Control of glycolysis in contracting skeletal muscle. II. Turning it off

GREGORY J. CROWATHER, WILLIAM F. KEMPER, MICHAEL F. CAREY, AND KEVIN E. CONLEY

Departments of Radiology, Physiology and Biophysics, and Bioengineering, University of Washington Medical Center, Seattle, Washington 98195-7115

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Crowther, Gregory J., William F. Kemper, Michael F. Carey, and Kevin E. Conley. Control of glycolysis in contracting skeletal muscle. II. Turning it off. Am J Physiol Endocrinol Metab 282:E74–E79, 2002.—Glycolytic flux in muscle declines rapidly after exercise stops, indicating that muscle activation is a key controller of glycolysis. The mechanism underlying this control could be 1) a Ca2+-mediated modulation of glycolgenolysis, which supplies substrate (hexose phosphates, HP) to the glycolytic pathway, or 2) a direct effect on glycolytic enzymes. To distinguish between these possibilities, HP levels were raised by voluntary 1-Hz exercise, and glycolytic flux was measured after the exercise ceased. Glycolytic H+ and ATP production were quantified from changes in muscle pH, phosphocreatine concentration, and Pi concentration as measured by 31P magnetic resonance spectroscopy. Substrate (HP) and metabolite (Pi, ADP, and AMP) levels remained high when exercise stopped because of the occlusion of blood flow with a pressure cuff. Glycolytic flux declined to basal levels within ∼20 s of the end of exercise despite elevated levels of HP and metabolites. Therefore, this flux does not subside because of insufficient HP substrate; rather, glycolysis is controlled independently of glycolgenolytic HP production. We conclude that the inactivation of glycolysis after exercise reflects the cessation of contractile activity and is mediated within the glycolytic pathway rather than via the control of glycogen breakdown.

We therefore conclude that the decline in glycolytic flux is a direct control; muscle energetics; human tibialis anterior

WHY DOES GLYCOLYTIC FLUX1 decline sharply at the end of exercise? An answer may lie in the mechanisms governing the onset of this flux at the start of exercise. We have previously shown that this onset is governed by a “dual-control” mechanism in which both metabolite levels and muscle activation affect glycolytic flux (9). The role of metabolite levels is consistent with the conventional model of glycolytic control, which holds that the metabolites generated by use of ATP increase flux by acting as substrates and allosteric activators of glycogenolycic and glycolytic enzymes (7). However, the decline in glycolytic flux at the end of exercise cannot simply be the result of changes in metabolites such as Pi, ADP, and AMP, because flux essentially ceases, even when these metabolites are kept high after exercise (24, 27). Instead, the inactivation of glycolysis accompanies the termination of muscle activity and therefore is consistent with a decline in a signal such as Ca2+.

Ca2+ could alter glycolytic flux by acting at two sites. First, a Ca2+-activated cascade converts glycogen phosphorylase (GP) from its less active b form to its more active a form (7). This conversion accelerates the glycogenolytic production of glucose 6-phosphate (G-6-P), the substrate for glycolysis; thus Ca2+ could modulate glycolytic flux through the supply of G-6-P to the pathway. In this scenario, a drop in intracellular Ca2+ concentration ([Ca2+]i) at the end of exercise would then inactivate glycolysis by limiting substrate supply.

A second possibility is that Ca2+ (or another contraction-related signal) controls glycolysis by acting directly on the glycolytic pathway. For example, this direct control could occur via Ca2+-initiated phosphorylation of glycolytic enzymes (23) and/or via Ca2+-mediated binding of these enzymes to the cytoskeleton (1). A fall in [Ca2+] after exercise would then lead to a reversal of these changes and a fall in glycolytic flux.

