Glycogen phosphorylase and pyruvate dehydrogenase transformation in white muscle of trout during high-intensity exercise

Jeff G. Richards, George J. F. Heigenhauser and Chris M. Wood

Am J Physiol Regul Integr Comp Physiol 282:828-836, 2002. First published Nov 15, 2001;
doi:10.1152/ajpregu.00455.2001

Am J Physiol Regulatory Integrative Comp Physiol 282: R828–R836, 2002.
First published November 15, 2001; 10.1152/ajpregu.00455.2001.

Glycogen phosphorylase and pyruvate dehydrogenase transformation in white muscle of trout during high-intensity exercise

JEFF G. RICHARDS,[1] GEORGE J. F. HEIGENHAUSER,[2] AND CHRIS M. WOOD[1]
[1]*Departments of Biology and* [2]*Medicine, McMaster University, Hamilton, Ontario, Canada L8S 4K1*

Received 1 August 2001; accepted in final form 7 November 2001

Richards, Jeff G., George J. F. Heigenhauser, and Chris M. Wood. Glycogen phosphorylase and pyruvate dehydrogenase transformation in white muscle of trout during high-intensity exercise. *Am J Physiol Regulatory Integrative Comp Physiol* 282: R828–R836, 2002. First published November 15, 2001; 10.1152/ajpregu.00455.2001.—We examined the regulation of glycogen phosphorylase (Phos) and pyruvate dehydrogenase (PDH) in white muscle of rainbow trout during a continuous bout of high-intensity exercise that led to exhaustion in 52 s. The first 10 s of exercise were supported by creatine phosphate hydrolysis and glycolytic flux from an elevated glycogenolytic flux and yielded a total ATP turnover of 3.7 μmol·g wet tissue^{-1}·s^{-1}. The high glycolytic flux was achieved by a large transformation of Phos into its active form. Exercise performed from 10 s to exhaustion was at a lower ATP turnover rate (0.5 to 1.2 μmol·g wet tissue^{-1}·s^{-1}) and therefore at a lower power output. The lower ATP turnover was supported primarily by glycolysis and was reduced because of posttransformational inhibition of Phos by glucose 6-phosphate accumulation. During exercise, there was a gradual activation of PDH, which was fully transformed into its active form by 30 s of exercise. Oxidative phosphorylation, from PDH activation, only contributed 2% to the total ATP turnover, and there was no significant activation of lipid oxidation. The time course of PDH activation was closely associated with an increase in estimated mitochondrial redox (NAD^+-to-NADH concentration ratio), suggesting that O_2 was not limiting during high-intensity exercise. Thus anaerobiosis may not be responsible for lactate production in trout white muscle during high-intensity exercise.

lactate; adenosine 5′-triphosphate turnover; mitochondrial redox; cytoplasmic redox; oxygen limitation; rainbow trout

THE CLASSICAL SCHEME OF SUBSTRATE use during high-intensity exercise involves the temporally separate utilization of creatine phosphate (CrP) followed by activation of glycolysis and then oxidative phosphorylation (16). In this scheme, the initial depletion of CrP at the onset of exercise results in the accumulation of P_i, free ADP (ADP_f), free AMP (AMP_f), inosine monophosphate (IMP), and NH_3, which are thought to activate glycolysis for further ATP production. Subsequently, the accumulation of pyruvate and lactate from glycolysis stimulates pyruvate dehydrogenase (PDH) for ATP production via oxidative phosphorylation. Trout white muscle is known (14) to rely primarily on CrP and glycogen to support high-intensity exercise. However, the majority of research in trout muscle metabolism has focused on elucidating the pattern of metabolite recovery after high-intensity exercise (2, 14, 17, 18, 28, 39) and the factors that regulate recovery metabolism (30). As pointed out in numerous studies (e.g., 21, 35, 39), little information is available on the dynamics of substrate selection and the integrated mechanisms that regulate fuel use in trout white muscle during high-intensity exercise.

Dobson et al. (5) examined the dynamics of "anaerobic" ATP production in trout swum at exercise intensities ranging from a 10-s sprint (~150% critical swimming speed; U_{crit}) to 30 min of burst swimming leading to exhaustion. Ten-second sprints were solely supported by CrP hydrolysis. During longer (>10 min) and slower swimming (~120% U_{crit}), glycogen was the primary fuel, utilized through the activation of glycolysis. Control points in glycolysis were identified primarily at phosphofructokinase-1 (PFK-1). Evidence from mammalian studies (3, 22) further implicates the rate-limiting enzyme glycogen phosphorylase (Phos) in regulating glycolytic flux by setting the upper limit for glycogen entry into glycolysis. Phos is regulated at the transformational level by reversible phosphorylation events and at the posttransformational level through substrate availability (P_i), product inhibition (glucose 6-phosphate; G-6-*P*), and changes in allosteric modulators (ADP_f and AMP_f; 10, 27). Phosphorylation of Phos by Phos kinase transforms the low-activity form of Phos ($Phos_b$) into the high-activity form ($Phos_a$), whereas the dephosphorylation by Phos phosphatase converts $Phos_a$ to $Phos_b$ (15). The transformation between $Phos_b$ and $Phos_a$ is regulated at the contractile level through Ca^{2+} release from the sarcoplasmic reticulum. Decreases in intracellular pH (pH_i) affect the transformation of Phos by inhibiting Phos kinase (4) and affect substrate availability by shifting the speciation of P_i (12). To our knowledge, the transformation of

Address for reprint requests and other correspondence: J. G. Richards, Dept. of Biology, McMaster Univ., 1280 Main St. West, Hamilton, Ontario, Canada L8S 4K1 (E-mail: richarjg@mcmaster.ca).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Phos has not been examined in trout white muscle during high-intensity exercise.

PDH is the rate-limiting enzyme that sets the rate at which glycolytically derived pyruvate is decarboxylated for entry into the tricarboxylic acid (TCA) cycle and oxidative phosphorylation. The catalytic rate of PDH is regulated by covalent modification (phosphorylation and dephosphorylation) and product inhibition (37). Dephosphorylation of PDH by PDH phosphatase transforms the inactive PDH (PDH_b) into the active PDH (PDH_a) whereas phosphorylation of PDH by PDH kinase transforms PDH_a into PDH_b. PDH kinase is allosterically stimulated by acetyl-CoA, NADH, and ATP and is inhibited by ADP, free CoA (CoA-SH), NAD^+, and pyruvate. In mammalian muscle, PDH phosphatase is stimulated by Ca^{2+} release from the sarcoplasmic reticulum and hormonally by insulin (26). Elevations in mitochondrial NADH inhibit PDH phosphatase and reduce its transformation. In trout white muscle, Richards et al. (28) demonstrated that PDH was maximally activated at exhaustion. Richards et al. suggested that this pathway could have contributed up to 14% of the total ATP production during a bout of high-intensity exercise. Furthermore, the relative transformation patterns and catalytic rates of Phos and PDH have recently been implicated (22, 31) in lactate production in human muscle.

The goal of the present study was to determine the role of the rate-limiting enzymes, Phos and PDH, in regulating white muscle metabolism during a continuous bout of high-intensity exercise. To accomplish these objectives, we measured the transformation states of Phos and PDH, as well as their allosteric regulators (e.g., ADP_f, AMP_f, P_i, and pH_i), in white muscle of trout during a typical high-intensity exercise regime. Insights into cellular O_2 limitations during high-intensity exercise were made through estimates of cytoplasmic and mitochondrial redox states (NAD^+-to-NADH concentration ratio; [NAD^+]/[NADH]).

