Regulation of PDH activity and isoform expression: diet and exercise

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Abstract
During exercise in human skeletal muscle, the proportion of carbohydrate derived acetyl-CoA is determined at least in part by the activity of the PDH (pyruvate dehydrogenase) complex. Inhibition of the complex is achieved through reversible phosphorylation of the E1 subunit by a family of PDH kinase isoforms (PDK1–4) while dephosphorylation and activation of the complex is catalysed by a pair of intrinsic PDH phosphatases (PDP1 and 2). In general, the relative activity of the kinases and phosphatases is determined by a host of intramitochondrial effectors which signal energy charge, substrate and product accumulation, muscle contraction and nutritional status. This review focuses on advances in our understanding in human skeletal muscle of the regulatory signals and changes in gene expression which are important during acute exercise and exercise training, as well as in prolonged situations of altered nutritional status.

Introduction
Carbohydrate and fat are the predominant fuels used to produce ATP in skeletal muscle at rest and during exercise. However, the complex regulation that determines the partitioning of these two fuels is different depending on the metabolic demand. PDH (pyruvate dehydrogenase) plays a pivotal role in determining the proportion of acetyl-CoA that is derived from carbohydrate sources, thereby regulating the flux rate of carbohydrate oxidation. The earliest studies delineated the classical regulation of the PDH complex by reversible phosphorylation, catalysed by an intrinsic phosphatase and kinase [1]. Shifting reliance from carbohydrate to fat oxidation was achieved through modulation of the relative kinase and phosphatase activities by intramitochondrial effectors which were linked to the metabolic state of the resting cell [2]. However, recent work has questioned the importance of some of these regulators during exercise in human skeletal muscle. This review will take a historical perspective, beginning 10 years ago with the regulation of PDH during exercise following a high-fat, LC (low-carbohydrate) diet. The intramitochondrial effectors which are considered important during exercise of different intensities will be highlighted, as well as recent advances in gene expression of the enzymes of the complex including the PDK (PDH kinase) isoforms.

Regulation of PDH
PDH is a multi-enzyme complex which catalyses the irreversible oxidative decarboxylation of pyruvate to acetyl-CoA. The E1 subunit has three known phosphorylation sites, with the first site being necessary for inactivation of the complex, and the other two sites acting as barrier sites to hinder phosphatase activation [3]. Phosphorylation of the complex is catalysed by a family of four PDKs (PDK1–4) which differ in their responsiveness to allosteric inhibition by pyruvate or allosteric activation by energy charge (ATP/ADP ratio), redox (NADH/NAD+ ratio), and acetyl-CoA to free CoA ratios (as reviewed by Sugden and Holness [4]). In addition, the kinases differ in their specificity for the different phosphorylation sites (as reviewed in [4]). Therefore, the relative activities of the kinase isoform population will determine the response of the complex in acute situations. An intrinsic pair of PDH phosphatases (PDP1 and 2) catalyse the activation of the PDH complex to its active form (PDHa) [5]. PDP1 is the phosphatase which is activated in the presence of increased Ca2+ ions, while PDP2 is activated when insulin levels are increased [5]. In addition to these regulators and intramitochondrial effectors, it has been suggested that the kinases and phosphatases have different pH optima, and therefore increasing intramitochondrial concentrations of H+ ion may modulate the activity of the complex by decreasing kinase activity and simultaneously increasing phosphatase activity [6].

In addition to the importance of acute regulation in the first few seconds or minutes, a long-term or chronic regulation has been documented under prolonged reliance on fat metabolism (e.g. hours to days). This chronic regulation is characterized by a ‘stable’ increase in PDK activity which persists in the absence of acute intramitochondrial regulators, and results in increased phosphorylation and inactivation of the complex ([7], and as reviewed in [4]). However, until recently the relative importance of these regulatory mechanisms had not been studied in human skeletal muscle, or during exercise.