In the present study, we determined whether substrate supply governs glycolytic flux. Specifically, is glycolysis turned off after exercise because glycogenolysis stops providing it with sufficient G-6-P? We tested this hypothesis by quantifying flux during postexercise ischemia, when hexose phosphates (HP) and thus G-6-P remain elevated. Our data show that glycolytic flux declines even when HP concentrations ([HP]) remain high. This finding suggests that Ca2+ or another contraction-related factor stimulates glycolytic enzymes directly rather than through the glycogenolytic supply of substrate.

1We consider glycogenolysis to be the conversion of glycogen to glucose 6-phosphate and glycolysis to be the conversion of glucose 6-phosphate to lactate acid.

Address for reprint requests and other correspondence: K. E. Conley, Dept. of Radiology, Box 357115, Univ. of Washington Medical Center, Seattle, WA 98195-7115 (E-mail: kconley@u.washington.edu).

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METHODS

The subjects and exercise protocols are reported in the accompanying paper (9). Key details and differences are noted below.

Subjects, experimental setup, data acquisition, and analysis of spectra. Eight adult men aged 25–61 yr and recruited from a population of normal volunteers were studied. Each subject lay supine in the bore of a 1.5-Tesla spectrometer; the right leg and foot were held in place with a plastic holder; and a strain gauge was used to measure force exerted by the ankle dorsiflexors. A surface coil placed over the anterior compartment of the leg was used to acquire 31P magnetic resonance spectra of the ankle dorsiflexors.

A high-resolution control spectrum of the resting muscle was acquired under conditions of fully relaxed nuclear spins (interpulse delay: 16 s). Sequential spectra were then obtained under partially saturating conditions (interpulse delay: 1.5 s) throughout the experimental protocol described below. The spectrum for each time point consisted of four summed acquisitions taken over 6 s.

Free-induction decays (FIDs) were summed, baseline corrected, zero filled, apodized with an exponential filter matched to the line width of the unfiltered phosphocreatine (PCr) peak (3–5 Hz), and Fourier transformed into spectra. Fully relaxed spectra were analyzed with the “MacFID” program (Tecmag, Houston, TX), whereas partially saturated spectra were analyzed with the “Fit-to-Standard” (17) and “MRUI” (28) programs. Relative areas of the PCr, P\textsubscript{i}, and HP peaks were converted to absolute concentrations, assuming ATP concentration to be 8.2 mM in human muscle (16). Free ADP concentration and AMP concentration were calculated assuming the creatine kinase and adenylate kinase reactions to be at equilibrium (22), with adjustments made for pH and Mg\textsuperscript{2+} concentration (15). The chemical shift of the P\textsubscript{i} peak relative to PCr was used to calculate muscle pH (27).

Experimental protocol. Subjects exercised by performing voluntary isometric ballistic dorsiflexions (9, 11, 12) against the resistance of a plastic foot holder. Subjects maintained a contraction frequency of 1 Hz and a peak contraction force of 100–200 N. The time course of a typical ballistic contraction is shown in Fig. 1 of our previous paper (9). Included in this time course is the “fall time,” the time taken by the muscle to relax after the attainment of peak force. Average fall times in our experiments were 0.3 s (data not shown). Because force is a sensitive measure of intracellular [Ca\textsuperscript{2+}] under nonfatiguing conditions, a fall time of 0.3 s indicates a very rapid decline in [Ca\textsuperscript{2+}] at the end of exercise.

Calculating glycolytic flux. Glycolytic flux under ischemic conditions was quantified as glycolytic H\textsuperscript{+} production (since the lactic acid produced by glycolysis cannot be removed from the muscle by the circulatory system or oxidized to CO\textsubscript{2}) and as glycolytic ATP production (since anoxia prevents mitochondrial ATP synthesis; see Ref. 5).