METHODS

Animal care. Adult rainbow trout (*Oncorhynchus mykiss*, Walbaum; 211 ± 8 g, n = 40) were obtained from Humber Springs Trout Hatchery (Orangeville, ON, Canada). Trout were transported to our freshwater holding facility and held in 800-liter tanks supplied with aerated, dechlorinated tap water (from the city of Hamilton) at 15°C for 2 mo before experimentation. Fish were fed daily to satiation with commercial trout pellets. One day before an experiment, fish were placed individually into dark, aerated, 2.5-l acrylic boxes supplied with 100 ml/min freshwater at 15°C. All experimental procedures fully complied with the "Guiding Principles for Research Involving Animals and Human Beings" of the American Physiology Society and the guidelines of the Canadian Council of Animal Care.

Experimental protocol. To exercise fish, we transferred individual fish without air exposure from the acrylic box to a 150-liter circular tank filled with aerated freshwater at 15°C. Fish were chased with almost constant body contact to avoid burst-glide swimming. White muscle samples were terminally sampled at rest (see below), at 10.5 ± 0.3, 20.4 ± 0.3, and 30.0 ± 0.2 s (n = 8) during high-intensity exercise, and at exhaustion (51.8 ± 3.2 s; n = 8). Fish were considered exhausted when they did not attempt to escape from manual chase. To sample muscle as quickly as possible, fish were removed from the tank by hand (with no struggle by the fish) at a prespecified time and killed by concussion. A 0.5- to 1-cm-thick cross section of the fish was taken posterior to the dorsal fin and immediately freeze clamped between two aluminum blocks cooled in liquid N_2. This sampling position is consistent with that used previously in other studies (e.g., 5, 18, 30, and 35). The entire sampling procedure took <5 s.

To obtain resting white muscle samples, we terminally anesthetized trout in their boxes by adding 0.5 g/l 2-aminobenzoic acid ethyl ester (methanesulfonate salt; MS-222) to the surrounding water from a neutralized stock solution. At complete anesthesia (~1 min), the fish was removed from the water and two muscle samples were taken. The first muscle sample was immediately freeze clamped as described above. The second muscle sample was freeze clamped after a 1-min delay to obtain resting Phos activities (27). All muscle tissues were stored under liquid N_2 until analyzed.

Analytic techniques. The frozen muscle was broken into pieces (50–100 mg) in an insulated mortar and pestle cooled in liquid N_2. As in other studies (e.g., 5, 18, 30, and 35), only white muscle dorsal of the lateral line was used. Several aliquots of muscle were stored separately in liquid N_2 for determination of PDH_a and total PDH activity as previously described by Richards et al. (28). A portion of the frozen muscle (~50 mg) was ground into a fine powder under liquid N_2 and used for pH_i measurements as described by Pörtner et al. (24). Total muscle NH_3 was determined on frozen muscle by the glutamate dehydrogenase method as described by Wang et al. (36). The remaining muscle was lyophilized for 72 h, dissected free of connective tissue, powdered, and stored dry at −80°C for subsequent analysis.

An aliquot of lyophilized muscle was used for determination of Phos activity. Briefly, a known weight (5–10 mg) of dry muscle was homogenized at −25°C in 200 μl of buffer containing 100 mM Tris, 50 mM KCl, and 10 mM EDTA in 60% glycerol at pH 7.5. Homogenates were then diluted with 800 μl of the above buffer without glycerol and homogenized further at 0°C. Total Phos activity (in the presence of 3 mM AMP) and $Phos_a$ (in the absence of AMP) were measured by following the production of glucose 1-phosphate (G-1-*P*) spectrophotometrically at 15°C. Maximal velocity (V_{max}) and the mole fraction of $Phos_a$ were calculated as described by Chasiotis et al. (3).

For the determination of muscle glycogen, ~20 mg of lyophilized muscle were digested in 1 ml 30% KOH at 100°C. Glycogen was isolated as described by Hassid and Abraham (9), and free glucose was determined after digestion with amyloglucosidase (1).

For the extraction of metabolites from white muscle, aliquots of lyophilized muscle (~20 mg) were weighed into borosilicated tubes, with 1 ml of ice-cold 1 M $HClO_4$ added, and homogenized at the highest speed of a Virtis hand-held homogenizer for 20 s at 0°C. Homogenates were transferred to 1.5-ml bullet tubes and centrifuged for 5 min at 20,000 *g* at 4°C, and the supernatant was neutralized with 3 M K_2CO_3. These extracts were assayed spectrophotometrically for ATP, CrP, creatine, lactate, pyruvate, glucose, G-6-*P*, fructose 6-phosphate (F-6-*P*), G-1-*P*, glycerol 3-phosphate (Gly-3-*P*), glycerol, L-glutamate, and α-ketoglutarate, using the methods previously described by Bergmeyer (1). Muscle acetyl-CoA, CoA-SH, and acetyl-, free-, short-chain fatty acyl- (SCFA), long-chain fatty acyl- (LCFA), and total carnitine were determined on neutralized extracts by radiometric methods previously described in Richards et al. (28).

Calculations. [ADP_f] and [AMP_f] were calculated according to Dudley et al. (7) using constants calculated from Schulte et al. (30). [P_i] was calculated as the difference between [resting PCr] and [exercise PCr], less the accumulation of G-6-*P*, F-6-*P*, G-1-*P*, and Gly-3-*P*. Values for [resting P_i] were estimated from Wang et al. (35) by subtracting [resting PCr] and [ATP] from measured total phosphate and were ~0.75 μmol/g wet tissue, a value that agrees well with the low resting P_i values reported by van den Thillart et al. (33).

The rates of glycogenolysis and glycolysis (in μmol glycosyl units·g wet weight^{-1}·s^{-1}) were estimated as described by Spriet et al. (32) from the accumulation of glycolytic intermediates plus the flux of pyruvate through PDH_a during each time interval. The equations were as follows

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

$$\text{glycolysis} = [\Delta([\text{Gly-3-}P] + [\text{lactate}] + [\text{pyruvate}])/2 \div \text{time}] + \text{PDH}_a/2$$

where Δ represents a change in tissue metabolite concentration over a specified time period and PDH represents the catalytic rate of PDH_a (in μmol glycosyl units·g wet weight^{-1}·s^{-1}). The catalytic rate of PDH_a has been demonstrated to equal flux of pyruvate into the TCA cycle in mammalian muscle (8, 11).

ATP turnover from CrP was calculated from the breakdown of CrP assuming that 1 mol of ATP is produced per mole of CrP consumed. ATP turnover from glycolysis was calculated from the accumulation of lactate and the flux of pyruvate through PDH_a assuming 1.5 mol of ATP produced per lactate. The rate of ATP turnover from oxidative phosphorylation was calculated as the total acetyl-CoA production from the PDH_a catalytic rate for each time period, assuming 1 mol of acetyl-CoA from glycogenolysis would yield 15 mol of ATP.