Diet and regulation of PDH during exercise
Although the reversible phosphorylation of the PDH complex was delineated decades ago, it was many years before
regulation of the complex was examined during exercise in human skeletal muscle. Early studies documented activation of the PDH enzyme with low-intensity and heavy aerobic exercise, but the exact mechanism of its complex regulation was poorly understood [8–10]. Then, 10 years ago, Putman and co-workers [11] undertook a landmark study to examine the importance of acetyl group accumulation in PDH regulation in human skeletal muscle. In this study, LC and HC (high-carbohydrate) diets were used as a perturbation to shift reliance from the two extremes, either towards fat or towards carbohydrate. Subjects completed muscle-glycogen-depleting exercise and then consumed for 3 days either a LC diet (<3% energy from carbohydrate) or a HC diet (86% carbohydrate), followed by exercise at 75% of their $\dot{V}O_{2\text{max}}$. At rest, results showed a 3-fold higher acetyl-CoA/CoA ratio, which resulted in lower PDHa activity as classically defined (Figure 1). However, during exercise the muscle acetyl-CoA concentrations dropped following the LC diet and increased following the HC diet, while free CoA remained unaltered between the conditions. In spite of the observation that acetyl-CoA/CoA ratio decreased in the LC condition and increased in the HC condition, PDHa activation was observed in both trials, but was attenuated following the LC condition (Figure 1). Therefore, although the accumulation of acetyl groups appeared to be important in inhibiting the complex at rest, the decreased activation of the complex during exercise after the LC diet was completely opposite to what would be expected based on changes in acetyl-CoA concentration. This was an important study which raised many questions and prompted a rich body of work to explore more fully the regulation of carbohydrate oxidation by dietary factors and during exercise. First, this study really questioned the importance of the classical acute regulators during exercise and preceded a number of studies aimed at delineating the mechanism(s) responsible during exercise. Second, this was the first study to suggest that, independent of power output, PDHa activation was equivalent to oxidative flux of carbohydrates. Finally, an explanation was required for the attenuated increase in PDHa activity during exercise after the LC diet, and led to studies exploring both chronic or adaptive regulation as well as acute regulation.

Adaptive regulation of PDH by diet

In an effort to explain the blunted increase in PDHa activation following a high-fat LC diet, adaptive regulation of the complex in human skeletal muscle was examined. As previously observed in rodent models [7,12], following 28 days, there was a 3–5-fold increase in PDK activity during exercise after the LC diet, which increased linearly over the 6 days [13,14]. As observed previously in rat skeletal muscle during a high-fat...
diet [15], this increase in PDK activity was dependent on the composition of the dietary fat, and was attenuated when the high-fat diet was enriched with long-chain \( \omega-3 \) fatty acids [16]. In the first 24 h, the increase in PDK activity could be explained by increased PDK4 isoform protein [14]. However, PDK4 protein concentrations were maximal in 24 h and did not increase further during the 3-day diet, while PDK2 protein was unchanged throughout the diet period (Figure 2). Therefore, the mechanisms for increased PDK activity still remain unclear. Although the possibility exists that increased PDK1 or PDK3 protein could contribute to the ongoing increase in activity, recent work using real-time quantification of PDK isoform mRNA has indicated that concentrations of the PDK1 and PDK3 transcripts in skeletal muscle were only a fraction of the predominant PDK2 isoform concentration (PDK4, PDK1 and PDK3 were 15, 1 and 1% of PDK2 respectively) [17]. In addition, PDK1 and PDK3 remained unchanged during starvation in human skeletal muscle, suggesting that the genes are not induced with dietary perturbations [17]. If increased protein synthesis of the PDK isoforms cannot explain the linear increase in PDK activity after the first 24 h, then there must be a stable increase in specific activity of the existing kinases which requires further study. Evidence from in vitro studies has indicated that association of PDK2 and PDK3 with the lipoyl residues of the E2 core of the complex increased intrinsic activity (as reviewed by Roche et al. [18]). However, whether this association with the core would happen in a stable manner in vivo has yet to be examined.

Recently, the changes in gene expression which occur during re-feeding of either carbohydrate or fat following a 20 h fast have been examined in human skeletal muscle. Although there was a 9-fold increase in PDK4 transcription rate following the 20 h fast, re-feeding either a HC meal or a high-fat meal resulted in a 60–80-fold increase in PDK4 gene transcription. This unexpected result suggested that PDK4 is a highly responsive gene, and in many situations regulation must be post-transcriptional [19]. Recent results from our laboratory have demonstrated that maximal PDK activity is decreased less than 1 h after re-feeding of carbohydrates following a 6-day high-fat LC diet, and completely returns to baseline in 3 h [19a]. Taken together, these data highlight the fact that a stable increase or decrease in maximal PDK activity is not necessarily related to PDK isoform gene expression.