Glycolytic H\textsuperscript{+} production was calculated from changes in muscle pH, PCr concentration ([PCr]), and P\textsubscript{i} concentration ([P\textsubscript{i}]; the “pH method”) as previously described (see Fig. 3 in Ref. 5). In brief, H\textsuperscript{+} generation by glycolysis (H\textsubscript{glycol}\textsuperscript{+}) equals the observed change in [H\textsuperscript{+}] plus the H\textsuperscript{+} consumed in the breakdown of PCr

\[
\Delta \text{H}_{\text{glycol}} = \Delta \text{pH} \times \beta_{\text{mot}} + (\gamma) \times \Delta [\text{PCr}] 
\]

where \(\Delta \text{pH}\) is the change in muscle pH, \(\beta_{\text{mot}}\) is the total muscle buffer capacity (5), \(\gamma\) is the proton stoichiometric coefficient of PCr hydrolysis (21), and \(\Delta [\text{PCr}]\) is the change in [PCr].

Glycolytic ATP production was calculated from postexercise changes in PCr (the “PCr method”). Net PCr resynthesis during postexercise ischemia reflects glycolytic ATP production in excess of ATP consumption. Postexercise ATP consumption is represented by the PCr decline after the 12-s exercise bout, since there is no apparent glycolytic PCr resynthesis during that time (see Fig. 6A). Thus glycolytic PCr resynthesis (\(\Delta \text{PCr}_{\text{glycol}}\)) after the 60-s bout was taken to be the observed change in PCr (\(\Delta \text{PCr}_{\text{tot}}\) plus the PCr consumed by basal metabolism (\(\Delta \text{PCr}_{\text{basal}}\)) as estimated from the 12-s postexercise data

\[
\Delta \text{PCr}_{\text{glycol}} = \Delta \text{PCr}_{\text{abs}} + \Delta \text{PCr}_{\text{basal}} 
\]

This flux was then compared with the flux calculated by the pH method (Eq. 1) using the stoichiometry that the breakdown of G-6-P to lactate produces 1.5 PCr molecules for every H\textsuperscript{+} released (26).

Glycolytic rates (see Fig. 6) were calculated by dividing point-by-point changes in cumulative glycolytic flux by the time interval between points (6 s, in this case).

Statistics. Reported values are means ± SE. Glycolytic fluxes and postexercise changes in PCr and pH were compared with zero using t-tests, with \(\alpha\)-levels adjusted for multiple comparisons using a sequential Bonferroni correction (25).

RESULTS

Force production dynamics. Subjects performed ballistic 1-Hz contractions throughout all exercise bouts. The time course of a typical ballistic contraction is shown in Fig. 1 of our previous paper (9). Included in this time course is the “fall time,” the time taken by the muscle to relax after the attainment of peak force. Average fall times in our experiments were 0.3 s (data not shown). Because force is a sensitive measure of intracellular [Ca\textsuperscript{2+}] under nonfatiguing conditions, a fall time of 0.3 s indicates a very rapid decline in [Ca\textsuperscript{2+}] at the end of exercise.

Metabolite dynamics. Figure 1 shows 31P spectra of the ankle dorsiflexors at rest and after 60 s of ischemic exercise. Exercise caused a decrease in [PCr] and an increase in [P\textsubscript{i}] and [HP]. Figure 2 shows the time course of muscle [PCr], [HP], and pH changes during and after exercise bouts lasting 12 and 60 s. No change in [HP] was apparent in the brief (12-s) bout, indicating that little glycogenolysis occurred during this bout. However, [HP] rose during the longer bout, presumably because of the onset of glycogenolysis. This elevation in [HP] persisted during postexercise ischemia. Similarly, [P\textsubscript{i}], ADP, and AMP levels increased during exercise and remained elevated above baseline levels during postexercise ischemia after both bouts (data not shown; see Ref. 9).

Figure 3 shows the postexercise changes in PCr relative to the end-exercise values. [PCr] declined after the 12-s exercise bout. Of the values plotted, only the point at 57 s was significantly less than zero (baseline); however, a linear regression of these data revealed a slope significantly less than zero (\(P < 0.05\)). Because there was no glycolytic flux (see Fig. 6A) and thus no glycolytic ATP production during this time, the mag-
The magnitude of the slope (0.019 mM PCr/s) reflects the rate of ATP use by the postexercise muscle. This rate is higher than that of 0.008 mM/s found in resting forearm muscle (2). Thus the ATP costs of muscle recovering from exercise may exceed those of resting muscle (10), possibly because of the increased ion-pumping activity after exercise (13).

