMP and at the contractile level by Ca²⁺ release from the sarcoplasmic reticulum (9, 43). In the present study, the transformation of Phos to its more active a form was rapid and reached a peak (46.8 ± 5.3%) within the initial 6 s of bout 1 and remained elevated until 15 s. This rapid transformation was probably due to Ca²⁺ release from the sarcoplasmic reticulum at the onset of contraction.

Table 3. Muscle high energy phosphate content in the vastus lateralis at rest and during maximal intermittent isokinetic cycling

<table>
<thead>
<tr>
<th>Measure</th>
<th>Rest</th>
<th>6 s</th>
<th>15 s</th>
<th>30 s</th>
<th>Rest</th>
<th>6 s</th>
<th>15 s</th>
<th>30 s</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP, mmol/kg dry wt</td>
<td>20.8 ± 1.6</td>
<td>22.4 ± 0.5</td>
<td>20.4 ± 2.0</td>
<td>16.2 ± 4.9</td>
<td>17.6 ± 1.3</td>
<td>16.4 ± 2.0</td>
<td>18.1 ± 0.9</td>
<td>17.8 ± 1.4</td>
</tr>
<tr>
<td>Free ADP, µmol/kg dry wt</td>
<td>101.0 ± 8.7</td>
<td>208.8 ± 21.6</td>
<td>274.8 ± 17.3*</td>
<td>651.2 ± 13.8*</td>
<td>49.2 ± 6.4</td>
<td>168.9 ± 38.5</td>
<td>295.4 ± 58.0*</td>
<td>455.4 ± 95.9*††</td>
</tr>
<tr>
<td>Free AMP, µmol/kg dry wt</td>
<td>0.5 ± 0.1</td>
<td>1.9 ± 0.4</td>
<td>3.8 ± 0.6</td>
<td>20.0 ± 0.3*</td>
<td>0.1 ± 0.0</td>
<td>1.8 ± 0.6</td>
<td>5.1 ± 1.6</td>
<td>15.6 ± 4.1*††</td>
</tr>
<tr>
<td>PCr, mmol/kg dry wt</td>
<td>88.2 ± 4.7</td>
<td>46.2 ± 4.0*</td>
<td>28.2 ± 2.6*</td>
<td>7.6 ± 3.2*</td>
<td>69.7 ± 4.3*</td>
<td>25.7 ± 3.9*</td>
<td>14.9 ± 4.5*</td>
<td>9.8 ± 1.8*</td>
</tr>
<tr>
<td>Total Pi, mmol/kg dry wt</td>
<td>10.8</td>
<td>36.7 ± 3.4*</td>
<td>45.5 ± 9.4*</td>
<td>47.8 ± 6.5*</td>
<td>18.8 ± 10.4</td>
<td>55.7 ± 6.5*</td>
<td>62.1 ± 7.4*</td>
<td>63.7 ± 8.6*†</td>
</tr>
<tr>
<td>HPO₄²⁻, mmol/kg dry wt</td>
<td>7.8 ± 0.0</td>
<td>22.1 ± 1.6*</td>
<td>24.1 ± 5.3*</td>
<td>22.1 ± 2.9*</td>
<td>7.7 ± 4.2</td>
<td>20.8 ± 2.0*</td>
<td>25.4 ± 2.9*</td>
<td>24.3 ± 3.5*</td>
</tr>
<tr>
<td>H₂PO₄⁻, mmol/kg dry wt</td>
<td>3.0 ± 0.0</td>
<td>14.6 ± 1.8*</td>
<td>21.4 ± 4.2*</td>
<td>25.7 ± 3.6*</td>
<td>11.2 ± 6.5</td>
<td>35.0 ± 5.5*</td>
<td>36.7 ± 4.6*†</td>
<td>39.4 ± 5.8*†</td>
</tr>
</tbody>
</table>

Data are means ± SE. PCr, phosphocreatine. *Different from rest of same bout. †Different between bouts 1 and 3. ††Different from previous time point in same bout.

0.58 mmol glucosyl units·kg dry wt⁻¹·s⁻¹ in the final 15 s (Fig. 5). The initial glycogenolytic rates obtained in the present study are similar to those that have previously been reported for maximal short-term exercise (7, 18, 26). Additionally, the decline in glycogenolytic rate observed over the first bout is in agreement with a previous study from this laboratory (26) and a study from Boobis et al. (7) in which glycogenolysis occurred, for the most part, within the first 6–10 s of a 30-s sprint. Furthermore, there was a decrease in glycogen utilization over the first 6–10 s of a 30-s sprint. Additionally, there was a decrease in glycogen utilization over the first 6–10 s of a 30-s sprint. Posttransformational regulation of Phos activity and glycogenolysis occurs via the availability of substrate (Pi and glycogen) and the presence of positive (free AMP and IMP) and negative (G-6-P) allosteric modulators. The Michaelis constant (Kₘ) values of Phos a and a + b for Pi are 26.2 and 6.8 mM, respectively (11). Furthermore, the presence of 0.01 mM free AMP reduces the Kₘ of Phos a for Pi to 11.8 mM (42). Phos b is also active in the presence of high levels of free AMP (>0.02 mM) and IMP (1). In response to the high demand for ATP turnover during the initial 6 s of maximal exercise, the rapid hydrolysis of PCr resulted in significant accumulation of free Pi, which falls within the range of the Kₘ for Phos.

