

# Regulation of Pyruvate Dehydrogenase (PDH) Activity in Human Skeletal Muscle During Exercise

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SPRIET, L.L., and G.J.F. HEIGENHAUSER. Regulation of pyruvate dehydrogenase (PDH) activity in human skeletal muscle during exercise. *Exerc. Sport Sci. Rev.*, Vol. 30, No. 2, pp. 91–95, 2002. *Pyruvate dehydrogenase is a mitochondrial multienzyme complex that catalyzes the conversion of pyruvate to acetyl-coenzyme A and regulates the entry of carbohydrate into the tricarboxylic acid cycle for oxidation. During exercise, pyruvate dehydrogenase activation in human skeletal muscle is proportional to the relative aerobic power output (percent  $\dot{V}O_2\text{max}$ ) and is regulated by increases in  $Ca^{2+}$ , free ADP, and pyruvate concentrations.*

**Keywords:** carbohydrate oxidation, mitochondrion, PDH kinase, PDH phosphatase

## INTRODUCTION

Fat and carbohydrate (CHO) are the main fuels metabolized in the pathways that lead to ATP production, with CHO becoming the dominant fuel above power outputs of  $\sim 50\%$   $\dot{V}O_2\text{max}$ . Glucose taken up from the blood or derived from glycogen stored inside the muscle cell is metabolized in the glycolytic pathway with the production of pyruvate (Fig. 1). One of the major fates of pyruvate is to be transported into the mitochondria where the nonequilibrium enzyme pyruvate dehydrogenase (PDH) regulates the conversion of pyruvate to acetyl-coenzyme A (acetyl-CoA). This reaction regulates the entry of CHO into the tricarboxylic acid (TCA) cycle and is the first irreversible step in the oxidation of CHO-derived carbon. However, when pyruvate production exceeds its rate of oxidation by PDH, lactate is produced because of the near equilibrium nature of the lactate dehydrogenase reaction (Fig. 1). This occurs to a small extent during aerobic exercise but is most pronounced during intense aerobic and sprint exercise.

The acetyl-CoA produced in the PDH reaction enters the TCA cycle and reducing equivalents (e.g., NADH, FADH<sub>2</sub>) are produced. These reducing equivalents, along with those pro-

duced directly in the PDH reaction and the glycolytic pathway, donate electrons to the respiratory chain. The processes of electron transport and oxidative phosphorylation combine to produce ATP from free ADP and inorganic phosphate (P<sub>i</sub>) with the consumption of O<sub>2</sub> and the production of H<sub>2</sub>O. The oxidation of 1 mole of glucose produces 38–39 moles of ATP, whereas anaerobic metabolism (substrate phosphorylation) of glucose or glycogen to lactate produces 2–3 moles of ATP.

## REGULATION OF THE PDH COMPLEX

Pyruvate dehydrogenase is a multienzyme complex located in the matrix of the mitochondria that catalyzes the following reaction:

