Regulation of skeletal muscle glycogen phosphorylase and PDH at varying exercise power outputs

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Howlett, Richard A., Michelle L. Parolin, David J. Dyck, Eric Hultman, Norman L. Jones, George J. F. Heigenhauser, and Lawrence L. Spriet. Regulation of skeletal muscle glycogen phosphorylase and PDH at varying exercise power outputs. *Am. J. Physiol.* 275 (*Regulatory Integrative Comp. Physiol.* 44): R418–R425, 1998.—This study investigated the transformational and posttransformational control of skeletal muscle glycogen phosphorylase and pyruvate dehydrogenase (PDH) at three exercise power outputs [35, 65, and 90% of maximal oxygen uptake ($\dot{V}O_{2max}$)]. Seven untrained subjects cycled at one power output for 10 min on three separate occasions, with muscle biopsies at rest and 1 and 10 min of exercise. Glycogen phosphorylase in the more active (*a*) form was not significantly different at any time across power outputs (21.4–29.6%), with the exception of 90%, where it fell significantly to 15.3% at 10 min. PDH transformation increased significantly from rest (average 0.53 mmol·kg wet muscle^{-1}·min^{-1}) to 1 min of exercise as a function of power output (1.60 ± 0.26, 2.77 ± 0.29, and 3.33 ± 0.31 mmol·kg wet muscle^{-1}·min^{-1} at 35, 65, and 90%, respectively) with a further significant increase at 10 min (4.45 ± 0.35) at 90% $\dot{V}O_{2max}$. Muscle lactate, acetyl-CoA, acetylcarnitine, and free ADP, AMP, and P_i were unchanged from rest at 35% $\dot{V}O_{2max}$ but rose significantly at 65 and 90%, with accumulations at 90% being significantly higher than 65%. The results of this study indicate that glycogen phosphorylase transformation is independent of increasing power outputs, despite increasing glycogenolytic flux, suggesting that flux through glycogen phosphorylase is matched to the demand for energy by posttransformational factors, such as free P_i and AMP. Conversely, PDH transformation is directly related to the increasing power output and the calculated flux through the enzyme. The rise in PDH transformation is likely due to increased Ca^{2+} concentration and/or increased pyruvate. These results demonstrate that metabolic signals related to contraction and the energy state of the cell are sensitive to the exercise intensity and coordinate the increase in carbohydrate use with increasing power output.

glycogenolysis; energy state; carbohydrate; transformation; pyruvate dehydrogenase

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SINCE THE CLASSICAL STUDIES of the 1930s [as reviewed by Dill (18)], it is has been recognized that a shift toward greater carbohydrate (CHO) use occurs with increasing aerobic exercise intensities. Muscle glycogen becomes the primary fuel at intensities above 50–60% maximal oxygen uptake ($\dot{V}O_{2max}$; see reviews in Refs. 10 and 34). Two key enzymes that regulate glycogen use are glycogen phosphorylase and pyruvate dehydrogenase (PDH). Flux through these enzymes can be controlled by covalent modification [transformation between less active (*b*) and more active (*a*) forms], allosteric regulation of the *a* and *b* forms, and changes in the concentrations of substrates and products. Evidence suggests that glycogen phosphorylase, the rate-limiting step in glycogenolysis, is regulated by a two-stage control system. First, Ca^{2+}-activated phosphorylation by phosphorylase kinase causes the initial transformation of glycogen phosphorylase from the *b* to *a* form, setting the potential upper limit for glycogenolytic flux (5, 15, 17). Posttransformational regulators linked to the energy state of the cell appear to then determine the actual flux (7, 8, 30).

PDH catalyzes the decarboxylation of pyruvate to acetyl-CoA for the entry of CHO into the pathways of oxidative metabolism. PDH transformation is controlled by PDH kinase, which phosphorylates and inactivates the complex, and PDH phosphatase, which dephosphorylates and activates the complex (24, 26). The balance between these controlling regulatory enzymes determines the proportion of PDH that will be in the active form. Although glycogen phosphorylase and PDH activities have been studied separately under many conditions, little is known regarding the interrelationship between the two enzymes in controlling glycogen use over a range of exercise intensities. Given that glycogen phosphorylase sets the rate of glycolytic flux at moderate to high power outputs and that PDH is responsible for determining the rate of pyruvate flux into the mitochondria, it seems likely that the activities of the two enzymes are coordinately regulated during aerobic exercise.

During exercise at 35% $\dot{V}O_{2max}$, the majority of energy is provided by plasma free fatty acid and glucose, with little or no reliance on muscle glycogen (31). At 65% $\dot{V}O_{2max}$, muscle glycogenolysis increases, but fat still provides a large proportion of the required energy. At 90%, the glycogenolytic rate increases (34), and CHO from glycogen provides the majority of the required substrate (31). The present study was designed to investigate the transformation state of PDH and glycogen phosphorylase and the accumulation of several regulators that may influence the transformation state and/or flux through the enzymes at the following three aerobic power outputs: 35, 65, and 90% $\dot{V}O_{2max}$. Three separate exercise trials were performed to study the transition from rest to exercise at the desired power output and to eliminate the interactive effects of previous exercise. Our hypotheses were that posttransformational regulation of glycogen phosphorylase, and not glycogen phosphorylase transformation, would be im-

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portant in regulating glycogenolysis, whereas PDH transformation would be related to the power output. This coordinated regulation would thus balance the need for CHO provision and oxidation with increasing power outputs.