The role of glycolytic flux and pyruvate

Although chronic regulation of PDH activity through adaptive increase in PDK activity appeared to be important, the acute regulation of the complex by glycolytic flux during exercise following a high-fat LC diet could not be ignored. A confounding factor in the Putman diet study was the 3-fold difference in muscle glycogen contents between the low and HC conditions, which resulted in very different glycogenolytic rates (\( \approx 9.5 \) compared with 3.5 mmol/min per kg of dry weight in HC compared with the LC diet) [11]. To further explore the importance of pyruvate concentration in regulation of the PDH complex, the diet study was repeated without depleting the glycogen prior to the LC diet [20]. Subjects were instructed to refrain from any intense exercise in an effort to conserve muscle glycogen stores. Although glycogen stores were still slightly lower following the LC diet (\( \approx 300 \) compared with 500 mmol of glycose units/kg of dry weight), during exercise at 65% \( \dot{V}O_2\text{max} \) the glycolytic flux and resulting muscle pyruvate concentrations were not different between the conditions. Similarly, the increase in PDHa activity was not different between the conditions, in spite of the 3–5-fold increase in PDK activity previously observed by Peters et al. [13,14]. Therefore, although there is an adaptive increase in PDK activity in response to a high-fat LC diet, this can be overridden during exercise as long as there is sufficient glycolytic flux and increased pyruvate concentrations.
Regulation of PDH during exercise

Submaximal exercise at different intensities
Earlier work had determined that PDH activation was correlated with exercise intensity and was independent of increasing acetyl group accumulation during incremental exercise [8]. However, the relative importance of other acute regulators had not been established at different power outputs. Howlett et al. [21] chose three representative power outputs. At 30% VO\textsubscript{2max}, most of the energy produced is derived from plasma glucose and free fatty acids. During exercise at 65% VO\textsubscript{2max} glycogen utilization increases, but the muscle still relies heavily on fat metabolism, while at 90% VO\textsubscript{2max} glycolysis provides almost all of the energy to support the high power output [22]. These authors observed a stepwise increase in PDH activation in the first 10 min of exercise, which correlated with power output (Figure 3A). Furthermore, maximal PDHs matched exactly with calculated flux rates of pyruvate oxidation for the three power outputs (Figure 3B). In good agreement with previous work, this incremental increase in PDH activity occurred independent of incremental increases in acetyl-CoA concentration which would be expected to inactivate the complex [8,11]. However, calculated changes in free ADP (and AMP) correlated very well with PDH activation (Figure 3C). The authors concluded that while increasing intramitochondrial Ca\textsuperscript{2+} concentration can stimulate the Ca\textsuperscript{2+}-sensitive PDH phosphatase to incrementally activate PDH at increasing exercise workloads, other factors may have been important in modulating PDH activity to match oxidative flux through the enzyme. With the stepwise increase in power output, the increasing free ADP down-regulated kinase activity, while higher pyruvate concentration (due to increased glycolytic flux) increased PDH activation and provided more substrate for flux through the enzyme.

Watt and co-workers [23] observed an 18% increase in carbohydrate oxidation during 20 min of moderate exercise (≈58% VO\textsubscript{2max}) in response to adrenaline infusion compared with the control. This increase was accounted for by increased glycogenolytic flux and ≈14% increase in PDH activation. The increase in PDH activity was not mediated by changes in energy charge or concentrations of muscle pyruvate which were not different between the control and adrenaline trials. This novel finding suggested that adrenaline may play a role in regulation of PDH activity, similar to that observed in perfused rat heart [24]. Although further work is needed to confirm this possibility, it is possible that adrenaline exerts its action through changes in intramitochondrial Ca\textsuperscript{2+} content [25].