Previous studies using submaximal exercise intensities ranging from 30 to 90% VO₂max have shown that activation of Phos is in excess of the estimated flux through the enzyme and that glycogenolytic flux through the sarcolemmal reticulum at the onset of contraction.

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The high glycogenolytic rate attained during the first 6 s of bout 1 and maintained during the next 9 s would require a Phos activity of 4.04 mmol glucosyl units·kg dry wt⁻¹·s⁻¹. The calculated Vₐ₅₀ of Phos a at 6 s was not sufficient to accommodate such a high glycogenolytic rate. This suggests that complete activation of Phos a + b would be required to achieve this flux. Due to the rapid reversal of Phos a to b (41), the 10-s delay from muscle sampling to complete freezing in our study may have led to an underestimation of Phos a activity and hence Phos activation.

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Phos is mediated primarily through posttransformational regulation (substrate and allosteric; see Refs. 12, 16, 22). In the present study, the estimated glycogenolytic flux was equal to the total Phos activity, and the peak glycogenolytic rates were ~20-fold higher than rates of 0.17–0.25 mmol glucosyl units·kg dry wt⁻¹·min⁻¹ observed at 80–90% VO₂max (12, 16, 22). The power outputs generated during the first 15 s of exercise in bout 1 were 3.5-fold greater than that obtained in the study by Howlett et al. (22) in which the average power output was 229 ± 19 W at 90% VO₂max. Thus it appears that, during the first 15 s of maximal exercise, total Phos activation is required to maintain glycogenolytic flux. This involves upregulation of Phos activity by both transformational and posttransformational mechanisms.

Compared with the first 15 s of bout 1, glycogenolysis was reduced by 86–95% in the latter one-half of bout 1 and throughout bout 3. This larger reduction in glycogenolysis coincided with Phos a to b reversion and the inability to reactivate Phos transformation in bout 3. In addition, the predicted flux through Phos during the last 15 s of bout 1 and throughout bout 3 corresponded more closely to the observed glycogenolytic fluxes. At the end of the first bout, Vₘₐₓ for Phos a corresponded more closely to the glycogenolytic rate estimated over the last 15 s of bout 1 (Table 1 and Fig. 5). Similarly, during the third bout, the estimated glycogenolytic rates were in the range of the calculated Vₘₐₓ for Phos a during each time interval (Fig. 5 and Table 1). In contrast, the maximal Phos a + b activity remained stable during the entire exercise protocol (Table 1). These observations imply that Phos b was minimally activated during this time despite elevations in free AMP. It is conceivable that the accumulation of G-6-P, a known allosteric inhibitor of Phos b (17), could have overridden positive modulation by free AMP.

The reduction in Phos transformation observed at the end of the first bout and throughout bout 3 suggests that the mechanism of activation by Ca²⁺ was being overridden. Krebs et al. (30) have shown in vitro that elevated [H⁺] inhibited Phos kinase, thus reducing the transformation of Phos to its a form. During the last 15 s of the first bout, [H⁺] increased significantly to 182.9 ± 12.4 nM (pH 6.74) and up to 254.5 ± 45.8 nM (pH 6.59) at 6 s in the third bout (Table 2). Chasiotis et al. (10) also observed in human skeletal muscle that the mechanism of activation by Ca²⁺ was overridden during this time despite elevations in free AMP. It is conceivable that the accumulation of G-6-P, a known allosteric inhibitor of Phos b (17), could have overridden positive modulation by free AMP.

Increases in [H⁺] may also have a dual inhibitory effect by influencing the concentration of the substrate for Phos. An increase in [H⁺] increases the prevalence of the diprotonated Pᵢ, H₂P₀₄⁻, over the monoprotonated form, HPO₂⁻. The latter is considered to be the only active substrate of Phos (28). The fall in pH from 6.74 at the end of bout 1 to 6.61 at the end of the third bout implies that the proportion of Pᵢ in the diprotonated form increases from 25.7 mmol/kg dry wt at the end of bout 1 to 39.4 mmol/kg dry wt at the end of bout 3 in association with the increase in the concentration of total Pᵢ, nullifying the change in the monoprotonated form. Thus, although the total Pᵢ was higher in the third bout, the availability of the monoprotonated form was similar in both bouts (Table 3). The availability of substrate for Phos does not appear to be the factor that downregulates glycogenolysis in the latter part of bout 1 and in bout 3.