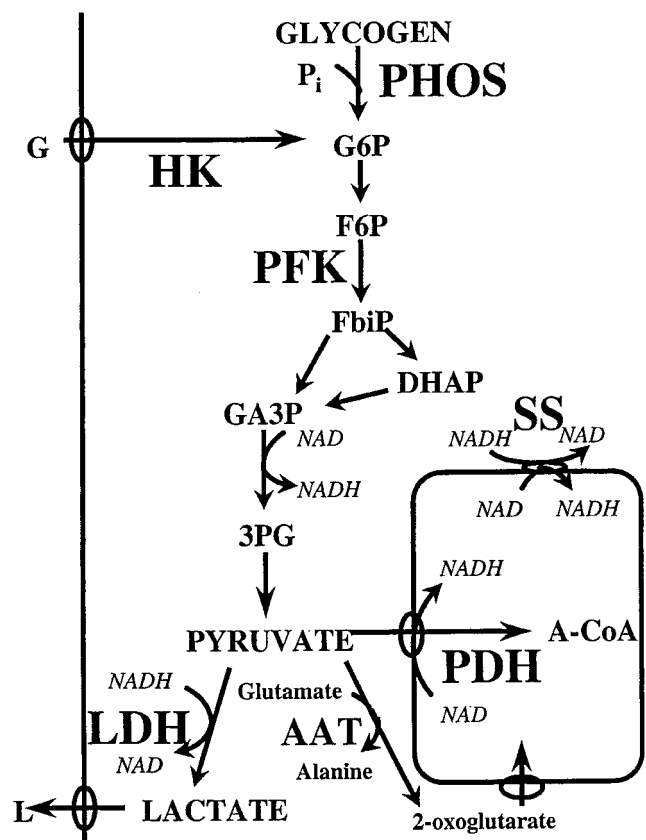


The reaction is catalyzed sequentially by a complex of three enzymes: E1 (pyruvate dehydrogenase), E2 (lipoate acetyltransferase), and E3 (dihydrolipoyl dehydrogenase). The PDH complex is covalently regulated by a phosphorylation-dephosphorylation cycle acting on the E1 catalytic subunits (Fig. 2). Phosphorylation is catalyzed by PDH kinase, which inactivates the enzyme (PDH<sub>b</sub>) whereas PDH phosphatase removes phosphate and returns the enzyme to the active form (PDH<sub>a</sub>) (5). The relative activities of the phosphatase and kinase determine the proportion of the complex in the active form at any point in time.

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**Figure 1.** Schematic of the glycogenolytic/glycolytic pathways highlighting aerobic fate of pyruvate. Figure depicts muscle membrane (vertical line), mitochondrion (rectangle), transport processes (ellipses), and the direction of net flux (arrows). G, glucose; L, lactate; A-CoA, acetyl-Coenzyme A; PHOS, glycogen phosphorylase; HK, hexokinase; PFK, phosphofructokinase; SS, malate-aspartate shuttle system; LDH, lactate dehydrogenase; G6P and F6P, glucose and fructose 6-phosphate; FbiP, fructose 1,6 bisphosphate; DHAP, dihydroxyacetone phosphate; GA3P, glyceraldehyde 3-phosphate; 3PG, 3-phosphoglycerol phosphate; and AAT, alanine amino transferase.

The activities of PDH phosphatase and kinase both require  $Mg^{2+}$  and are in turn regulated by several allosteric regulators (Fig. 2). At rest, PDH kinase activity is acutely stimulated by acetyl-CoA, NADH, and ATP, or high ratios of acetyl-CoA/CoA, ATP/ADP, and NADH/NAD, and inhibited by pyruvate. Phosphatase activity is acutely and powerfully stimulated by  $Ca^{2+}$ . There are no known allosteric regulators of the PDHa and b subunits. Although the availability of substrates is required for flux, increases in the concentrations of the substrates (pyruvate,  $NAD^+$ , CoA) or decreases in the products of the reaction (acetyl-CoA,  $CO_2$ , and NADH) may also stimulate flux. However, substrate/product concentrations usually have minor influences on the regulation of nonequilibrium enzymes, as covalent and allosteric control dominates. As discussed below, only pyruvate appears to play a dual role in activating PDH allosterically and stimulating flux as a substrate.

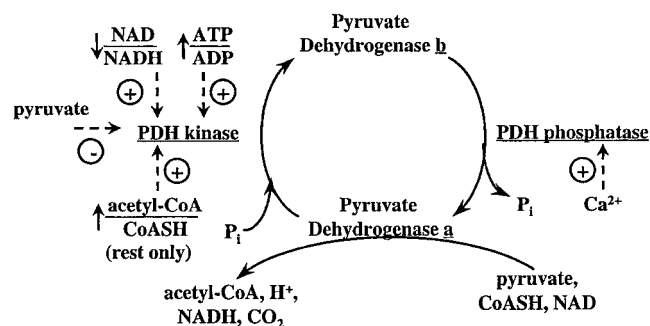
## PDH ACTIVATION AND FLUX IN HUMAN SKELETAL MUSCLE DURING EXERCISE

The majority of the work summarized in the previous section examining the regulation of PDH activity was per-