The redox state (i.e., [NAD^+]/[NADH]) of the cytoplasm was estimated from the apparent equilibrium of the lactate dehydrogenase reactions using measurements of pH_i, lactate, and pyruvate and the equilibrium constant (K_{eq}) from Wang et al. (35). Mitochondrial redox was estimated from the glutamate dehydrogenase reaction using whole cell measurements of NH_3, glutamate, α-ketoglutarate, K_{eq} from Williamson et al. (38), and estimates of mitochondrial pH from Moyes et al. (19) for carp muscle.

Data presentation and statistical analysis. All data are presented as means ± SE (n). All metabolite concentrations determined on lyophilized tissues were converted back to wet weights by taking into account a wet-to-dry ratio of 4:1 (35). Statistical analysis consisted of a one-way ANOVA followed by a least significant difference method of pairwise multiple comparisons. Results were considered significant at $P < 0.05$.

RESULTS

In response to manual chasing, trout swam vigorously for the first 20 s of exercise, then slowed but maintained burst activity until exhaustion at 51.8 ± 3.2 s (n = 8).

Adenylates, CrP, and cellular energy status. White muscle [ATP] decreased by 28% during the first 10 s of exercise, then remained stable until 20 s and further decreased to 23% of resting [ATP] at exhaustion (Fig. 1). During the initial 10 s of exercise, there was a 63% decrease in [CrP] that remained lower than resting values and more or less stable for the duration of the exercise (Fig. 1). These decreases in [CrP] were matched by stoichiometric increases in [Cr], and these relative differences account for the majority of the calculated increase in [P_i] (Table 1). The calculated [ADP_f] and [AMP_f] both increased rapidly within the first 10 s of exercise and then gradually decreased until exhaustion at which point [ADP_f] and [AMP_f] were not significantly higher than resting values (Table 1). As a result, the ATP-to-ADP_f ratio (ATP/ADP_f) decreased during the first 10 s of exercise and remained depressed until exhaustion. pH_i decreased during the initial 10 s of exercise, stabilized, and then further decreased at exhaustion (Table 1). White muscle total NH_3 increased gradually over the entire time of exercise (Table 1).

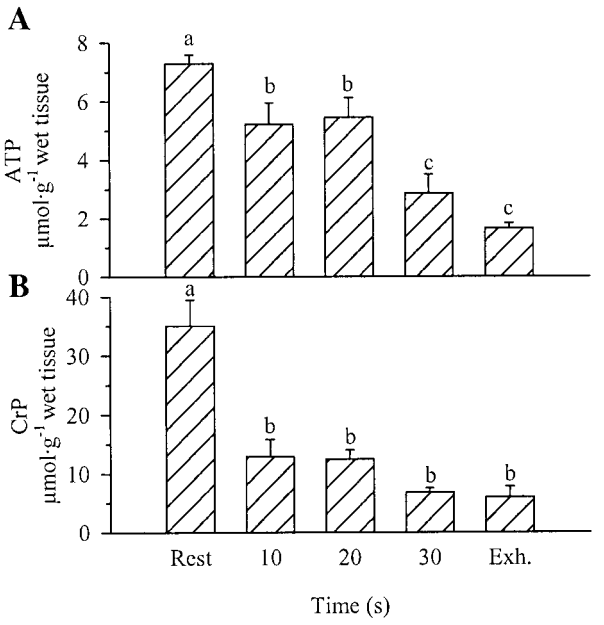

Fig. 1. White muscle ATP (*A*) and creatine phosphate (CrP; *B*) concentrations at rest and during high-intensity exercise. Exhaustion (Exh) occurred at 51.8 s of manual chasing. Values are means ± SE; n = 8. Values with different letters are significantly different ($P < 0.05$).

Phos. The maximum total Phos activity (V_{max}) was 0.85 ± 0.09 μmol·g wet tissue^{-1}·s^{-1} (n = 8) at rest and did not change during exercise. In contrast, $Phos_a$ activity increased from 0.16 ± 0.02 μmol·g wet tissue^{-1}·s^{-1} (n = 8) at rest to 0.42 ± 0.02 μmol·g wet tissue^{-1}·s^{-1} (n = 8; $P < 0.05$) at 10 s and then decreased significantly to 0.34 ± 0.04 μmol·g wet tissue^{-1}·s^{-1} at exhaustion (n = 8; $P < 0.05$). As a result, the calculated transformation of $Phos_b$ into $Phos_a$ increased rapidly during the first 10 s of exercise, remained elevated until 30 s of exercise, and then decreased at exhaustion to levels that remained elevated compared with resting values (Fig. 2).

PDH. The total PDH activity in white muscle of trout was 2.9 ± 0.3 nmol·g wet tissue^{-1}·s^{-1} (n = 8). In contrast, PDH_a activity increased gradually from resting values of 0.3 ± 0.1 (n = 8) to 3.0 ± 0.3 nmol·g wet tissue^{-1}·s^{-1} (n = 8) at 30 s and remained elevated

Table 1. *White muscle creatine, P_i, adenylate, pH_i, and total NH_3 content in trout at rest and during high-intensity exercise*

Measure	Time				
	Rest	10 s	20 s	30 s	Exh
Cr	23.2 ± 2.2*	45.4 ± 5.2†	45.8 ± 1.5†	53.3 ± 1.8†	52.2 ± 1.8†
P_i	1.0	22.3	21.2	27.5	28.1
ADP_f	15.6 ± 1.9*	56.0 ± 9.6†	40.9 ± 4.3†‡	36.0 ± 6.3‡§	22.4 ± 4.8*§
AMP_f	0.9 ± 0.2*	19.0 ± 5.5†	7.1 ± 2.0*§	13.1 ± 3.7†§	7.6 ± 3.6*§
ATP/ADP_f	516 ± 118*	190 ± 49†	173 ± 25†	87 ± 13†	102 ± 29†
pH_i	7.30 ± 0.03*	7.01 ± 0.04†	6.97 ± 0.04†	6.95 ± 0.06†§	6.85 ± 0.04§
NH_3	0.91 ± 0.28*	2.12 ± 0.40*	3.63 ± 0.72†	4.47 ± 0.65†	6.21 ± 0.51§

Values are means ± SE (n = 8). Cr, free creatine; ADP_f, free ADP; AMP_f, free AMP; pH_i, intracellular pH; NH_3, total ammonia; Exh, exhaustion. Cr, P_i, and total NH_3 are given in μmol/g wet tissue, and ADP_f and AMP_f are given in nmol/g wet tissue. Resting P_i was taken from Wang et al. (35), and changes in P_i due to exercise were estimated from changes in creatine phosphate (see Fig. 1), glucose 6-phosphate (G-6-*P*), fructose 6-phosphate (F-6-*P*), glucose 1-phosphate (G-1-*P*), and glycerol 3-phosphate (Gly-3-*P*) (Table 2; see METHODS for more details). Values with different superscripts are significantly different ($P < 0.05$).

until exhaustion. Consequently, the transformation of PDH_b into PDH_a increased gradually during the first 20 s of exercise, reaching 100% transformation at 30 s and exhaustion (Fig. 2).