METHODS

Subjects. Seven (3 female, 4 male) healthy, active subjects volunteered to participate in this study. Their mean age, weight, and $\dot{V}O_{2max}$ were 22.1 ± 0.3 yr, 77.7 ± 3.6 kg, and 47.1 ± 2.3 $ml \cdot kg^{-1} \cdot min^{-1}$, respectively. None of the subjects was well trained, but all participated in some form of regular activity. Subjects were informed of possible risks involved in participation, and informed consent was received from all subjects. The study was approved by the ethics committees of both universities.

Preexperimental protocol. Subjects underwent a continuous incremental exercise test on a bicycle ergometer to determine $\dot{V}O_{2max}$ using a metabolic cart (Quinton Q-Plex 1; Quinton Instruments, Seattle, WA). From this test, power outputs eliciting 35, 65, and 90% of $\dot{V}O_{2max}$ were calculated. Subjects then reported on two separate occasions to practice cycling for the required 10 min at these power outputs and to confirm that the correct percentage of $\dot{V}O_{2max}$ was reached. Mean power outputs for the trials were 58 ± 11, 164 ± 15, and 229 ± 19 W, resulting in percentages of 36.7 ± 1.5, 62.3 ± 1.5, and 90.1 ± 0.6% of $\dot{V}O_{2max}$, respectively.

Experimental protocol. On two separate experimental days (separated by 2–3 wk), subjects arrived at the laboratory at the same time of day, having eaten a meal within the previous 2–3 h. Subjects were asked to consume their normal mixed diet before the test days. To ensure subjects were in the same nutritional state for each test day, 48-h dietary records were kept before their first day and replicated for 48 h before the second day. The average diet composition was 60.0 ± 4.3% CHO, 25.0 ± 2.4% fat, and 15.0 ± 2.0% protein. On one day, subjects cycled for 10 min at the 35% power output, rested for at least 1 h, and cycled for 10 min at the 65% power output, with biopsies taken from separate legs. On the other day, subjects cycled for 10 min at 90% $\dot{V}O_{2max}$. The order of the two days was randomized. Before each exercise trial, while resting quietly on a bed, subjects had their legs prepared for needle biopsies (3), with three incisions made through the skin superficial to the vastus lateralis muscle under local anesthesia (2% lidocaine without epinephrine). After a resting biopsy had been taken, subjects moved to an electronically braked cycle ergometer (Excalibur; Quinton Instruments) and began pedaling at the prescribed power output. Exercise biopsies were taken on the cycle ergometer at 1 and 10 min; samples were immediately frozen in liquid N_2 (3–5 s from the insertion of the needle), removed from the needle, and stored in liquid N_2. Respired gases were collected to measure oxygen uptake and carbon dioxide production. At the lower (35 and 65%) power outputs, gas collection occurred between 4 and 8 min, when subjects were in a metabolic steady state. However, in anticipation of a significant oxygen uptake ($\dot{V}O_2$) drift, gas collection for the 90% $\dot{V}O_{2max}$ trial occurred at 2–4 and 7–9 min of the trial.

Analyses. A small piece of frozen wet muscle (20–30 mg) was removed under liquid N_2 for the determination of the PDH transformation state (PDH *a*), as described by Constantin-Teodosiu et al. (12) and modified by Putman et al. (29). The remainder of the biopsy sample was freeze-dried, dissected of all visible blood, connective tissue, and fat, and powdered for subsequent analysis.

One aliquot (3–4 mg) of freeze-dried muscle was used for determination of the percentage of glycogen phosphorylase in the more active *a* form (glycogen phosphorylase *a*) in the exercise biopsy samples (9, 39). The maximal velocity (V_{max}) of total ($a + b$) and *a* forms and the mole fraction of glycogen phosphorylase in the *a* form were calculated from the measured activities (5, 7, 9). Glycogen phosphorylase *a* was not measured for resting time points, because an accurate resting glycogen phosphorylase *a* value is obtainable only if the biopsy is held from liquid N_2 freezing for >30 s (7), requiring a separate biopsy. Resting glycogen phosphorylase *a* has been measured previously and is ~10% (7, 27).

A second aliquot of freeze-dried muscle was extracted with 0.5 M $HClO_4$ (containing 1 mM EDTA) and neutralized with 2.2 M $KHCO_3$. This extract was used for determination of creatine, phosphocreatine (PCr), ATP, glucose 6-phosphate (G-6-*P*), lactate, and glucose by enzymatic spectrophotometric assays (2, 22). Acetyl-CoA and acetylcarnitine were determined by radiometric measures (4).

Muscle glycogen was determined on a third aliquot of freeze-dried muscle at rest and 10 min at the 90% power output to quantify the glycogenolytic rate (22).