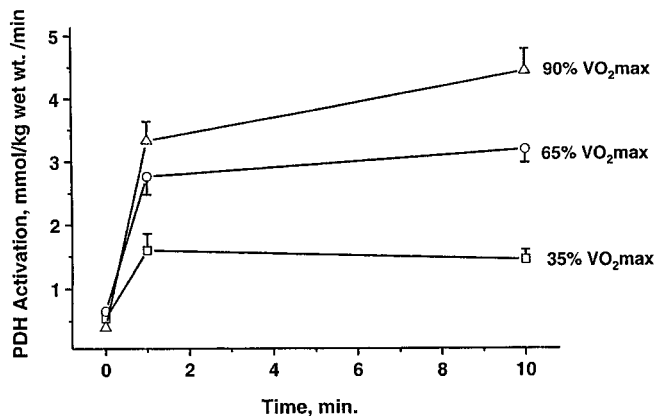
formed using rodent skeletal muscle extracts *in vitro* or isolated skeletal muscle in resting and contracting conditions. Although the regulation appears to be similar in human skeletal muscle at rest, differences have been reported during exercise. Ward *et al.* (14) were the first to measure total PDH activity and PDH activation during exercise in human skeletal muscle, sampled from the vastus lateralis with the needle biopsy technique. They demonstrated that PDH activation increased during aerobic, sprint, and isometric exercise. More recently, other investigators have measured PDH activation during a variety of exercise paradigms in human skeletal muscle and have examined the factors believed to regulate its activity (1,3,5,7,8,10,12,15). For example, in one study active subjects exercised for 10 min at either 35, 65, or 90%  $\dot{V}O_{2max}$  on three separate occasions with muscle biopsies sampled at rest and at 1 and 10 min of exercise (3). At all intensities, PDH was rapidly activated at 1 min of exercise and linearly correlated with the relative power output (Fig. 3). At 35 and 65%  $\dot{V}O_{2max}$ , the increase in PDH activation was maintained throughout the 10 min of exercise. At 90%  $\dot{V}O_{2max}$ , PDH activation continued to increase in line with a continual upward drift in  $\dot{V}O_2$ , such that maximal  $O_2$  uptake and maximal PDH activation were reached after 10 min of exercise (Fig. 3). Separate measures of total PDH activity confirmed that the enzyme was maximally activated to the a form after 10 min at the high power output.

In a second study, the time course of PDH activation was examined during a series of 30-s maximal sprints on an isokinetic cycle ergometer at a power output of ~250–300% of that required to elicit  $\dot{V}O_{2max}$  (7). Subjects cycled maximally at 100 rpm for 6, 15, or 30 s on 3 separate days with muscle biopsies sampled before and after each sprint. PDH was rapidly activated within 6 s and maximal activation was already reached by 15 s (Fig. 4). During repeated sprints, each separated by a 4-min rest, PDH activation before the successive sprints remained elevated from the previous bout (7). The rate of PDH activation also increased during the successive sprints (Fig. 4). Collectively, these changes in PDH activation with repeated sprints resulted in increased  $O_2$  uptake and energy production from CHO oxidation.

Estimates of PDH flux or CHO flux through the TCA cycle in exercising skeletal muscle at various power outputs correspond closely with PDH activation or the catalytic rate of PDHa (3,10) (Fig. 5). PDH flux estimates at the lower power outputs (35 and 65%  $\dot{V}O_{2max}$ ) were based on calcu-



**Figure 2.** Schematic diagram of proposed regulation of PDH. Abbreviations as in Figure 1.



**Figure 3.** PDH activation at rest and during cycling at various power outputs. (Reprinted from Howlett, R.A., M.L. Parolin, D.J. Dyck, E. Hultman, N.L. Jones, G.J.F. Heigenhauser, and L.L. Spriet. Regulation of skeletal muscle glycogen phosphorylase and pyruvate dehydrogenase at different exercise power outputs. *Am. J. Physiol.* 275:R418-R425, 1998. Copyright © 1998 American Physiological Society. Used with permission.)

lations of whole body CHO oxidation ( $\dot{V}O_2$  and RER measurements) and predictions of the active muscle mass. At the intense power outputs (90 and ~250–300%  $\dot{V}O_{2\max}$ ), PDH flux was calculated from the predicted active muscle mass and the difference between total glycogenolysis and glucose uptake minus the accumulated muscle lactate, the lactate that escaped the muscle, and the accumulation of glycolytic intermediates. A close match between measured PDH activation and calculated PDH and TCA cycle flux during leg knee extensor exercise at 100% of the leg  $\dot{V}O_{2\max}$  was also reported (2). Muscle CHO oxidation was estimated from direct measurements of leg  $O_2$  uptake and active muscle mass and the assumption that CHO was the primary fuel for oxidation at this intensity.