[PCr] increased after the 60-s bout (Fig. 3); all data points from 9 s postexercise onward were significantly greater than zero (baseline). The Pi, ADP, and AMP levels were elevated at or above the levels that activated glycolysis (see Table 2 of Ref. 9). Therefore, postexercise glycolytic ATP production exceeded postexercise ATP use after the 60-s bout. Overall, Fig. 3 suggests that there was no glycolytic ATP resynthesis after the short (12-s) exercise bout but significant glycolytic ATP production after the longer (60-s) bout.

pH dynamics. An initial alkalinization of pH was apparent during both exercise bouts (Fig. 2), but a subsequent acidification occurred in the 60-s bout, indicating the onset of glycolytic H\(^+\) production in the longer bout. Figure 4 displays the postexercise changes in pH relative to the end-exercise values. No pH change was found after the 12-s bout; none of the postexercise points differed significantly from zero (i.e., baseline). However, a decline of ~0.1 pH units was seen after the 60-s bout; in this case, all postexercise points were significantly less than zero (baseline). Thus significant postexercise acidification was seen only after the 60-s exercise bout, indicating that glycolytic H\(^+\) production occurred after the longer bout but not after the shorter bout.

Calculating glycolytic flux. Figure 5 shows that our two methods of quantifying postexercise glycolytic flux (see METHODS) yielded similar estimates of flux. The pH method (Eq. 1) calculated glycolytic H\(^+\) production as the H\(^+\) accumulation apparent in the pH change plus the H\(^+\) consumed by the creatine kinase reaction. The PCr method (Eq. 2) calculated glycolytic PCr synthesis as the postexercise increase in PCr plus the PCr consumed by postexercise metabolism (i.e., basal ATPase).

The glycolytic PCr synthesis was then converted to H\(^+\) production using the stoichiometry of 1.5 PCr/H\(^+\) and yielded values that corresponded closely to those of the pH method.

Postexercise glycolytic flux. Figure 6 reports glycolytic fluxes at the end of and after exercise as calculated by the pH method. Essentially no flux occurred during or after the 12-s exercise bout (Fig. 6A). However, a substantial flux occurred during and immediately after the 60-s bout (Fig. 6B). These data indicate that glycolytic flux persists beyond the end of exercise if the flux becomes elevated during exercise. After the 60-s exercise bout, the glycolytic flux rate remained at end-exercise levels for the first ~3 s of ischemic recovery and remained significantly greater than zero after 9 s but dropped to baseline levels within ~20 s (Fig. 6B). These results show that glycolytic flux persists seconds beyond the cessation of force production but subsequently declines despite high [HP] (Fig. 2). Thus we can reject the hypothesis that a reduction in [HP] causes the termination of glycolytic flux.

DISCUSSION

The key finding of this study is that glycolytic flux ceases rapidly after exercise despite elevations in glycolytic substrate (HP) and metabolites (Pi, ADP, and AMP) thought to act as feedback activators of the glycolytic pathway. Our data thus show that high levels of HP and metabolite activators are not sufficient to sustain high glycolytic fluxes. This finding rejects the hypothesis that a decline in substrate supply by glycogenolysis is responsible for the cessation of glycolytic flux at the end of exercise. The fact that flux declines when exercise stops supports the alternative hypothesis, i.e., that a muscle activation-related factor such as Ca\(^{2+}\) controls the glycolytic pathway independently of its effect on glycogenolysis. In addition, the gradual time course of this decline suggests that, if cytosolic Ca\(^{2+}\) is indeed the key factor, it affects glycolytic flux through a slow-to-reverse process.
Our primary goal was to determine whether a depletion of substrate is responsible for the cessation of glycolytic flux after exercise. The substrate for glycolysis is G-6-P, which is supplied mainly by glycogenolysis during the first few minutes of exercise (14, 19). We cannot measure G-6-P concentration ([G-6-P]) directly; however, changes in [HP] have been found to correspond to changes in [G-6-P] (4). Our exercise protocol raised [HP] to >3 mM, well above the level (1.5–2 mM) at which glycolytic flux began (Fig. 2). Although [HP] (and thus [G-6-P]) remained high throughout postexercise ischemia (Fig. 2), glycolytic flux declined to basal levels within ~20 s of the end of exercise (Fig. 6B). Thus glycolytic flux is not simply a mass-action response to the glycogenolytic generation of substrate. Instead, the glycolytic pathway is controlled at one or more sites downstream of glycogenolysis.