Calculations. Free ADP and AMP concentrations were calculated by assuming equilibrium of the creatine kinase and adenylate kinase reactions as previously described (19). Free ADP was calculated using the measured ATP, PCr, and creatine content (19) and a H^+ concentration estimated from the muscle lactate content according to the regression equation of Sahlin et al. (33). Free AMP was calculated from the ATP concentration and the estimated free ADP. Free P_i was calculated by adding the estimated resting free phosphate of 10.8 mmol/kg dry muscle (19) to the ΔPCr − ΔG-6-*P* between rest and each exercise time point. All metabolites and the activities of glycogen phosphorylase (V_{max} *a* and V_{max} total) and PDH were normalized to the highest total creatine measurement in the nine biopsies from each subject.

Statistics. All data are presented as means ± SE. For all dependent variables, a two-way ANOVA (time × power output) with repeated measures was employed. Significance was set at $\alpha = 0.05$, and, when obtained, the Tukey post hoc test was used to identify where significant differences occurred.

RESULTS

$\dot{V}O_2$ and respiratory exchange ratio. Exercise $\dot{V}O_2$ for the 35 and 65% power outputs was 1.32 ± 0.12 and 2.18 ± 0.19 l/min, respectively. $\dot{V}O_2$ at 90% was 2.75 ± 0.26 l/min (2–4 min) and increased to 3.29 ± 0.31 l/min (7–9 min). Respiratory exchange ratios (RER) for the 35 and 65% power outputs were 0.84 ± 0.02 and 0.92 ± 0.02. RER for the 90% $\dot{V}O_{2max}$ trial was continuously above 1.0 and therefore is not a true representation of substrate use but indicated substantial use of CHO.

Phosphorylase. Phosphorylase V_{max} total was similar between trials at all time points, whereas V_{max} *a* was decreased only at 10 min at 90% (Table 1). The resulting percentage of phosphorylase in the more active *a* form was unchanged (21.4–29.6%) with both power output and time, with the exception of 10 min at 90%, when glycogen phosphorylase *a* was lower (15.3%) than at 1 min (Fig. 1).

PDH a. Resting PDH activity was similar before all exercise trials (0.40 ± 0.08 to 0.65 ± 0.18 $mmol \cdot kg$ wet $muscle^{-1} \cdot min^{-1}$). PDH activity increased above rest after 1 min of exercise at all three power outputs with

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Table 1. *Skeletal muscle phosphorylase activities at different exercise power outputs*

Time, min	V_{max} *a*	V_{max} Total
	35% $\dot{V}O_{2max}$	
1	35.5 ± 5.3	150.7 ± 17.0
10	41.2 ± 11.0	152.9 ± 19.6
	65% $\dot{V}O_{2max}$	
1	30.9 ± 5.1	144.0 ± 15.7
10	39.0 ± 10.2	167.3 ± 25.4
	90% $\dot{V}O_{2max}$	
1	45.6 ± 10.1	151.4 ± 20.3
10	23.4 ± 4.8*	161.7 ± 18.3

Values are means ± SE in mmol · kg dry muscle^{-1} · min^{-1}. V_{max} *a*, calculated maximal phosphorylase *a* activity; V_{max} total, calculated total (*a* + *b*) phosphorylase activity; $\dot{V}O_{2max}$, maximal oxygen uptake. * Significantly different from 1 min.

65 and 90% being higher than 35% (Fig. 2). PDH at the two lower power outputs did not subsequently change from 1 to 10 min, whereas at 90% it increased.

Muscle metabolites. Muscle glycogen averaged 360.1 ± 50.1 mmol/kg muscle at rest before the 90% $\dot{V}O_{2max}$ trial and decreased to 235.7 ± 41.3 mmol/kg dry muscle after 10 min of cycling, resulting in a net utilization of 124.4 ± 14.0 mmol/kg dry muscle. Glycogen was not determined at the lower power outputs as the expected change in muscle glycogen in those trials would be below the detection limits of the assay.

Resting metabolite contents were similar before each trial (Table 2). During exercise, PCr was unchanged at 35% $\dot{V}O_{2max}$ but was lower at both 1 and 10 min of exercise at 65 and 90% $\dot{V}O_{2max}$, with 90% being lower than 65%. Exercise at 90% $\dot{V}O_{2max}$ also resulted in PCr contents that were lower at 10 min than at 1 min. ATP was unchanged by exercise at all time points and all power outputs (Table 2). Muscle lactate was unchanged at 35% but increased after 1 and 10 min of exercise at both 65 and 90%, with 90% being higher than 65% at 10 min (Table 2). G-6-*P* was unchanged by exercise at 35 and 65% $\dot{V}O_{2max}$ but increased significantly at 1 and 10 min of 90% $\dot{V}O_{2max}$. Free glucose increased only at 10 min at 90% $\dot{V}O_{2max}$.

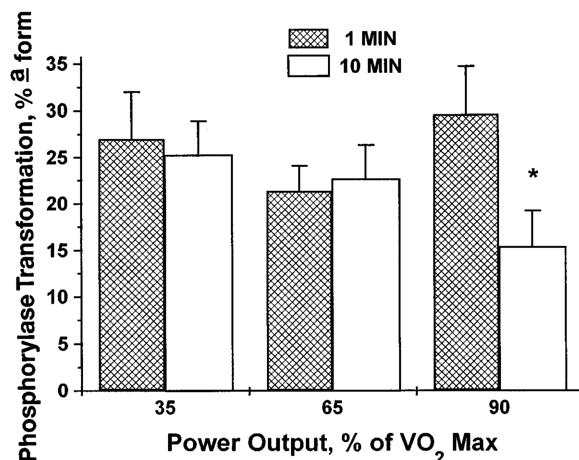

Fig. 1. Phosphorylase transformation during cycling at the various power outputs. Values are means ± SE. $\dot{V}O_{2max}$, maximal oxygen uptake; *a*, active. * Significantly different from 1 min.