Muscle metabolites. White muscle [glycogen] decreased by 35% during the first 10 s of exercise, remained more or less stable until 30 s, and then decreased at exhaustion to levels that were 16% of resting values (Fig. 3). These changes in [glycogen] were matched by reciprocal increases in [lactate] and [pyruvate] (Fig. 3). During the entire bout of exercise, there was a gradual accumulation of the glycolytic intermediates, G-6-*P*, F-6-*P*, and G-1-*P*, all of which peaked in concentration at 20 s (Table 2). Muscle [Gly-3-*P*] remained stable for the first 30 s of exercise and then increased at exhaustion (Table 2). Muscle [glycerol] increased over the first 20 s of exercise and then remained unchanged until exhaustion (Table 2). There were no changes in L-glutamate or α-ketoglutarate during exercise (Table 2). The calculated rates of glycogenolysis and glycolysis were both high during the first 10 s of exercise then decreased by ~60% during subsequent exercise (Fig. 4). There was higher glycogenolytic flux compared with glycolytic flux be-

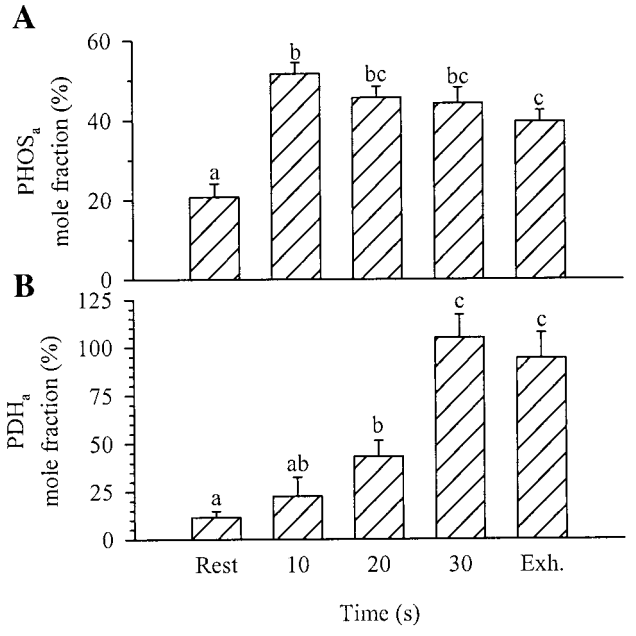

Fig. 2. White muscle glycogen phosphorylase (Phos; *A*) and pyruvate dehydrogenase (PDH; *B*) mole fraction at rest and during high-intensity exercise. $Phos_a$, high-activity form of Phos; PDH_a, high-activity form of PDH. Values are means ± SE; n = 8. Values with different letters are significantly different ($P < 0.05$).

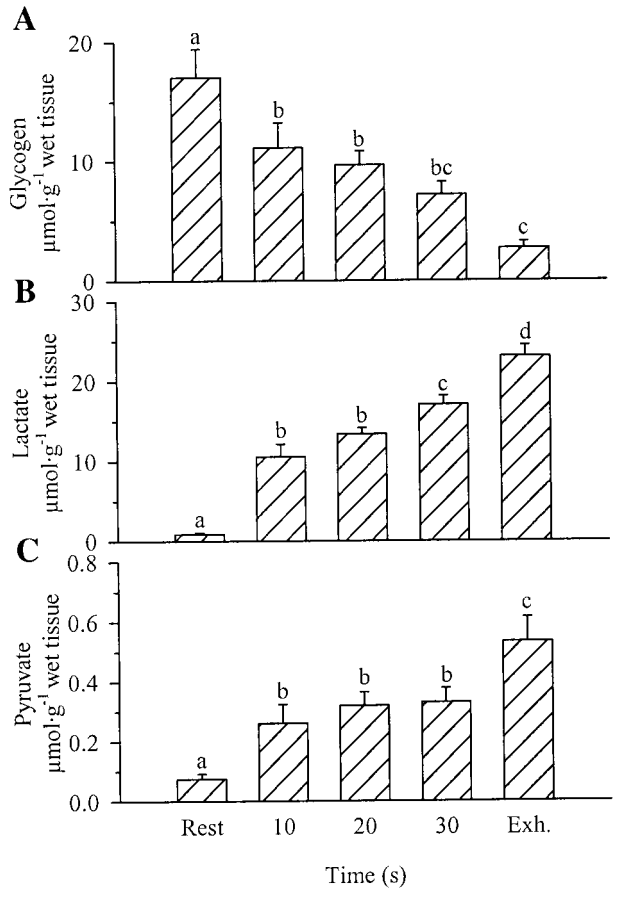

Fig. 3. White muscle glycogen (*A*), lactate (*B*), and pyruvate (*C*) concentrations at rest and during high-intensity exercise. Glycogen is expressed in glycosyl units. Values are means ± SE; n = 8. Values with different letters are significantly different ($P < 0.05$).

Table 2. *White muscle glycolytic intermediates, glycerol, glutamate, and α-ketoglutarate content at rest and during high-intensity exercise*

Measure	Time				
	Rest	10 s	20 s	30 s	Exh
G-6-*P*	0.01 ± 0.01*	0.45 ± 0.12*†	1.33 ± 0.39‡	0.91 ± 0.29†‡	0.94 ± 0.23†‡
F-6-*P*	0.01 ± 0.01*	0.16 ± 0.09*†	0.40 ± 0.15†‡	0.13 ± 0.05*†	0.36 ± 0.13†‡
G-1-*P*	0.23 ± 0.05*	0.40 ± 0.13*†	0.62 ± 0.11†‡	0.37 ± 0.08*†	0.41 ± 0.08*†
Glucose	0.19 ± 0.05*	0.36 ± 0.04‡	0.39 ± 0.04‡	0.53 ± 0.09‡	0.71 ± 0.05†
Gly-3-*P*	0.63 ± 0.09*	0.76 ± 0.14*	0.95 ± 0.08*‡	0.85 ± 0.14*‡	1.18 ± 0.16‡
Glycerol	0.28 ± 0.05*	0.54 ± 0.02‡	0.63 ± 0.02†	0.66 ± 0.03†	0.62 ± 0.03†‡
Glutamate	2.66 ± 0.40*	2.51 ± 0.35*	2.12 ± 0.13*	2.85 ± 0.23*	2.68 ± 0.39*
α-Ketoglu	0.05 ± 0.01*	0.06 ± 0.01*	0.08 ± 0.02*	0.14 ± 0.07*	0.07 ± 0.01*

Values are means ± SE (n = 8) in μmol/g wet tissue. α-Ketoglu, α-ketoglutarate. Values with different superscripts are significantly different ($P < 0.05$).

tween 10 and 20 s, a trend that reversed between 20 and 30 s. Between 30 s and exhaustion, calculated glycogenolytic and glycolytic fluxes were equal (Fig. 4).

White muscle [acetyl-CoA] did not change during high-intensity exercise, except at 30 s when it decreased slightly (Table 3). However, muscle [CoA-SH] decreased by ~60% during the first 10 s of exercise and remained lower than resting values until exhaustion (Table 3). There was no effect of exercise on muscle [acetyl-carnitine] (Table 3). Muscle [total carnitine] and [free carnitine] were variable, with a trend for a gradual decrease over the exercise period (Table 3). [SCFA-carnitine] and [LCFA-carnitine] did not change during exercise (Table 3).

Cytoplasmic redox did not change during exercise or at exhaustion (Fig. 5), but mitochondrial redox increased during high-intensity exercise (Fig. 5).