The close relationship between PDH activation and flux through the PDH reaction can be divorced in situations of substrate lack. One study examined the effects of glycogen-depleting exercise followed by a 3-d high-fat/low-CHO diet on PDH activation and flux during exercise at 75%  $\dot{V}O_{2\max}$  (10). Although the activation of PDH was decreased slightly compared with the CHO-fed trial, it was much higher than the estimated flux through PDH. Because the muscle glycogen store was low after the preceding exercise and high-fat/low-CHO regimen, the provision of pyruvate was also low, decreasing PDH activation and limiting the flux through PDH.

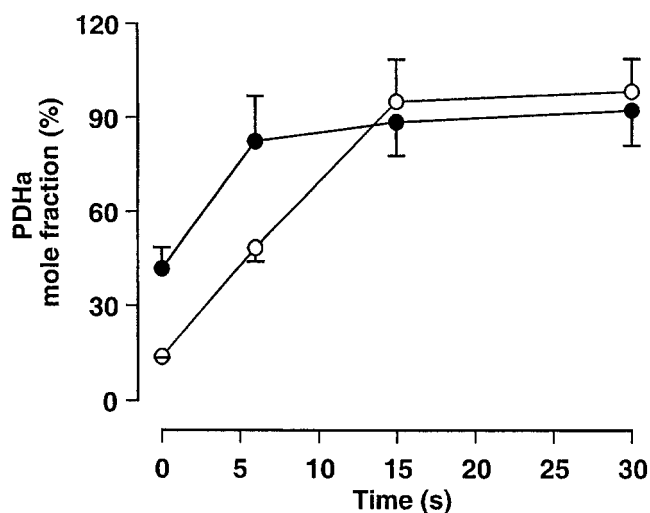
### REGULATION OF PDH ACTIVATION IN HUMAN SKELETAL MUSCLE DURING EXERCISE

At rest, high ATP/ADP, NADH/NAD, and acetyl-CoA/CoA ratios and a low pyruvate concentration maintain a high PDH kinase activity, and a low  $Ca^{2+}$  concentration keeps PDH phosphatase activity low (Fig. 2). This ensures that PDH is mainly in the inactive *b* form. At the onset of exercise, increases in  $Ca^{2+}$ , pyruvate and free ADP contribute to the activation of PDH (1,3,5,7,10).  $Ca^{2+}$  appears to be the initial and most powerful signal for PDH activation, increasing as a function of the power output to activate PDH

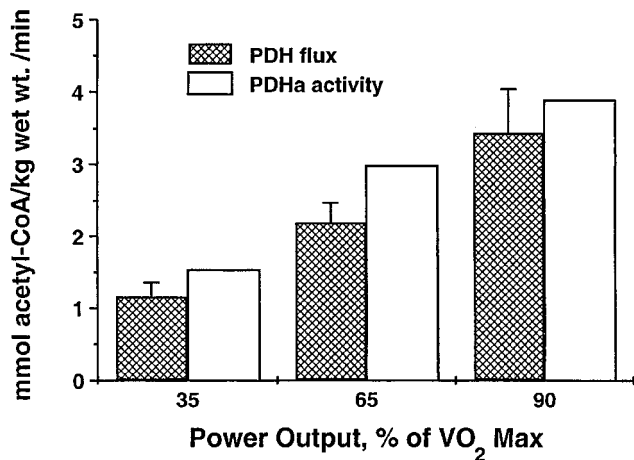
phosphatase activity. The control by  $Ca^{2+}$  is an early-warning or feed-forward system that coarsely regulates PDH activation to the intensity of exercise. Glycogenolytic/glycolytic flux also increases at the onset of exercise and the resulting increase in pyruvate inhibits PDH kinase activity to aid PDH activation and provides substrate for the reaction to increase flux. An increase in the free ADP concentration during exercise coupled with no change in the total ATP concentration also decreases the ATP/ADP ratio, which removes the normal stimulation of PDH kinase activity and contributes to the activation of PDH. The control by pyruvate and the ATP/ADP ratio work as feedback systems to fine-tune the activation of PDH to the need for ATP in contracting skeletal muscle.