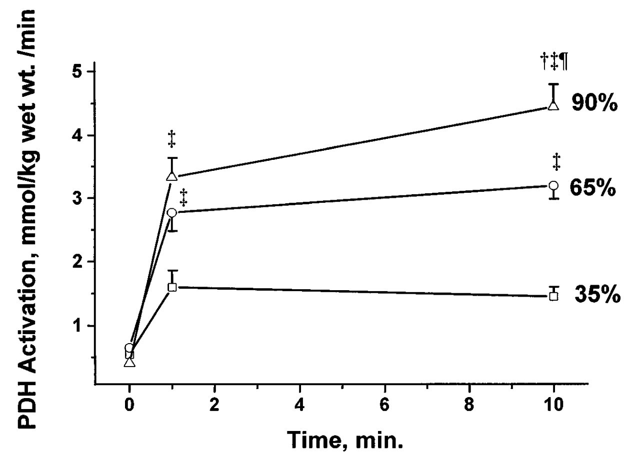

Fig. 2. Pyruvate dehydrogenase (PDH) transformation during cycling at the various power outputs. Values are means ± SE. All exercise values were significantly greater than rest at a given power output. † Significantly different from 1 min at a given power output. ‡ Significantly different from 35% at a given time point. ¶ Significantly different from 65% at a given time point. Error bars were omitted at 0 min for clarity.

Muscle acetyl-CoA was unchanged during exercise at 35% $\dot{V}O_{2max}$ but increased after 10 min at 65% and 1 and 10 min at 90% (Fig. 3*A*). At 10 min, 90% was also higher than at 65%. Acetylcarnitine generally followed the same pattern as acetyl-CoA (Fig. 3*B*).

Free ADP, AMP, and P_i. Resting free ADP contents were not different between trials (89.7 ± 8.7 to 105.8 ± 15.2 µmol/kg dry muscle) and were unchanged at any time during exercise at 35% $\dot{V}O_{2max}$ (Fig. 4*A*). At 65%, free ADP was elevated at 10 min. At 90%, free ADP at both 1 and 10 min was higher than rest and 35%, whereas the 10-min value was also higher than 65%. Free AMP contents were not different at rest and did not change after exercise at both 35 and 65% (Fig. 4*B*). At 90%, free AMP was greater than both 35 and 65% at 1 and 10 min. Free AMP was also greater at 10 min of 90% than at 1 min. Free P_i accumulation during exercise at 65% $\dot{V}O_{2max}$ was greater than at 35% at both time points (Table 1). At 90% $\dot{V}O_{2max}$, free P_i contents were greater than at 35 and 65% at both time points, and the 10-min value was higher than at 1 min.

DISCUSSION

This study investigated the control of skeletal muscle glycogen metabolism at increasing exercise power outputs by examining the regulation of two key enzymes (glycogen phosphorylase and PDH). The primary findings of the study were that glycogen phosphorylase transformation is always well in excess of glycogenolytic flux, whereas PDH transformation is a good reflection of CHO flux into the mitochondria. Our data suggest that, although exercise induces transformation of glycogen phosphorylase, the magnitude of transformation is always sufficient for the required flux and is independent of aerobic power output. Posttransformational regulators linked to the power output (demand

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Table 2. *Muscle metabolite contents at rest and during cycling at the various power outputs*

	35% $\dot{V}O_{2max}$			65% $\dot{V}O_{2max}$			90% $\dot{V}O_{2max}$		
	0 min	1 min	10 min	0 min	1 min	10 min	0 min	1 min	10 min
PCr	88.4 ± 1.2	80.3 ± 1.7	80.2 ± 1.8	89.4 ± 2.3	55.5 ± 2.44*†	47.1 ± 5.3*†	87.8 ± 2.4	42.0 ± 4.7*†‡	17.2 ± 2.0*†‡§
ATP	26.1 ± 1.6	23.8 ± 1.2	24.3 ± 2.3	28.8 ± 2.1	22.5 ± 1.7	22.4 ± 2.1	25.0 ± 2.0	23.9 ± 1.4	19.6 ± 1.7
Free P_i	ND	18.2 ± 2.1	18.4 ± 2.1	ND	43.9 ± 3.0†	51.8 ± 6.6†	ND	52.6 ± 4.8†	74.1 ± 2.7†‡§
Glucose	2.4 ± 0.5	3.8 ± 0.9	3.4 ± 0.7	3.6 ± 1.2	4.2 ± 0.6	4.9 ± 1.0	3.0 ± 1.2	3.7 ± 0.9	13.8 ± 0.9*†‡§
G-6-*P*	0.3 ± 0.1	1.0 ± 0.2	0.9 ± 0.2	0.5 ± 0.2	1.3 ± 0.3	1.7 ± 0.6	0.4 ± 0.2	4.4 ± 1.3*†‡	7.7 ± 1.2*†‡§
Lactate	4.7 ± 1.3	5.2 ± 1.2	4.7 ± 0.6	2.4 ± 0.87	23.1 ± 2.6*†	37.5 ± 10.0*†	6.27 ± 3.9	37.0 ± 4.3*†	107.9 ± 10.1*†‡§
pH	7.04 ± 0.01	7.04 ± 0.01	7.04 ± 0.004	7.05 ± 0.004	6.98 ± 0.02	6.93 ± 0.05*†	7.03 ± 0.02	6.91 ± 0.02*†	6.61 ± 0.04*†‡§