DISCUSSION

The present study examined the regulation of substrate use in white muscle of rainbow trout during a continuous bout of high-intensity exercise leading to exhaustion. During the first 10 s of burst activity, high ATP turnover rates (Fig. 6) were supported primarily by CrP hydrolysis and glycolysis (Fig. 1 and 4), yielding dramatic decreases in muscle CrP (Fig. 1) and glycogen with corresponding increases in lactate and pyruvate (Fig. 3). The entry of glycogen into glycolysis was the consequence of a very large and rapid transformation of $Phos_b$ into $Phos_a$ (Fig. 2). Oxidative phosphorylation through PDH made only minor contributions to total ATP turnover during the first 10 s of exercise. Total ATP turnover rate during the first 10 s of exercise was 3.7 μmol·g wet tissue^{-1}·s^{-1}, which is in close agreement with the maximum ATP turnover rates estimated by Dobson et al. (5) for trout white muscle (3.1 μmol·g wet tissue^{-1}·s^{-1}).

During longer periods of exercise (>10 s), Phos remained significantly transformed compared with resting values (Fig. 2), but glycogenolytic and glycolytic fluxes were reduced (Fig. 4), therefore leading to a lower ATP turnover from glycolysis (Fig. 6) and likely a correspondingly reduced power output. However, glycolysis still supported the majority of the total ATP turnover throughout the exercise period (Fig. 6). CrP hydrolysis made only minor contributions to total ATP

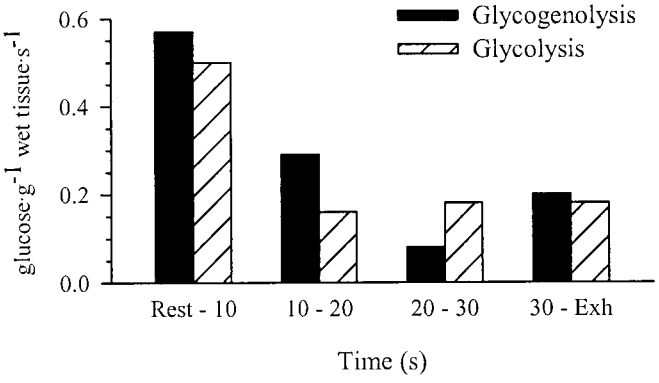

Fig. 4. Estimated white muscle glycogenolytic and glycolytic flux rates at rest and during high-intensity exercise.

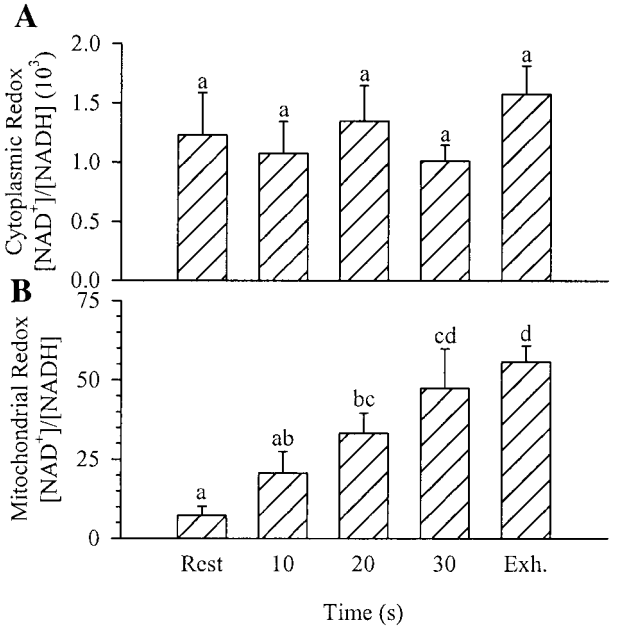

Fig. 5. Estimated white muscle cytoplasmic redox (*A*) and mitochondrial redox states (*B*) at rest and during high-intensity exercise. Values are means ± SE; n = 8. Values with different letters are significantly different ($P < 0.05$).

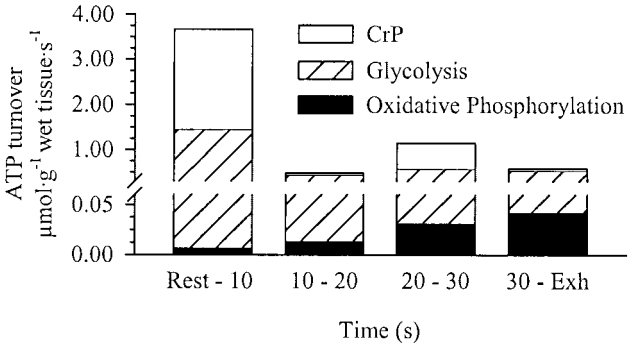

Fig. 6. White muscle ATP turnover rates during high-intensity exercise.

turnover after the initial 10 s of exercise. As exercise duration increased, there was a gradual transformation of PDH_b into PDH_a that was complete by 30 s of exercise; however, oxidative phosphorylation from carbohydrate only contributed ~ 2% to the total ATP turnover during the bout of exercise (Fig. 6).

Adenylates and CrP. Considerable debate surrounds the sequence of substrate utilization by muscle during maximal contraction. It is generally believed that CrP hydrolysis is tightly linked to myofibrilliar ATPase activity through high-affinity, high-activity creatine kinase (16). Once the ATP-buffering capacity of CrP is exceeded, endogenous ATP is utilized, yielding increases in ADP_f, AMP_f, IMP, NH_3, and P_i, all of which act to stimulate glycogenolysis and ATP production via glycolysis. Indeed, during the first 10 s of exercise CrP was depleted by 63% whereas ATP only decreased by 28% (Fig. 1), indicating that a greater proportion of CrP was hydrolyzed before endogenous ATP. However, during the first 20 s of exercise, muscle [ATP] remained elevated compared with [ATP] at exhaustion, suggesting that complete depletion of ATP was not required to activate glycolysis. Decreasing [ATP] was associated with stoichiometric increases in NH_3 (Table 1), indicating the deamination of adenylates during high-intensity exercise and the formation of IMP (30).

Regulation of Phos. It has long been recognized (2, 14, 17, 28, 35) that glycogen is an important substrate for ATP production in fish white muscle during bouts of burst activity. During the initial 10 s of exercise, glycogenolytic flux and glycolytic flux supported ~40% of the ATP turnover (Fig. 6). Subsequently, glycogenolytic and glycolytic flux both decreased by about two-thirds but continued to contribute the largest portion to ATP turnover from 10 s of exercise to exhaustion.

The high glycogenolytic flux observed during the first 10 s of exercise (Fig. 4) was achieved primarily by a large transformation of $Phos_b$ into $Phos_a$ (Fig. 2), probably caused by Ca^{2+} release from the sarcoplasmic reticulum activating Phos kinase (15). The calculated V_{max} of $Phos_a$ during the initial 10 s of exercise was 0.42 μmol·g wet tissue^{-1}·s^{-1}; therefore the increase in activity due to Phos transformation was nearly sufficient to completely explain the observed glycogenolytic flux (Fig. 4). In addition to increased Phos activity due to the transformational modification, posttransformational modification of $Phos_a$ also contributed to increased glycogenolysis. Accumulation of P_i from CrP hydrolysis (Table 1; Fig. 1) increases substrate availability during the first 10 s of exercise, and the accumulation of AMP_f (Table 1) is thought to decrease the K_m of $Phos_a$ for P_i and thus increase the catalytic rate (27). In addition, the accumulation of the allosteric activators (ADP_f; Table 1), decreasing cellular energy status (ATP/ADP_f; Table 1), and lack of product accumulation (G-6-*P*; Table 2) would further allosterically increase $Phos_a$ activity to allow for the observed glycogenolytic flux.

