The relationship between human skeletal muscle pyruvate dehydrogenase phosphatase activity and muscle aerobic capacity

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Love LK, LeBlanc PJ, Inglis JG, Bradley NS, Chotipany J, Heigenhauer GJ, Peters SJ. The relationship between human skeletal muscle pyruvate dehydrogenase phosphatase activity and muscle aerobic capacity. J Appl Physiol 111: 427–434, 2011. First published May 19, 2011; doi:10.1152/japplphysiol.00672.2010.—Pyruvate dehydrogenase (PDH) is a mitochondrial enzyme responsible for regulating the conversion of pyruvate to acetyl-CoA for use in the tricarboxylic acid cycle. PDH is regulated through phosphorylation and inactivation by PDH kinase (PDK) and dephosphorylation and activation by PDH phosphatase (PDP). The effect of endurance training on PDK in humans has been investigated; however, to date no study has examined the effect of endurance training on PDP in humans. Therefore, the purpose of this study was to examine differences in PDP activity and PDP1 protein content in human skeletal muscle across a range of muscle aerobic capacities. This association is important as higher PDP activity and protein content will allow for increased activation of PDH, and carbohydrate oxidation. The main findings of this study were that 1) PDP activity ($r^2 = 0.399, P = 0.001$) and PDP1 protein expression ($r^2 = 0.153, P = 0.039$) were positively correlated with citrate synthase (CS) activity as a marker for muscle aerobic capacity; 2) E1α ($r^2 = 0.310, P = 0.002$) and PDK2 protein ($r^2 = 0.229, P = 0.012$) are positively correlated with muscle CS activity; and 3) although it is the most abundant isoform, PDP1 protein content only explained ~18% of the variance in PDP activity ($r^2 = 0.184, P = 0.033$). In addition, PDP1 in combination with E1α explained ~38% of the variance in PDP activity ($r^2 = 0.383, P = 0.005$), suggesting that there may be alternative regulatory mechanisms of this enzyme other than protein content. These data suggest that with higher muscle aerobic capacity (CS activity) there is a greater capacity for carbohydrate oxidation (E1α), in concert with higher potential for PDH activation (PDP activity).

carbohydrate oxidation; PDH; PDP1; E1α; E2; PDK2

PYRUVATE DEHYDROGENASE (PDH) is a multienzyme complex consisting of multiple copies of E1 (α and β), E2 (the core of the PDH complex), and E3 subunits, along with an E3 binding protein (E3BP), which serves to bind E3 to the complex (as reviewed by 1, 39). Each of these subunits (with the exception of E3BP) is involved in the conversion of pyruvate to acetyl-CoA in a stepwise manner. The complex is regulated largely via covalent modification by the addition of a phosphate group to at least one of its three serine residues located on the E1α subunit of the complex (15, 31, 33, 34, 37). Phosphorylation and inactivation are accomplished by a group of specific PDH kinases (PDK1–4), while dephosphorylation and activation are accomplished by a pair of PDH phosphatases (PDP1 and -2; Refs. 21, 34, 37). Each of these regulatory enzyme isoforms has different specificities and tissue expressions, with PDK2 and PDP1 being the most abundant isoforms in skeletal muscle (3, 11). Both isoforms of PDP require Mg2+ for activation, but PDP1 is stimulated by Ca2+ (16), while PDP2 is Ca2+ insensitive and may instead respond to insulin (11). The activity of these regulatory enzymes in skeletal muscle is of particular importance as this tissue represents a major site for glucose disposal in the body.

Recent research has focused on the chronic regulation of PDH, with the vast amount of this literature focusing on nutritional regulation of PDK, with little research available on the changes in PDP activity with endurance training. It has been found that in states of restricted carbohydrate availability there is an increase in skeletal muscle PDH activity (5, 6, 26, 27, 28) and PDK4 protein content (26, 27, 36), while similar nutritional states also cause a decrease in PDP2 protein content in rat heart and kidney (12) and skeletal muscle, with this decrease leading to a decrease in PDP activity (17). As a result of these adaptations there is an overall decrease in the active form of PDH (PDHa; 6, 28, 30), allowing for the conservation of carbohydrates, which is essential during these carbohydrate-deficient perturbations.