Values are means ± SE in mmol/kg dry muscle (except pH). Resting values for each metabolite are not different between power outputs. PCr, phosphocreatine; G-6-*P*, glucose 6-phosphate. * Significantly different from rest at any power output. † Significantly different from 35% at a given time point. ‡ Significantly different from 65% at a given time point. § Significantly different from 1 min within a given power output. ND, not determined.

for ATP) determine the actual rate of glycogenolysis. Conversely, the regulators that control both the transformation of PDH to its more active form and the flux through the enzyme are related to the exercise power output.

For many years, the classical explanation of enzyme activation was that transformation of the enzyme into the more active form was synonymous with flux through the enzyme. However, for glycogen phosphorylase, the simple conversion of *b* to *a* forms alone cannot account for the glycogenolytic rate, as illustrated by epinephrine infusion and caffeine ingestion studies. In the case

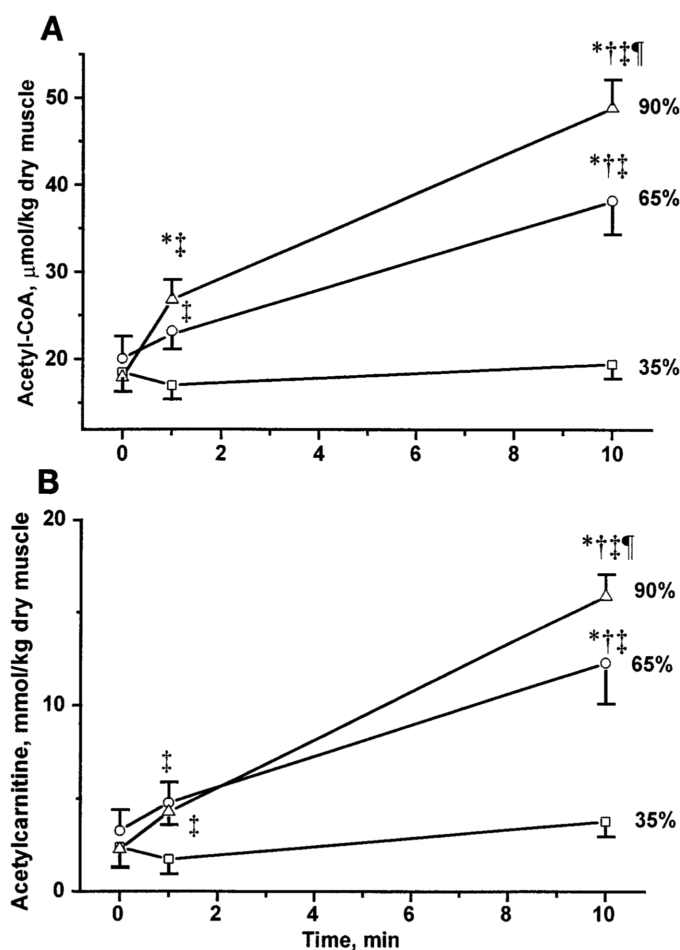

Fig. 3. Muscle acetyl-CoA (*A*) and acetylcarnitine (*B*) contents during cycling at the various power outputs. Values are means ± SE. * Significantly different from rest at any power output. † Significantly different from 1 min within a given power output. ‡ Significantly different from 35% at a given time point. ¶ Significantly different from 65% at a given time point.