Glycogenolytic flux decreased linearly during the first 30 s of high-intensity exercise (Fig. 4). During this period of decreased glycogenolysis, Phos remained significantly transformed into $Phos_a$ (Fig. 2), and the calculated V_{max} of $Phos_a$ was two- to threefold larger than the calculated glycogenolytic flux. As a result, after the initial 10 s of exercise, Phos activity was primarily mediated via posttransformational modification. The decreased flux through $Phos_a$ was likely mediated by an increase in product, G-6-*P* (Table 2), acting to inhibit $Phos_a$ in addition to the decrease in AMP_f observed at 20 s compared with 10 s (Table 1), which would reduce the activation of $Phos_a$. G-6-*P* remained elevated and AMP_f remained at lower than peak levels until exhaustion. Decreasing pH_i inhibits Phos kinase and would reduce the transformation of Phos (4). In addition, decreasing pH_i would shift the

Table 3. *White muscle acetyl-CoA, CoA-SH, and carnitine derivative content at rest and during high-intensity exercise*

	Time				
Measure	Rest	10 s	20 s	30 s	Exh.
Acetyl-CoA	1.13 ± 0.08*	1.15 ± 0.08*	1.11 ± 0.06*	0.92 ± 0.02†	1.11 ± 0.06*
CoA-SH	3.93 ± 0.66*	1.59 ± 0.26†	2.26 ± 0.31†	1.71 ± 0.39†	1.34 ± 0.19†
Acetyl-Carn	19.0 ± 2.1*	36.8 ± 10.2*	20.4 ± 2.7*	25.1 ± 6.0*	33.3 ± 9.4*
Total Carn	283.7 ± 28.4*	240.6 ± 15.4*†	233.8 ± 13.1*†	282.4 ± 16.2*	214.7 ± 19.0†
Free Carn	190.1 ± 19.4*	140.3 ± 13.3†‡	153.5 ± 10.0*†‡	150.1 ± 17.2*‡	119.3 ± 17.1†
SCFA-Carn	93.7 ± 11.4*	100.3 ± 10.8*	80.3 ± 8.0*	110.1 ± 10.8*	92.4 ± 13.9*
LCFA-Carn	31.8 ± 2.0*	31.7 ± 2.1*	33.9 ± 2.2*	31.6 ± 2.1*	48.5 ± 14*

Values are means ± SE (n = 8) in nmol/g wet tissue. CoA-SH, free coenzyme A; acetyl-Carn, acetyl-carnitine; total Carn, total carnitine; free Carn, free carnitine; SCFA-Carn, short-chain fatty acyl carnitine; LCFA-Carn, long-chain fatty acyl carnitine. Values with different superscripts are significantly different ($P < 0.05$).

chemical form of P_i from the monoprotonated form, which is the active substrate for $Phos_a$, to the diprotonated form, which does not act as a substrate for $Phos_a$ (12). Changes in muscle NH_3 suggest that at >10 s of exercise IMP was accumulating from the deamination of AMP, which is also thought to allosterically stimulate $Phos_a$ (3). However, it appears that G-6-*P* and AMP_f are the two important allosteric regulators of Phos activity in trout white muscle.

In general, the calculated fluxes through Phos and glycolysis were reasonably well matched throughout the exercise period (Fig. 4), although during the initial 20 s of exercise, there was greater glycogenolytic flux than glycolytic flux and this pattern was reversed between 20 and 30 s. This match between Phos and glycolysis suggests that Phos transformation and catalytic rate directly set the upper limit for glycolytic flux and therefore pyruvate and lactate production. The slightly lower glycolytic rate observed during the first 20 s of exercise suggests that PFK-1 modifies the glycolytic rate set by Phos, a conclusion reinforced by the accumulation of F-6-*P* and G-6-*P* during this period (Table 2). Dobson et al. (5) identified PFK-1 as a point of control in trout white muscle during exercise but did not critically examine the role of Phos. Considering the very close match between glycogenolytic flux and glycolytic flux observed in Fig. 4, it appears that the transformation state of Phos sets the upper limit for glycogenolytic and glycolytic flux in trout muscle, and flux is further modified by posttransformational modification of Phos and allosteric regulation of PFK-1 activity (6, 23).

Regulation of PDH. During exercise, the transformation of PDH_b into PDH_a was slow to occur, taking 30 s of intense activity before PDH_b was fully transformed into PDH_a (Fig. 2). The initial cue for the transformation of PDH is generally thought to be Ca^{2+} release from the sarcoplasmic reticulum (26). However, Ca^{2+} does not appear to be a potent stimulator of PDH phosphatase in trout white muscle because of the considerable delay in PDH transformation (Fig. 2). Within 10 s of exercise, the energy status (ATP/ADP_f) of the cells was substantially reduced with a large and significant increase in ADP_f and pyruvate (Table 1; Fig. 3), which should inhibit PDH kinase (37) and further allow Ca^{2+}-mediated activation of PDH phosphatase and thus PDH transformation. However, in trout white muscle there must be factors that override the stimulatory effects of Ca^{2+} on PDH and slow its transformation. Thus the reason for the delayed transformation of PDH appears, at first glance, to be paradoxical.

Two possible explanations exist to explain the delayed activation of PDH (Fig. 2) in trout muscle. First, PDH is located within the mitochondrial matrix and is therefore subject to regulation by changes in mitochondrial metabolites and energy status. During the transition from rest to 10 s of exercise there is a significant decrease in CoA-SH in the absence of any change in acetyl-CoA; thus the acetyl-CoA-to-CoA-SH ratio (acetyl-CoA/CoA-SH) increases substantially during the first 10 s of exercise. This increase in acetyl-CoA/CoA-SH could stimulate PDH kinase (10) and slow the transformation of PDH at the onset of exercise. The reason for decreasing CoA-SH is also paradoxical, but likely CoA-SH is bound by TCA cycle intermediates or fatty acids (34). In human muscle, acetyl-CoA/CoA-SH is thought to contribute to PDH regulation only at rest, and its role in regulating PDH during exercise is thought to be minimal (25). However, in fish muscle the importance of acetyl-CoA/CoA-SH maybe greater.

The second plausible reason for the delayed PDH activation in trout muscle is related to the estimated redox state of the mitochondrial matrix. The pattern of PDH transformation (Fig. 2) closely approximates the estimated changes in muscle mitochondrial redox (Fig. 5). This suggests that increases in mitochondrial $[NAD^+]$ act to stimulate PDH phosphatase and inhibit PDH kinase in trout muscle. Therefore, it appears that PDH transformation may be more closely related to the redox state of the cellular compartment within which PDH is found than to the general energy status of the cell or Ca^{2+} release from the sarcoplasmic reticulum.