Intermittent sprint exercise
Parolin and co-workers [26] examined the time course of regulation of PDH during three repetitive 30-s bouts of maximal isokinetic cycling sprints separated by 4-min rest periods. This study compared the first with the final bout of exercise for PDH regulation from rest to 6 s, 6–15 s and 15–30 s.
During bout 1, PDHa activity increased from 0.53 to 1.81 mmol/min per kg of muscle wet weight (ww) in the first 6 s, and was fully activated by 15 s (3.56 mmol/min per kg of ww). Activity was maintained for the duration of the 30-s sprint exercise, ending at 3.74 mmol/min per kg of ww (which represented 95.2% of the total PDH activity). Although the primary stimulus for activation for contraction was probably elevated levels of intramitochondrial Ca$^{2+}$, the increase in PDHa activity during the sprint correlated with increasing concentrations of H$^+$ and pyruvate. Prior to bout 3 of maximal cycling, resting PDH had ≈3-fold higher than rest for bout 1. During the sprint in bout 3, PDHa activation was accelerated in bout 3 compared with bout 1, achieving maximum activation by 6 s and remaining high for the duration of the 30-s sprint. The higher PDH activity prior to bout 3 could be explained by elevated pyruvate and H$^+$ concentrations at rest, which would override the higher acetyl-CoA accumulation. However, pyruvate concentration did not change throughout bout 3, and therefore could not contribute to the higher PDHa activation at 6 s in bout 3 compared with bout 1. Free ADP and AMP levels were also not involved at 6 s, since they were not different at this time point between the bouts. However, H$^+$ was higher in the rest to 6-s transition during bout 3, suggesting that decreased intramitochondrial pH could enhance the activity of the phosphatase in this early phase of maximal activity, and accelerate PDHa activation.

Controversy surrounds the role of intramitochondrial redox state on PDHa activation during maximal intermittent sprint exercise; however Putman et al. [27] estimated mitochondrial NADH and NAD$^+$ concentrations using the equilibrium constant for the glutamate dehydrogenase reaction. They observed a decreased NADH/NAD$^+$ ratio during bouts 1 and 2, with the ratio remaining low throughout bout 3. The authors concluded that although Ca$^{2+}$ appeared to be the major stimulus in PDHa activation during the successive sprints, decreased NADH played a supportive role in activation through withdrawal of kinase stimulation [27].

These studies clearly described a shift from anaerobic to aerobic energy production mediated both within a single bout, and throughout successive bouts of what is normally considered ‘anaerobic’ exercise. This shift in ATP production from substrate phosphorylation (glycolysis) to increasing ATP produced from oxidative phosphorylation is mediated in part through increased activation of PDHa. Important regulators were Ca$^{2+}$ and energy charge, with H$^+$ and NADH playing supportive roles.

In another maximal intermittent sprint study, Putman et al. [28] examined the regulation of PDH in the inactive deltoid muscle. Inactive muscle plays an important role in clearing plasma lactate to buffer the large lactate efflux from exercising muscle. The protocol for this study employed the same 30-s isokinetic sprint exercise: three bouts separated by 4-min rest periods. In spite of the fact that there was no contractile activity during the sprints in the deltoid muscle (which might involve changes in Ca$^{2+}$ or energy charge), PDHa activity increased 2.6-fold during bout 3, and remained high throughout the 15-min recovery. Acetyl-CoA was unchanged in the inactive muscle during the sprints, but increased lactate concentrations (reflecting lactate uptake by the muscle) and increased pyruvate concentrations probably contributed to the increase in PDHa activity. In addition, H$^+$ may have accelerated activation of the complex as in the previous study [26]. PDHa activity was comparable or slightly higher in the previously active muscle prior to bout 3 in the Parolin study [26]. This might be explained by the fact that there were no changes in the putative regulators (e.g. acetyl-CoA/CoA ratio) in the inactive muscle compared with the previously active muscle in the first study. Although inactive muscle had lower concentrations of some positive regulators (e.g. Ca$^{2+}$, energy charge), there was little opposition to the activation of the complex by pyruvate and H$^+$, due to the low acetyl-CoA/CoA ratio. Increased PDHa activity observed during the high-intensity sprint exercise supports an important role for inactive muscle in disposal and oxidation of lactate produced both during high-intensity sprint exercise and during recovery.