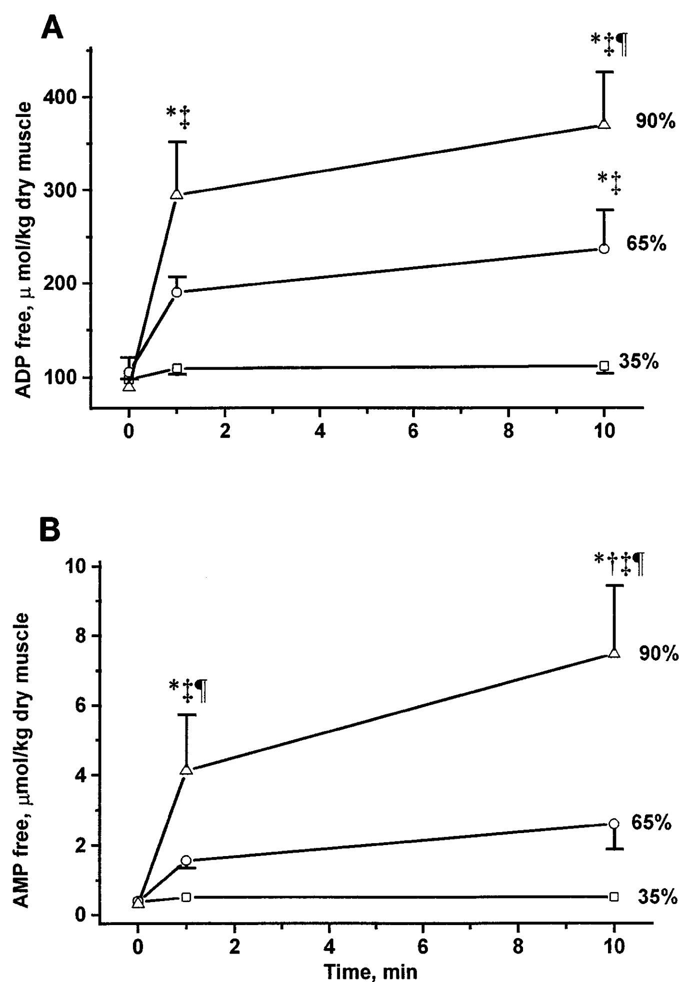

Fig. 4. Muscle free ADP (*A*) and free AMP (*B*) contents during cycling at the various power outputs. Values are means ± SE. * Significantly different from rest at any power output. † Significantly different from 1 min within a given power output. ‡ Significantly different from 35% at a given time point. ¶ Significantly different from 65% at a given time point. Error bars were omitted because they were smaller than the symbols.

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of epinephrine infusion at physiological levels, glycogen phosphorylase reaches nearly total conversion to the *a* form, yet flux through the enzyme is much lower than expected, suggesting that actual glycogenolysis is related to the energy state of the cell and not transformation (6, 9). Similarly, after caffeine ingestion, the percentage of glycogen phosphorylase in the *a* form is significantly increased, whereas glycogen is in fact "spared" during aerobic exercise compared with control (8, 35).

Conversely, PDH transformation has previously been shown to be more closely matched to PDH flux under most situations. Exercise studies have shown that the calculated flux through PDH is almost stoichiometric with the transformation of PDH to the *a* form (13, 28, 29). However, one study reported no further activation of PDH when comparing activities at 90 vs. 60% $\dot{V}O_{2max}$ while CHO oxidation and flux through PDH increased (11). The close match reported in most studies can be uncoupled under certain conditions. Subjects on a low-CHO diet showed similar PDH *a* values compared with high-CHO controls during exercise, but, due to the low muscle glycogen contents, flux through PDH was ~50% of PDH *a* during exercise in the low-CHO condition. These data suggest that transformation and flux are not always equivalent for this enzyme (29).

Control of glycogen phosphorylase. Several previous studies have demonstrated that the rate of glycogenolysis, and thus flux through glycogen phosphorylase, increases as a function of power output (10, 31, 34). Although resting glycogen phosphorylase *a* was not measured in this study, as separate biopsies would be required, it has been shown that resting glycogen phosphorylase *a* averages ~10% or less (7, 27). Therefore, in the present study, glycogen phosphorylase transformation to the *a* form increased from rest at all power outputs (Fig. 1). However, in accordance with our hypothesis, glycogen phosphorylase transformation during exercise did not differ with increasing power output, despite the required increase in flux through glycogen phosphorylase (glycogenolysis). It is important to note, however, that the potential flux through glycogen phosphorylase, based on the catalytic activity of the transformed enzyme (glycogen phosphorylase *a*), was always well in excess of the estimated flux through the enzyme. Potential glycogen phosphorylase activity (i.e., V_{max} of the more active *a* form) ranged from 23.4 ± 4.8 to 41.3 ± 11.0 mmol·kg dry muscle^{-1}·min^{-1} (Table 1), whereas the rate of glycogenolysis reached only 12.4 mmol·kg dry muscle^{-1}·min^{-1} at 90% $\dot{V}O_{2max}$ and was much less at the lower power outputs. Therefore, glycogen phosphorylase transformation was well in excess of what was needed at all power outputs. The disparity between the low rate of glycogen phosphorylase flux and its potential catalytic activity is due to the fact that glycogenolysis does not provide substrate solely for oxidative metabolism (10). To maintain high rates of anaerobic ATP turnover during very intense bouts of exercise, glycogenolysis must also be capable of reaching fluxes through glycogen phosphorylase that approach its maximum potential (i.e., ~150 mmol·kg dry muscle^{-1}·min^{-1}). Using an isokinetic cycle ergometer at very high power outputs, Parolin et al. (27) showed that glycogen phosphorylase transformation was ~60% within 6 s and that glycogenolytic flux approached the V_{max} total of glycogen phosphorylase.

It appears that transformation of glycogen phosphorylase by phosphorylation is obligatory to permit glycogenolysis during exercise, but the degree of transformation is not necessarily related to the actual flux through the enzyme. The transformation from the less active *b* to more active *a* form occurs with the onset of exercise, due to a stimulation of phosphorylase *a* kinase by Ca^{2+} (5, 17). A second stage of control, which serves to regulate the flux through glycogen phosphorylase, is related to the energy status of the cell. Increases in glycogen phosphorylase flux have been shown to correlate with increases in both free P_i and free AMP (glycogen phosphorylase substrate and allosteric modulator, respectively). Both free P_i and free AMP increased significantly with power output (Table 1), providing increased substrate for the glycogen phosphorylase reaction as the need for flux through the reaction increased. In support of the close relationship between glycogenolysis and free AMP, previous studies, utilizing short-term training, high-fat (Intralipid) infusion, and caffeine ingestion, have shown a blunted accumulation of free AMP during exercise when glycogen sparing occurs (7, 8, 20).