A second finding of this study was the discovery that glycolytic flux persists for several seconds beyond the end of exercise. The observed increase in PCr (Fig. 3) and decrease in pH (Fig. 4) after the 60-s exercise bout offer direct evidence of this persistence. We have previously shown that PCr and pH changes provide separate measures of the glycolytic flux (5), and in this study, the flux estimate based on pH changes agreed with the flux calculated from PCr changes when postexercise ATP use was taken into account (Fig. 5).
Furthermore, we have recently validated these methods of calculation by comparing them against direct measurements of lactate flux in rattlesnake tail shaker muscle (20). The calculations reported here indicate that glycolytic flux is maintained at the high rates seen during exercise for ~3 s after exercise ends but then drops to baseline within ~20 s (Fig. 6B). As noted above, this decline occurs despite elevated substrate (HP) and metabolite (P_i, ADP, and AMP) levels.

**Nature of control.** The fact that glycolysis stops soon after exercise stops, even when metabolites and HP remain high, suggests that a muscle stimulation-related signal controls glycolytic flux. The role of Ca^{2+} in initiating muscle contraction is an example of how this type of signal can alter enzyme activity. Cytosolic Ca^{2+} activates the actomyosin ATPase by binding to tropomycin C; once [Ca^{2+}] drops, force production ceases. The time required to turn off contractile activity is < 0.3 s in ballistic contractions such as those studied here. However, in contrast to this extremely rapid turn-off time, glycolytic flux requires 15–20 s to return to basal levels (Fig. 6B). Clearly, an effect of Ca^{2+} analogous to the triggering of muscle contraction cannot be responsible for the inactivation of glycolysis.

Ca^{2+} can also affect enzymes via slower-to-reverse processes such as that by which GP is interconverted between its a and b forms. Ca^{2+} causes phosphorylase kinase to convert GP_b to GP_a, a phosphatase converts GP_a back to GP_b once [Ca^{2+}] drops to resting levels. The reversion of GP_a to GP_b occurs with a half-time of 12 s in frog muscle at 30°C (18) and 2.5 s in rat muscle at 37°C (3), similar to the time course of the decline in glycolytic flux reported here (Fig. 6B). This reversion is thus a useful example of how a fall in cytosolic Ca^{2+} may gradually inactivate a flux-generating enzyme over a period of several seconds. This particular enzyme conversion cannot account for our results because, as discussed above, glycolysis appears to be controlled independently of glycogenolysis. However, it is possible that Ca^{2+} directly influences glycolytic flux by phosphorylating glycolytic enzymes (23) and/or promoting binding of these enzymes to the cytoskeleton (1).

In conclusion, this study has investigated the possible role of glycogenolytic substrate supply in the control of glycolytic flux. We have found that HP levels remain high when exercise ceases, yet glycolytic flux subsides within ~20 s. We conclude that muscle activation controls glycolysis at a site downstream of glycogenolysis. The gradual time course of the postexercise decline in flux suggests that a Ca^{2+}-mediated enzyme cascade may underlie this activation-related control.
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Portions of this work have been previously presented in abstract form (8).

Current address for M. F. Carey: Exercise Metabolism Unit, Victoria University, Footscray 3011, Australia.

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