The regulation of the PDH complex is pivotal in determining the partitioning of carbohydrate compared with fat oxidation. Many models have been used to perturb this partitioning, such as starvation (12, 17, 26, 36), and a high-fat diet (27, 28, 30). One such perturbation previously used has been endurance training (17, 19, 20), which in general causes a shift toward increased fat oxidation and decreased carbohydrate utilization. During submaximal exercise following aerobic training, the body adapts to rely more heavily on fat as a fuel for the production of ATP, thereby decreasing the need for pyruvate production and carbohydrate oxidation. Previous studies have shown that training results in an attenuated activation of PDH during submaximal exercise in human (18) and rat skeletal muscle (23, 24), with this attenuation appearing to be at least in part due to increased PDK activity, and increased PDK2 protein content (20).

However, 8 wk of aerobic training has also been found to increase total PDH activity and E1α protein expression (20). This increase facilitates higher capacity for carbohydrate oxidation generally required during maximal exercise and high ATP turnover rates. In the only previous study to investigate the effect of training on PDP it was shown that PDP activity increased following 8 wk of aerobic training in skeletal muscle of obese rats, with a trend for an increase in lean rats (19). It...
was also found that PDP1 protein expression increased with aerobic training in soleus and red gastrocnemius of obese rats (19). Therefore, during maximal exercise, this upregulation in PDP activity and PDP1 protein would result in increased pyruvate oxidation and ATP production by offsetting the increase in PDK activity and PDK2 protein content, allowing for increased activation of the PDH complex.

Given the absence of explicit information in the literature, the aim of this study is to examine human skeletal muscle PDP activity and protein content and how it correlates with muscle aerobic capacity. It is expected that maximal PDP activity and PDP1 protein content will be higher in individuals who possess a higher muscle aerobic capacity. Since only one longitudinal study has been done in humans (males only) to investigate the effects of training on the various PDH components (including E1α, E2, E3 binding protein, and PDK2), our study will also act to extend the work of this previous study to both males and females. Specifically, we expected that E1α and PDK2 protein contents would positively correlate with measures of muscle aerobic capacity, and E2 would be unchanged.

METHODS

Subjects. Male (n = 18) and female (n = 12) subjects volunteered for this study (at McMaster University; Hamilton, ON). All subjects were healthy nonsmokers. This research project was given human research ethics approval from both McMaster University and Brock University, and was in accordance with the Declaration of Helsinki. All subjects provided written informed consent to participate in the study.

Experimental protocol. Subjects reported to the laboratory on three occasions. In the first meeting the study was explained, and the subjects filled out a questionnaire regarding current health status and submitted a detailed description of their physical activity levels over the past few months. During the second visit subjects were screened using their peak oxygen consumption (V\text{\textsubscript{O}2peak}) to be sure that the sample had a wide range of aerobic capacities represented. V\text{\textsubscript{O}2peak} was measured on an electromagnetically braked cycle ergometer (Lode Excalibur, Quinton Instruments, Seattle, WA) using a metabolic measurement system (Quinton Q-Plex 2, Quinton Instruments) following a protocol with incremental workloads to maximal exercise. At least 1 wk later subjects reported back to the laboratory to have two resting muscle biopsies performed on the vastus lateralis muscle with the first placed in buffer at 4°C for mitochondrial extraction and the second immediately frozen in liquid nitrogen for later analysis of citrate synthase activity and Western blotting for specific proteins. Subjects refrained from alcohol, caffeine, and exercise for at least 24 h before the biopsies and consumed their habitual diet in that time period to ensure that all subjects had an equal baseline for measurement. Their last habitual meal was consumed approximately 3–5 h before the biopsies were done to ensure that insulin levels were at baseline and therefore would not interfere with PDP activity.