Glycogen phosphorylase *a* was significantly reduced after 10 min at 90% $\dot{V}O_{2max}$. This fall may have been caused by the increasing acidosis of the muscle at this power output and duration as the calculated muscle pH fell to 6.61 ± 0.04 (Table 2). Low pH inhibits phosphorylase *b* kinase and would lower transformation into the *a* form (5). It has been shown that glycogen phosphorylase transformation is inhibited with repeated intense bouts of exercise when muscle pH approached 6.4 (27).

PDH regulation. Transformation of PDH is regulated by the balance between PDH kinase (deactivating) and PDH phosphatase (activating; see Refs. 26 and 38). PDH kinase is believed to be inhibited by pyruvate and high CoASH/acetyl-CoA or NAD-to-NADH ratios and stimulated by high ATP/ADP (14, 23, 38). Conversely, PDH phosphatase is stimulated by Ca^{2+} (1).

In contrast to glycogen phosphorylase, PDH transformation rose as a function of increasing power output in the present study. Constantin-Teodosiu et al. (11) had subjects cycle at 30, 60, and 90% $\dot{V}O_{2max}$ consecutively and reported increases in PDH transformation at 30 and 60% $\dot{V}O_{2max}$. There was no further increase at 90% $\dot{V}O_{2max}$, as was observed in the present study. However, we feel that the increase in PDH at the high power output is necessary to account for the increased CHO oxidation that occurs when moving from 60 to 90% $\dot{V}O_{2max}$. To test whether the increase in PDH transformation was equal to the flux through the enzyme, we estimated PDH flux by different methods, depending on the power output. For the 35 and 65% $\dot{V}O_{2max}$ power outputs, PDH flux was estimated from $\dot{V}O_2$ and RER, and an estimate of active muscle mass. The total amount of CHO oxidized during the 10 min was calcu-

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lated (21) and converted to mmol pyruvate $\cdot kg^{-1} \cdot min^{-1}$, assuming an active muscle mass of 10 kg (16, 29). This resulted in calculated PDH fluxes of 1.17 ± 0.19 and 2.20 ± 0.27 mmol·kg wet muscle^{-1}·min^{-1} for 35 and 65% $\dot{V}O_{2max}$, respectively, compared with measured PDH *a* activities of 1.53 and 2.98 mmol·kg wet muscle^{-1}·min^{-1} for the two power outputs. At 90% $\dot{V}O_{2max}$, total glycogenolysis was determined as the difference between resting and 10-min glycogen measurements. The PDH flux was estimated as (total glycogenolysis + glucose uptake) − (muscle lactate and glycolytic intermediate accumulation + blood lactate efflux), assuming an active muscle mass of 10 kg. Glucose uptake was estimated based on the data of Katz et al. (25). Lactate efflux was estimated, assuming that 20% of the total lactate produced left the muscle. This calculation resulted in a PDH flux of 3.45 ± 0.59 mmol·kg wet muscle^{-1}·min^{-1}. Measured PDH *a* activity at this power output averaged 3.89 mmol·kg wet muscle^{-1}·min^{-1} between 1 and 10 min.

When the activity of PDH at different power outputs is compared with the estimated PDH flux (Fig. 5), it is apparent that PDH transformation is closely matched to the flux through the enzyme, as suggested previously (1, 28, 29). The probable stimuli for the increase in PDH *a* are the increases in Ca^{2+} concentration and pyruvate concentration observed with increasing power output (1, 11, 28, 32). Ca^{2+} increases during muscle contraction would activate PDH phosphatase and stimulate PDH transformation to the active form. Likewise, because pyruvate is both an inhibitor of PDH kinase, and a substrate for PDH, an increase in pyruvate would increase the transformation of PDH and flux through the PDH reaction.

The rise in acetyl-CoA and acetylcarnitine with increasing power output is similar to that shown by others (11, 32). However, despite its proposed inhibitory effects, the rise in acetyl-CoA levels did not suppress the increase in PDH *a* with increasing power output. It is very likely that other activating factors override the acetyl-CoA inhibition that may occur in a resting situation (28). To prevent the rise in acetyl-CoA from trapping all of the available free CoA, thereby slowing PDH flux and several other reactions, the acetyl groups are transferred to carnitine, increasing the acetylcarnitine concentration and buffering the removal of free CoA from the system (28, 32).