If the acetyl-CoA/CoA ratio were important for increasing PDH activation and flux during exercise, a decrease in the ratio would be required to remove the activation of PDH kinase. This would also increase the substrate concentration (CoA) and decrease the product concentration (acetyl-CoA) for the reaction. However, a large increase in the acetyl-CoA/CoA ratio during exercise has been reported (1,3) and it has been argued that the activators of PDH ( $Ca^{2+}$ , pyruvate, free ADP) are far more powerful during exercise and override the inhibitory effect of acetyl-CoA to ensure PDH activation. Therefore, although the acetyl-CoA/CoA ratio appears to be a significant inhibitor of PDH kinase activity at rest, it is not important during exercise. This example of differential regulation between rest and exercise is commonly seen in the regulation of the nonequilibrium enzymes controlling ATP production in human skeletal muscle.

Lastly, the role of the redox state in the regulation of PDH activation during exercise is presently unclear given the uncertainty surrounding the methods used to estimate the NADH/NAD ratio in human skeletal muscle. We, and oth-



**Figure 4.** PDH activation (PDHa) at rest and during the first bout (open circles) and third bout (closed circles) of maximal isokinetic cycling. Mole fraction represents percentage of the enzyme in the active (a) form. (Reprinted from Parolin, M.L., A. Chesley, M.P. Matsos, L.L. Spriet, N.L. Jones, and G.J.F. Heigenhauser. Regulation of human skeletal muscle phosphorylase and pyruvate dehydrogenase during maximal intermittent exercise. *Am. J. Physiol.* 277: E890-E900, 1999. Copyright © 1999 American Physiological Society. Used with permission.)



**Figure 5.** Comparison of measured PDH activation and estimated PDH flux during cycling at various power outputs. ((Reprinted from Howlett, R.A., M.L. Parolin, D.J. Dyck, E. Hultman, N.L. Jones, G.J.F. Heigenhauser, and L.L. Spriet. Regulation of skeletal muscle glycogen phosphorylase and pyruvate dehydrogenase at different exercise power outputs. *Am. J. Physiol.* 275: R418-R425, 1998. Copyright © 1998 American Physiological Society. Used with permission.)

ers, have argued that muscle NADH concentration increases during moderate and intense aerobic exercise (5,6), a change that would increase PDH kinase activity and inhibit PDH activation. An increased NADH and decreased NAD may also inhibit PDH flux, as the reaction substrate NAD is decreased and the product NADH is increased. However, if the reaction is saturated with NAD at all times, changes in the NAD and NADH concentrations over the physiological range would not affect PDH flux and it may be that the redox couple is an important regulator only at rest. This situation already exists in the regulation of PDH with the acetyl-CoA/CoA ratio as discussed above. On the other hand, if the NADH decreases and NAD increases during moderate and intense aerobic exercise, as reported by other investigators, the changes would activate PDH and enhance flux. Unfortunately, the role of the redox couple in PDH regulation and many other mitochondrial enzymes in human skeletal muscle during exercise will not be resolved until an accepted method for the measurement of NADH is established.

In summary,  $\text{Ca}^{2+}$  initially activates PDH during exercise, as is the case with many regulatory enzymes associated with energy metabolism. Further control is provided by the energy status of the cell (ATP/ADP). An increase in the free ADP concentration at the onset of exercise, due to increased ATP turnover, plays a key role in activating oxidative phosphorylation, glycogenolysis, glycolysis, PDH, and the TCA cycle enzymes. The resulting increase in glycolytic flux also produces pyruvate, which further activates PDH and provides substrate for the reaction (Fig. 2). Collectively, these events lead to the higher CHO oxidation rates that occur with increasing exercise power outputs.