In all studies that have examined PDH activation to date (e.g., 8, 11), the activity of PDH is very closely matched to the total flux through the TCA cycle and thus oxidative phosphorylation. In previous work examining recovery metabolism in trout white muscle, we (28) observed that immediately after high-intensity exercise PDH was fully transformed into PDH_a. In that study (28), we assumed an immediate transformation of PDH_b to PDH_a at the onset of exercise and calculated that oxidative phosphorylation from carbohydrate could contribute up to a maximum of 14% of the total ATP turnover during a 5-min bout of high-intensity exercise. However, in the present study, we have demonstrated that PDH transformation is slow to occur over a 52-s bout of exercise, therefore oxidative phosphorylation only contributes ~2% to total ATP turnover during high-intensity exercise in trout muscle. Despite the lack of ATP production via oxidative phosphorylation of carbohydrate in trout muscle, the pattern of PDH transformation has implications toward lactate metabolism.

Lactate metabolism in trout white muscle. Classically, lactate production was thought to occur during high-intensity exercise because of a limitation of O_2 supply to the mitochondria preventing oxidative phosphorylation, thus activating "anaerobic" glycolysis for ATP production. However, recent evidence (10, 31) suggests that O_2 may not be limiting in mammalian muscle during high-intensity exercise but rather that lactate production may be due to metabolic inertia whereby pyruvate production exceeds pyruvate oxidation. The present study contributes to the accumulating evidence (e.g., 22) that suggests lactate accumulation is due to an imbalance between the transformation pattern and catalytic rates of Phos and PDH. The catalytic rate of $Phos_a$ in trout white muscle is about 140 times greater than the catalytic rate of PDH_a (Phos, ~0.42 μmol·g wet tissue^{-1}·s^{-1}; cf. PDH, 0.003 μmol·g wet tissue^{-1}·s^{-1}; see RESULTS). The low mitochondrial content present within trout white muscle

and likely low copy number of PDH will accentuate the mismatch between glycolytic flux and pyruvate oxidation and will therefore yield lactate accumulation via lactate dehydrogenase.

Considerable controversy surrounds the issue of whether an O_2 limitation in muscle cells plays a role in lactate formation during high-intensity exercise (13). Measurements of whole cell [NADH] and [NAD^+] suggest that the cell becomes more reduced with increases in exercise intensity (29). However, as with the adenylates (ADP and AMP), changes in total [NADH] and [NAD^+] do not provide information regarding the regulation of metabolism; only changes in free NADH and NAD^+ provide insight into metabolic control. On the basis of the lactate dehydrogenase reaction, we have demonstrated that cytoplasmic redox remains constant during high-intensity exercise. This is in direct agreement with previous work from our laboratory (35) and further agrees with Dobson et al. (5) in trout swum for 10 min at 120% U_{crit}.

Estimates of cytoplasmic redox do not yield information regarding the redox state of the mitochondrial matrix. Our estimates of mitochondrial NAD^+/NADH in trout muscle during exercise suggest that the mitochondrial matrix becomes more oxidized as exercise progresses (Fig. 5) and that this is associated with the progressive activation of PDH (Fig. 2), supporting the idea that the electron transport chain is functioning. However, it should be noted that the use of the glutamate dehydrogenase equilibrium to estimate mitochondrial redox state remains highly controversial (13), especially in white muscle with low mitochondrial number (20). Therefore, the conclusion that O_2 is not limiting at the mitochondrion during high-intensity exercise in trout muscle must be considered tentative and awaits confirmation by other methods.

If O_2 is not limiting in white muscle mitochondria during high-intensity exercise, it is possible that other substrates are also used for oxidative ATP production. Because lipid oxidation is very important during post-exercise recovery (28), we wished to determine whether lipid oxidation could contribute significantly to ATP production in trout white muscle during high-intensity exercise. There was a small, transient decrease in free carnitine within the first 10 s of exercise, but this decrease did not correspond to an increase in either SCFA or LCFA-carnitine and there were no increases in acetyl-CoA (Table 3). These data indicate that there was not an increase in fatty acid transport into the mitochondria by carnitine palmitoyltransferase I.

In conclusion, we have demonstrated that during the first 10 s of high-intensity exercise CrP hydrolysis and glycolysis support ATP turnover. Subsequent exercise through to exhaustion is supported primarily by glycolytic ATP production. Entry of glycogen into glycolysis is regulated by the transformational and posttransformational modification of Phos. As exercise duration increased there was a near maximal activation of PDH, and the mismatch between Phos and PDH transformation can explain lactate production in fish muscle. Estimates of cytoplasmic and mitochondrial NAD^+/NADH, in addition to the activation pattern of PDH, suggest that O_2 is not limiting in white muscle cells during high-intensity exercise and is not the primary cause of lactate formation.

Perspectives. Many studies have examined the effects of exhaustive exercise on trout white muscle metabolism and looked at the pattern of metabolite recovery. However, the present study is the first to document changes in muscle metabolites during a continuous bout of exercise. Measurements of the transformation state of regulating enzymes (Phos and PDH) and their allosteric modulators have added evidence to support the hypothesis that lactate production during exercise is not due to an O_2 limitation but rather to an imbalance between Phos and PDH transformation. The slow activation pattern of PDH in trout white muscle indicates that the major fate of lactate is not oxidation. Instead, lactate production in white muscle could be considered integral to white muscle design in fish, so that accumulated lactate is conserved as the substrate for glycogen synthesis during recovery (17, 28, 30). Further research should focus on isolating the role of PDH in white muscle lactate production through manipulation of PDH activity with dichloroacetate (a pharmacological analog of pyruvate), hyperoxia, and hypoxia.

We gratefully acknowledge E. Fitzgerald for the excellent technical assistance.

This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) (C. M. Wood) and the Medical Research Council of Canada (G. J. F. Heigenhauser). J. G. Richards was supported by an NSERC postgraduate scholarship, and C.M. Wood is supported by the Canada Research Chair Program.

REFERENCES

1. **Bergmeyer HU.** *Methods of Enzymatic Analysis*. New York: Academic, 1983.
2. **Black EC, Connor AR, Lam KC, and Chiu WG.** Changes in glycogen, pyruvate and lactate in rainbow trout (*Salmo gairdneri*) during and following muscular activity. *J Fish Res Bd Can* 19: 409–435, 1962.
3. **Chasiotis D, Sahlin K, and Hultman E.** Regulation of glycogenolysis in human muscle at rest and during exercise. *J Appl Physiol* 53: 708–715, 1982.
4. **Connett RJ and Sahlin K.** Control of glycolysis and glycogen metabolism. In: *Handbook of Physiology. Exercise: Regulation and Integration of Multiple Systems*. Bethesda, MD: Am Physiol Soc, 1996, sect. 12, chapt. 19, p. 870–911.
5. **Dobson GP, Parkhouse WS, and Hochachka PW.** Regulation of anaerobic ATP-generating pathways in trout fast-twitch skeletal muscle. *Am J Physiol Regulatory Integrative Comp Physiol* 253: R186–R194, 1987.
6. **Dobson GP, Yamamoto E, Hochachka PW.** Phosphofructokinase control in muscle: nature and reversal of pH-dependent ATP inhibition. *Am J Physiol Regulatory Integrative Comp Physiol* 250: R71–R76, 1986.
7. **Dudley GA, Tullson PC, and Terjung RL.** Influence of mitochondrial content on the sensitivity of respiratory control. *J Biol Chem* 262: 9109–9114, 1987.
8. **Gibala M, MacLean DA, Graham TE, and Saltin B.** Tricarboxylic acid cycle intermediate pool size and estimated cycle flux in human muscle during exercise. *Am J Physiol Endocrinol Metab* 275: E235–E242, 1998.
9. **Hassid WZ and Abraham S.** Chemical procedures for analysis of polysaccharides. In: *Methods Enzymology*, edited by Colowick SP and Kaplan NO. New York: Academic, 1957, vol. 3, p. 34–37.