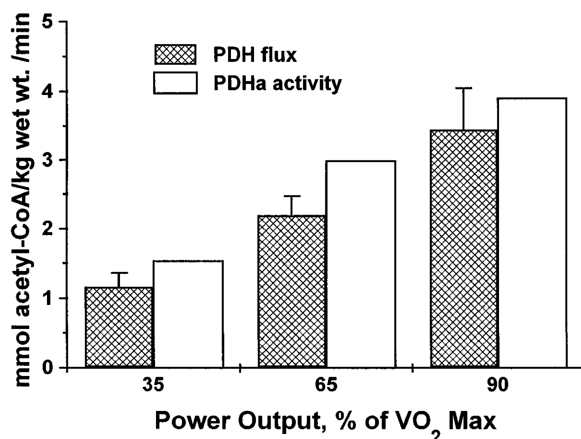

Fig. 5. Comparison of measured PDH transformation and estimated PDH flux across exercise power outputs. SE bars were omitted from transformation data because they represent the mean of 2 time points for each power output.

The muscle ATP-to-ADP ratio decreased with increasing power output, as ATP did not change across power outputs (Table 2), but free ADP rose markedly with increased power output (Fig. 4*A*). The fall in the ATP-to-ADP ratio would be expected to increase the transformation of PDH to the active form due to inhibition of PDH kinase. The relationship between the NADH-to-NAD ratio and PDH transformation is difficult to quantify due to the difficulty in measuring the mitochondrial redox state during exercise. One study, using the direct, bioluminescent measurement of NADH, demonstrated that PDH transformation was independent of mitochondrial redox (13). Conversely, using the glutamate dehydrogenase equilibrium method to estimate NADH/NAD, a second group found that the fall in NADH was consistent with stimulation of PDH transformation (28). Until a proven method of measuring mitochondrial redox during exercise is established, this relationship will remain unclear.

Coordinate regulation of glycogen phosphorylase and PDH. Increases in exercise power output require increased rates of ATP degradation and increased rates of ATP provision from oxidative metabolism. As the power output increases, a greater mismatch between energy demand and provision occurs at the onset of exercise. The present data demonstrate that muscle cells can provide the required ATP with very few homeostatic changes from rest at 35% $\dot{V}O_{2max}$. However, at 65 and 90%, a greater mismatch between ATP demand and provision is reflected by the increased breakdown of PCr and the accumulation of free ADP, AMP, and P_i after 1 min of exercise (Table 2). This fall in the energy state of the cell stimulates tricarboxylic acid (TCA) cycle activity, mitochondrial respiration, and the enzymes of glycogen metabolism (glycogen phosphorylase and PDH). Likewise, increases in cellular free Ca^{2+} that occur with increasing power outputs increase the transformation of both glycogen phosphorylase (17) and PDH (1) to their more active forms, especially at the onset of exercise.

The results of the present study suggest that the increased concentrations of free AMP and P_i exert a positive feedback effect on the flux through glycogen phosphorylase *a* to increase glycogen breakdown and pyruvate concentration. The initial rise in pyruvate during the transition to steady-state exercise "feeds forward" on PDH to increase PDH *a* and allows for the increased flux required to support oxidative metabolism. Therefore, the rates of pyruvate delivery and oxidation can be adjusted to maintain oxidative ATP production as power output increases, but at the cost of a reduced energy state of the cell.

One consequence of the increased pyruvate required to stimulate PDH is an increased accumulation of lactate as power output increases. Because the equilib-

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rium enzyme lactate dehydrogenase favors the production of lactate, a small rise in pyruvate causes a marked rise in lactate. At 90% $\dot{V}O_{2\,max}$, PDH is fully transformed and increases in glycolytic flux, or pyruvate cannot be oxidized through increased PDH flux, resulting in a greater lactate accumulation. However, it is somewhat unclear why lactate accumulates at 65% $\dot{V}O_{2\,max}$, since PDH is not fully transformed and could potentially support greater flux. It would be interesting to examine whether increased PDH transformation from dichloroacetate (DCA) infusion would result in greater PDH flux (and lower lactate) at this power output. DCA has been shown to increase PDH *a* and subsequently improve contractile function in ischemic canine muscle (36, 37). Given that subjects are using almost exclusively muscle glycogen at 90% $\dot{V}O_{2\,max}$, PDH flux (and acetyl-CoA formation) would set the rate of TCA cycle flux (24) and thus the rate of NADH production for oxidation by the electron transport chain. Timmons et al. (36) hypothesized that TCA cycle flux could be limited by substrate (acetyl-CoA) availability at the start of exercise. By increasing PDH *a* transformation at rest, they were able to provide more acetyl-CoA from acetylcarnitine at the transition from rest and subsequently increase the initial rate of oxidative metabolism.

In summary, from this investigation it appears that several signals act to coordinate glycogen utilization over a wide range of aerobic power outputs. First, as the intensity of contractions increases, the amount of cellular free Ca^{2+} increases, causing transformation of both glycogen phosphorylase and PDH. Second, an increasing fall in the cellular energy state occurs with increasing power output. This fall in energy state fine tunes the rate of glycogenolysis through substrate (P_i) and allosteric (free AMP) control. Third, pyruvate production from glycogenolysis feeds forward to further regulate the transformation of and flux through PDH. In conclusion, the present data demonstrate mechanistically how a rise in CHO use could occur based on metabolic signals related to exercise intensity.

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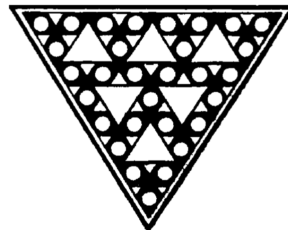

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