**Prolonged (4 h) moderate-intensity exercise**

During prolonged exercise at a moderate intensity (50–55% $\dot{V}$O$_{2\text{max}}$), there is a progressive shift in reliance from carbohydrate metabolism to fat metabolism [22,29]. The regulation of this shift in fuel utilization had been studied at several exercise intensities and durations, but there was very little information regarding the relative proportion of fat and carbohydrate utilization during exercise lasting more than 2 h. Recently, Watt and co-workers [30] examined PDHa activation during 4 h of cycling exercise at 55% $\dot{V}$O$_{2\text{max}}$ in endurance-trained cyclists. Calculating whole-body oxidation of fat and carbohydrate from the respiratory exchange ratio confirmed a gradual shift to increased reliance on fat, with fat oxidation exceeding carbohydrate oxidation by 2.5 h and remaining high until the end of the exercise. PDH activity increased from ≈0.9 at rest to ≈2.4 mmol/min per kg of ww by 10 min. PDHa activity remained elevated at 2 h, but was reduced to ≈1.7 mmol/min per kg of ww at the end of 4 h of exercise. The exact mechanism(s) regulating the decrease in PDHa activation at the end of exercise remain unclear, although the authors put forth several hypotheses. While energy charge and acetyl-CoA concentrations were unchanged from 2 to 4 h, evidence from rodent models suggests that uptake of Ca$^{2+}$ by mitochondria is reduced during exhaustive exercise and this could have contributed to the attenuation in PDHa activity. Although no change in whole muscle pyruvate was observed at 4 h of exercise compared with 2 h, calculated glycolytic flux was marginally reduced, and could have contributed to the attenuation in PDH activity. In addition, the authors suggested that there could be up-regulation of gene expression during the 4 h of exercise, and therefore the contribution of increased PDK gene expression and activity needed to be explored.

Pilegaard et al. [31] demonstrated increased PDK4 gene transcription and mRNA content in human skeletal muscle following 4 h of cycling exercise at 50–60%...
Vo2max, suggesting that increased synthesis of PDK4 could increase inactivation of the complex late in the prolonged exercise. However, recent results from our laboratory have demonstrated that PDK4 and PDK2 protein were unchanged throughout 4 h of moderate cycling exercise [32]. This means that the increase in PDK4 mRNA observed was not translated into protein during that time period. However, there was a 2-fold increase in maximal PDK activity even in the absence of a change in PDK4 protein, and this may play a role in decreasing PDHa activity during the 4-h exercise. This provides another example of dissociation between PDK activity and gene expression, suggesting that the specific activities of the existing kinases are up-regulated. It is clear that our understanding of the regulation of PDH activity during prolonged exercise cannot be restricted to studies of gene expression, and further work will be required to fully elucidate the mechanism(s) responsible for decreased PDHa activity following 4 h of moderate exercise.

Effect of training

With aerobic training, skeletal muscle develops an increased oxidative potential, and the result is a better match between energy demand and ATP production. This, in turn, translates into decreased glycolytic flux and pyruvate production, mediated by decreased activation of glycogen phosphorylase by ADP and AMP. Following short-term training (7–8 days) decreased pyruvate production was observed, along with decreased pyruvate oxidation through PDH, although PDH activation was unchanged during submaximal exercise [33]. However, when the duration of the training was extended to 7 weeks, PDH activation was decreased by 27% after 15 min of exercise at 75% Vo2max [34]. Of the measured PDH regulators, only pyruvate and ATP/ADP ratio changed as a result of training which would contribute to increased activation of the complex through kinase inactivation, while acetyl-CoA was unaltered by training. Recent results have demonstrated that prolonged aerobic training increased PDK activity, as well as total PDH activity [35]. The increase in PDK activity is the result of increased PDK2, as well as PDK4 protein [35]. These studies contribute to our understanding of the shift following exercise training towards increased oxidative energy production, and the regulation of carbohydrate oxidation through PDH.

Summary

In general, PDHa activity increases proportionally to the power output during exercise and correlates very well with flux through the enzyme. Increased intramitochondrial Ca2+ concentrations are the initial feed-forward stimulus for activation, followed by finer modulation (co-ordination) with glycolytic flux) through changes in energy charge and pyruvate. Adrenaline (epinephrine) appears to increase PDH activation without changes in whole muscle pyruvate concentration, but the exact mechanism responsible requires further study. During intense sprinting exercise, increased intramitochondrial H+ ion correlates well with changes in activity and is likely to be responsible for modulation of the relative activities of the kinases and phosphatases. In general, increased PDHa activity is independent of changes in the acetyl-CoA/CoA ratio, and the role of NADH in regulation during exercise is unclear, but it appears to support activation of the complex during maximal exercise. During training, there is progressive shift in favour of fat oxidation, and this is mediated through decreased PDHα activity in the long term. A long-term ‘stable’ increase in PDK activity is observed during fasting, high-fat diet, and prolonged exercise, but the higher activity can only be partially explained by changes in PDK4 gene expression, indicating that the activity of the existing kinases may be up-regulated.

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References


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