10. **Heigenhauser GJF and Parolin ML.** Role of pyruvate dehydrogenase in lactate production in exercising human skeletal muscle. In: *Hypoxia: Into the Next Millennium*, edited by Roach RC, Wagner PD, and Hackett PH. New York: Kluwer Academic, 1999, p. 205–218.
11. **Howlett RA, Parolin ML, Dyke DJ, Hultman E, Jones NL, Heigenhauser GLF, and Spriet LL.** Regulation of skeletal muscle glycogen phosphorylase and PDH at varying exercise power outputs. *Am J Physiol Regulatory Integrative Comp Physiol* 275: R418–R425, 1998.
12. **Kasvinski PJ and Meyer WL.** The effect of pH and temperature on the kinetics of native and altered glycogen phosphorylase. *Arch Biochem Biophys* 181: 616–631, 1977.
13. **Katz A, Spencer MK, and Sahlin K.** Failure of glutamate dehydrogenase system to predict oxygenation state of human skeletal muscle. *Am J Physiol Cell Physiol* 259: C26–C28, 1990.
14. **Kieffer JD.** Limits to exhaustive exercise in fish. *Comp Biochem Physiol A Mol Integr Physiol* 126: 161–179, 2000.
15. **Krebs EG, Love DS, Bratwold GE, Trayserm KA, Meyerm WL, and Fishers EH.** Purification and properties of rabbit skeletal muscle phosphorylase b kinase. *Biochemistry* 3: 1022–1033, 1964.
16. **Meyer RA and Foley JM.** Cellular processes integrating the metabolic response to exercise. In: *Handbook of Physiology. Exercise: Regulation and Integration of Multiple Systems*. Bethesda, MD: Am Physiol Soc, 1996, sect. 12, chapt. 18, p. 841–869.
17. **Milligan CL and Girard SS.** Lactate metabolism in rainbow trout. *J Exp Biol* 180: 175–193, 1993.
18. **Milligan CL, Hooke GB, and Johnson C.** Sustained swimming at low velocity following a bout of exhaustive exercise enhances metabolic recovery in rainbow trout. *J Exp Biol* 203: 921–926, 2000.
19. **Moyes CD, Buck LT, and Hochachka PW.** Temperature effects on pH of mitochondria isolated from carp red muscle. *Am J Physiol Regulatory Integrative Comp Physiol* 254: R611–R615, 1988.
20. **Moyes CD, Schulte PM, and Hochachka PW.** Recovery metabolism of trout white muscle: role of mitochondria. *Am J Physiol Regulatory Integrative Comp Physiol* 262: R295–R304, 1992.
21. **Moyes CD and West TG.** Exercise metabolism of fish. In: *Biochemistry and Molecular Biology of Fishes*, edited by Hochachka PW and Mommsen TP. Amsterdam: Elsevier Science, 1995, vol. 4, p. 367–392.
22. **Parolin ML, Chesley A, Matsos MP, Spriet LL, Jones NL, and Heigenhauser GJF.** Regulation of skeletal muscle glycogen phosphorylase and PDH during maximal intermittent exercise. *Am J Physiol Endocrinol Metab* 277: E890–E900, 1999.
23. **Peters SJ and Spriet LL.** Skeletal muscle phosphofructokinase activity examined under physiological conditions in vitro. *J Appl Physiol* 78: 1853–1858, 1995.
24. **Pörtner HO, Boutlier RG, Tang Y, and Toews DP.** The use of fluoride and nitriloacetic acid in tissue acid-base physiology. II. Intracellular pH. *Respir Physiol* 81: 255–274, 1991.
25. **Putman CT, Jones NL, Lands LC, Bragg TM, Hollidge-Horvat MG, and Heigenhauser GJF.** Skeletal muscle pyruvate dehydrogenase activity during maximal exercise in humans. *Am J Physiol Endocrinol Metab* 269: E458–E468, 1995.
26. **Randle PJ.** Metabolic fuel selection: general integration at the whole-body level. *Proc Nutr Soc* 54: 317–327, 1995.
27. **Ren JM and Hultman E.** Phosphorylase activity in needle biopsy samples: factors influencing transformation. *Acta Physiol Scand* 133: 109–114, 1988.
28. **Richards JG, Heigenhauser GJF, and Wood CM.** Lipid oxidation fuels recovery from exhaustive exercise in white muscle of rainbow trout. *Am J Physiol Regulatory Interrative Comp Physiol* 282: R89–R99, 2002.
29. **Sahlin K.** NADH in human skeletal muscle during short-term intense exercise. *Pflügers Arch* 403: 193–196, 1985.
30. **Schulte PM, Moyes CD, and Hochachka PW.** Integrating metabolic pathways in post-exercise recovery of white muscle. *J Exp Biol* 166: 181–195, 1992.
31. **Spriet LL, Howlett RA, and Heigenhauser GJF.** An enzymatic approach to lactate production in human skeletal muscle during exercise. *Med Sci Sports Exerc* 32: 756–763, 2000.
32. **Spriet LL, Söderlund K, Bergström M, and Hultman E.** Skeletal muscle glycogenolysis, glycolysis, and pH during electrical stimulation in men. *J Appl Physiol* 62: 616–621, 1987.
33. **Van den Thillart G, van Waarde A, Muller HJ, Erklens C, Addink A, and Lugtenberg J.** Fish muscle energy metabolism measured by in vivo ^{31}P-NMR during anoxia and recovery. *Am J Physiol Regulatory Integrative Comp Physiol* 256: R922–R929, 1989.
34. **Van der Vusse GJ and Reneman RS.** Lipid metabolism in muscle. In: *Handbook of Physiology. Exercise: Regulation and Integration of Multiple Systems*. Bethesda, MD: Am Physiol Soc, 1996, sect. 12, chapt. 21, p. 954–994.
35. **Wang Y, Heigenhauser GJF, and Wood CM.** Integrated responses to exhaustive exercise and recovery in rainbow trout white muscle: acid-base, phosphogen, carbohydrate, lipid, ammonia, fluid volume and electrolyte metabolism. *J Exp Biol* 195: 227–258, 1994.
36. **Wang Y, Wilkie MP, Heigenhauser GJF, and Wood CM.** The analysis of metabolites in rainbow trout white muscle: a comparison of different sampling and processing methods. *J Fish Biol* 45: 855–873, 1994.
37. **Weiland OH.** The mammalian pyruvate dehydrogenase complex: structure and regulation. *Rev Physiol Biochem Pharmacol* 96: 123–170, 1983.
38. **Williamson DH, Lund P, and Krebs HA.** The redox state of free nicotinamide-adenine dinucleotide in the cytoplasm and mitochondria of rat liver. *Biochem J* 103: 514–527, 1967.
39. **Wood CM.** Acid-base and ion balance, metabolism, and their interactions, after exhaustive exercise in fish. *J Exp Biol* 160: 285–308, 1991.