The reduced rate of glycogenolysis during the last 15 s of the first bout and throughout the third bout appears to be complex as it is modulated by the cumulative effects of a multiplicity of factors. In bout 1, the reduction in glycogenolysis appears to be primarily due to reversion of Phos a to b form and inhibition of Phos b activity at the end of the bout. In bout 3, the inability to transform Phos a to b could be due to increased activity of the Phos phosphatase or decreased activity of the Phos kinase due to elevated [H⁺]. Although substrate was available, the inhibitory effect of G-6-P appears to override the positive allosteric effects of free AMP on flux through Phos.

Regulation of PDH

PDH is the rate-limiting enzyme that regulates the entry of glycolytically generated pyruvate into oxidative metabolism. The PDH complex is a mitochondrial enzyme that is also regulated by reversible phosphorylation. PDH kinase catalyzes the phosphorylation of PDH with concomitant inactivation, whereas PDH phosphatase dephosphorylates the enzyme, thereby activating PDH. The activity of PDH is determined by the proportion of the complex in the active form. PDH kinase is inhibited by pyruvate and elevated ratios of CoASH to acetyl-CoA and NAD to NADH and, conversely, is stimulated by an elevated ratio of ATP to ADP. PDH phosphatase is stimulated by Ca²⁺ (see Refs. 3, 40, and 51 for review).

Ca²⁺ released from the sarcoplasmic reticulum at the onset of exercise was probably the primary stimulus for the activation of PDH. The Ca²⁺ released activates PDH phosphatase (23) and may simultaneously inactivate PDH kinase (40), thus increasing the proportion of PDH⁻. Increases in Ca²⁺ concentration in rat heart mitochondria have previously been shown in vitro to result in 60% activation of PDH (14), which is similar to what we observed in the first 6 s of contraction. The remaining 40% of the activation of PDH may have been due to other allosteric activators of PDH. Although the effects of Ca²⁺ on PDH activation were studied in cardiac muscle (14), it is generally believed that Ca²⁺ is also important in PDH activation in skeletal muscle (51).

Pyruvate is a known activator of PDH and inactivates PDH kinase (23). Pyruvate increased linearly during the first 15 s, served as substrate for the reaction, and may also have functioned as an allosteric regulator that stimulated activation of PDH. The levels of pyruvate appear to be within the estimated range of the inhibitory constant of PDH kinase for pyruvate of 0.5–2.0 mM (51). Increases in [H⁺] followed the same time course as pyruvate and may have played a role in PDH activation. Again, the only available evidence has been demonstrated in cardiac muscle. A study in per-
fused levels of pyruvate and $[H^+]$ significantly elevated above resting values before bout 1. In a previous study, we observed a minimal utilization of glycogenolysis, resulting in lactate accumulation. The increased glycolytic rate translated into energy provision and oxidation resulted in the net accumulation of pyruvate and lactate in the first 15 s of exercise (Fig. 6). As bout 1 progressed, there was a better match between pyruvate production and oxidation due to decreased glycolgenolysis and increased PDH activation, which was evidenced by the reduced accumulation of pyruvate and thus lactate toward the end of the first bout (Fig. 6). Throughout the third bout, the rates of pyruvate production were considerably reduced to $<0.67 \text{ mmol} \cdot \text{kg dry wt}^{-1} \cdot \text{s}^{-1}$, whereas the rates of pyruvate oxidation through PDH were maintained at $0.17-0.25 \text{ mmol} \cdot \text{kg dry wt}^{-1} \cdot \text{s}^{-1}$, representing less than a fourfold difference. The better matching between the rates of pyruvate production and pyruvate oxidation in the third bout resulted in no further accumulation of pyruvate or lactate.

During the initial seconds of maximal exercise, large rates of glycolgenolysis and glycolysis are required to provide a rapid and immediate source of ATP and $Ca^{2+}$ appears to be the primary mechanism responsible for Phos and PDH activation. The rapid hydrolysis of ATP and PCr resulted in an accumulation of free P, and AMP that increased Phos activity and substrate for glycolysis. The increased glycolytic rate translated into enhanced pyruvate production, which may have exerted a positive allosteric effect on the activation of PDH. The increase in $[H^+]$ toward the end of the first bout may have inhibited Phos to a transformation and glycojenolysis, while simultaneously activating PDH. The elevated $[H^+]$ at the beginning of and throughout the third bout may have completely inhibited the transformation of Phos and glycojenolysis and may have simultaneously maintained a high level of PDH activity. This mechanism of regulation may have served to spare the glycogen stores, inhibit further accumulation of lactate, and limit further increases in $[H^+]$. Thus the increase in $[H^+]$ that accompanies intense exercise may be the primary mechanism responsible for limiting further increases in $H^+$ by inhibiting Phos and the accumulation of lactate while increasing PDH activity and the oxidation of lactate. The result was a tighter match between flux through Phos and PDH and a shift toward greater reliance on oxidative phosphorylation and less.
on substrate phosphorylation by PCr hydrolysis and glycolysis.

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