## THE ROLE OF PDH ACTIVATION AT THE ONSET OF EXERCISE

There has been controversy regarding the factors that determine the rate at which oxidative phosphorylation and

hence whole body  $\text{O}_2$  uptake increase at the onset of exercise. It has been suggested that the rate of oxidative phosphorylation may be a function of metabolic inertia, including lags in enzyme activation or substrate availability, or due to a limited  $\text{O}_2$  supply at the mitochondria in some muscle fibers. Recently, a series of studies (4,8,13) have tested the hypothesis that the rate of PDH activation (substrate availability) is important for determining how quickly aerobic ATP production begins at the onset of exercise. The administration of the pharmacological agent, dichloroacetate (DCA), maximally activated PDH during the rest period before exercise such that flux through PDH was greatly increased in the initial seconds of exercise. The contracting muscle relied more on oxidative phosphorylation and less on substrate level phosphorylation (anaerobic ATP production) from phosphocreatine degradation and glycogenolytic/glycolytic activity (decreased lactate production). The results suggested that substrate availability was partially responsible for the rate at which aerobic ATP production was activated at the onset of moderate-intensity aerobic exercise (e.g., ~65%  $\text{VO}_2$ max). In hypoxia, a situation in which  $\text{O}_2$  supply is traditionally thought to be limiting, PDH activation was delayed in the initial minute of exercise, resulting in greater lactate formation (8). When DCA was administered to activate PDH before exercise in hypoxia, oxidative phosphorylation was increased and lactate production was reduced. This suggested that substrate delivery through the PDH reaction at the onset of exercise was important even in a hypoxic environment.

## ALTERED FUEL STATES AND PDH REGULATION

The regulation of PDH activation during exercise has also been studied in a variety of altered fuel states. When the availability of free fatty acids was acutely increased via the infusion of a triacylglycerol and heparin solution, fat oxidation was increased and CHO oxidation and PDH activation were decreased, during low- and moderate-intensity exercise (6). The decreased PDH activation at the onset of exercise was attributed to a higher mitochondrial NADH level at rest and early in exercise and a decreased pyruvate concentration at rest. Using the opposite approach, increasing the availability of CHO during moderate exercise with the infusion of epinephrine increased both PDH activation and CHO oxidation (15). No changes in the conventional regulators of PDH (e.g., pyruvate) were reported to explain the increased activation, although the production of pyruvate was clearly increased. The authors speculated that epinephrine might have a currently unknown affect on PDH that is not mediated through the glycolytic pathway.

As discussed above, 3 days on a high-fat/low-CHO diet after glycogen-depleting exercise decreased PDH activation and flux during exercise (10). This effect was related to the availability of pyruvate, as the same dietary protocol without the glycogen-depleting exercise restored PDH activation and flux during moderate-intensity exercise (12). The restoration of PDH activation and flux occurred despite stable increases in the maximal activity of PDH kinase (9). The combination of dietary manipulation and acute exercise is complicated by



mechanisms related to longer-term regulation of the PDH complex. For instance, increases in insulin concentration postprandial are believed to activate PDH phosphatase over a longer time course than does exercise (minutes to hours vs seconds). In addition, situations of CHO deprivation lead to longer-term upregulation of PDH kinase activity (9). Lastly, little is known regarding the effects of increasing or decreasing physical activity (aerobic training and detraining) on the regulation of the PDH complex. Short-term (6-d) training has shown that PDH activation is reduced at the same absolute power output posttraining in concert with decreased CHO oxidation during submaximal exercise (11). To date, no studies have examined the effects of longer-term aerobic training on PDH regulation in human skeletal muscle. It is predicted that longer-term training would increase total PDH protein and maximal PDH activity and therefore allow for increased CHO oxidation at higher power outputs. There may also be other longer-term changes associated with the regulatory enzymes, PDH kinase, and PDH phosphatase.

## SUMMARY

In summary, PDH regulates CHO oxidation via its entry into the TCA cycle. PDH activation during exercise in human skeletal muscle is proportional to the relative aerobic power output and correlates with estimates of CHO oxidation. During exercise,  $\text{Ca}^{2+}$  initially activates PDH, as is the case with many regulatory enzymes associated with energy metabolism. Further control is provided by decreases in the energy status of the cell (ATP/free ADP). The increased ATP turnover at the onset of exercise increases free ADP, which assists in activating oxidative phosphorylation, glycogenolysis, glycolysis, PDH, and the TCA cycle enzymes. The resulting increase in glycolytic flux also produces pyruvate, which further activates PDH and provides substrate for the reaction. Together, these events activate PDH and explain the increase in CHO oxidation with increasing power outputs.

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## References

1. Constantin-Teodosiu, D., J.I. Carlin, G. Cederblad, R.C. Harris, and E. Hultman. Acetyl group accumulation and pyruvate dehydrogenase activity in human muscle during incremental exercise. *Acta. Physiol. Scand.* 143:367–372, 1991.
2. Gibala, M.J., D.A. MacLean, T.E. Graham, and B. Saltin. Tricarboxylic acid cycle intermediate pool size and estimated cycle flux in human muscle during exercise. *Am. J. Physiol.* 275:E235–E242, 1998.
3. Howlett, R.A., M.L. Parolin, D.J. Dyck, E. Hultman, N.L. Jones, G.J.F. Heigenhauser, and L.L. Spriet. Regulation of skeletal muscle glycogen phosphorylase and pyruvate dehydrogenase at different exercise power outputs. *Am. J. Physiol.* 275:R418–R425, 1998.
4. Howlett, R.A., G.J.F. Heigenhauser, E. Hultman, M.G. Hollidge-Horvat, and L.L. Spriet. Effects of dichloroacetate infusion on human skeletal muscle metabolism at the onset of exercise. *Am. J. Physiol.* 277:E18–E25, 1998.
5. Hultman, E. Pyruvate dehydrogenase as a regulator of substrate utilization in skeletal muscle. In: *Biochemistry of Exercise IX*, edited by R.J. Maughan and S.M. Shirreffs. Champaign, IL: Human Kinetics, 1996, p. 157–172.
6. Odland, L.M., G.J.F. Heigenhauser, and L.L. Spriet. Effects of high fat availability on muscle PDH activation and malonyl-CoA content during moderate exercise. *J. Appl. Physiol.* 89:2352–2358, 2000.
7. Parolin, M.L., A. Chesley, M.P. Matsos, L.L. Spriet, N.L. Jones, and G.J.F. Heigenhauser. Regulation of human skeletal muscle phosphorylase and pyruvate dehydrogenase during maximal intermittent exercise. *Am. J. Physiol.* 277:E890–E900, 1999.
8. Parolin, M.L., L.L. Spriet, E. Hultman, M.G. Hollidge-Horvat, N.L. Jones, and G.J.F. Heigenhauser. Effects of PDH activation by dichloroacetate in human skeletal muscle metabolism during exercise in hypoxia. *Am. J. Physiol.* 279:E752–E761, 2000.
9. Peters, S.J., T.A. St. Amand, R.A. Howlett, G.J.F. Heigenhauser, and L.L. Spriet. Human skeletal muscle pyruvate dehydrogenase kinase activity increases following a low carbohydrate diet. *Am. J. Physiol.* 276:E980–E986, 1998.
10. Putman, C.T., L.L. Spriet, E. Hultman, M.I. Lindinger, L.C. Lands, R.S. McKelvie, G. Cederblad, N.L. Jones, and G.J.F. Heigenhauser. Pyruvate dehydrogenase activity and acetyl group accumulation during exercise after different diets. *Am. J. Physiol.* 265:E752–E760, 1993.
11. Putman, C.T., N.L. Jones, E. Hultman, M.G. Hollidge-Horvat, A. Bonen, D.R. McConachie, and G.J.F. Heigenhauser. Effects of short-term submaximal training in humans on muscle metabolism in exercise. *Am. J. Physiol.* 275:E132–E135, 1998.
12. St. Amand, T.A., L.L. Spriet, N.L. Jones, and G.J.F. Heigenhauser. Pyruvate overrides inhibition of PDH during exercise following a low carbohydrate diet. *Am. J. Physiol.* 279:E275–E283, 2000.
13. Timmons, J.A., T. Gustafsson, C.J. Sundberg, E. Jansson, and P.L. Greenhaff. Muscle acetyl group availability is a major determinant of oxygen deficit in humans during submaximal exercise. *Am. J. Physiol.* 274:E377–E380, 1998.
14. Ward, G.R., J.R. Sutton, N.L. Jones, and C.J. Toews. Activation by exercise of human skeletal muscle pyruvate dehydrogenase in vivo. *Clin. Sci.* 63:87–92, 1982.
15. Watt, M., K.F. Howlett, M.A. Febbraio, L.L. Spriet, and M. Hargreaves. Adrenaline increases skeletal muscle glycogenolysis, PDH activation and carbohydrate oxidation during moderate exercise in humans. *J. Physiol.* 534:1: 269–278, 2001.