Mitochondrial extraction. Mitochondria were isolated from the first muscle biopsy by differential centrifugation, following a method originally described by Makinen and Lee (22) and modified by Jackman and Willis (13), which has been used previously in our lab (17, 19, 20, 27, 28), with the subsarcolemmal mitochondria being primarily targeted for isolation. Briefly, −75 mg muscle was minced and homogenized on ice by hand in 20 volumes of solution 1 (100 mM KCl, 40 mM Tris-HCl, 10 mM Tris base, 5 mM MgSO\textsubscript{4}, 5 mM Na\textsubscript{2}EDTA, 1 mM ATP, pH 7.5). The sample was then centrifuged at 700 g for 10 min at 4°C to pellet out contractile proteins, large membranes, and other cellular debris. The supernatant was retained and spun at 14,000 g for 10 min at 4°C to pellet out the mitochondria after which the supernatant was discarded and the pellet resuspended in 10 volumes of solution 2 (100 mM KCl, 40 mM Tris-HCl, 10 mM Tris base, 1 mM MgSO\textsubscript{4}, 0.1 mM Na\textsubscript{2}EDTA, 0.25 mM ATP, 1% BSA, pH 7.5) and centrifuged at 7,000 g for 10 min at 4°C. The supernatant was drawn off and discarded and the pellet was resuspended in solution 3 (same as solution 2, but with BSA omitted) and centrifuged at 7,000 g for 10 min at 4°C. The supernatant was drawn off and discarded and the pellet was resuspended in a 1:1 wt/vol ratio (1 mg/l μl) of sucrose and mannitol buffer (220 mM sucrose, 70 mM mannitol, 10 mM Tris-HCl, 0.1 mM Na\textsubscript{2}EDTA, pH 7.4) for use in PDP activity analysis. A 5-μl aliquot of this suspension was added to 100 μl of sucrose and mannitol buffer to measure total mitochondrial suspension citrate synthase activity to determine mitochondrial recovery as described previously (2). Mitochondria were stored at −80°C for later measures of PDP and citrate synthase activities.

Citrate synthase. A piece (~10 mg) of muscle was shipped from the frozen biopsy and homogenized on ice in 100 volumes of homogenizing buffer (0.1 M KH\textsubscript{2}PO\textsubscript{4}, 0.05% BSA, pH 7.3) and freeze/thawed twice using liquid nitrogen. Citrate synthase activity for the whole muscle homogenate or the mitochondrial suspension was determined using a spectrophotometer (Biochrom, Cambridge, UK) by measuring the formation of DTNB-CoA from dithiobis-2-nitrobenzoate (DTNB) and CoASH at 412 nm (32).

PDP activity assay. Pyruvate dehydrogenase phosphatase (PDP) activity was determined using the nonradioactive phosphate assay system from Promega (Madison, WI) and a synthetic peptide substrate (14 amino acid long segment of the E1α subunit surrounding the phosphorylation sites 1 and 2) from New England Peptide (Gardner, MA) as previously described (4, 12, 17). Briefly, mitochondria were diluted with sucrose and mannitol buffer to a final protein concentration of 2 μg/μl and a total volume of 30 μl, freeze-thawed three times to fracture mitochondria membranes, and centrifuged at 21,000 g for 1 h at 4°C to pellet out large protein and other insoluble materials. The supernatant was further purified through Sephadex G-25 spin columns (which were equilibrated with 10 ml Sephadex G-25 storage buffer; 10 mM Tris base, 1 mM EDTA, 0.02% sodium azide, pH 7.5) to remove excess inorganic phosphate from the supernatant. The prepared sample (5 μl) was then added to a plate well containing 10 μl reaction buffer (50 mM imidazole, 0.2 mM EDTA, 5 mM MgCl\textsubscript{2}, 0.01% β-mercaptoethanol, 1 mg/ml BSA, pH 7.2) pyrophosphatase inhibitor cocktail (Sigma, St. Louis, MO) to inhibit other phosphatases (alkaline phosphatases, protein phosphatases 1 and 2A) and incubated at 37°C for 30 min. The reaction was started with the addition of the phosphopeptide and was allowed to proceed at 37°C for 30 min. The activity was stopped by adding 50 μl of molybdate dye to each well. Optical density of the plate was read after 30 min incubation at room temperature by measuring light absorbance at 600 nm. Activity is expressed in nanomoles per minute per milligram mitochondrial protein and nanomoles per minute per gram wet tissue weight.

Activity per gram wet tissue weight was determined by calculating PDP activity (nmol/min) per microliter of mitochondrial solution in the assay and using this value to determine the PDP activity present in the extracted mitochondria sample by using the recovery of the mitochondria as calculated as the ratio of citrate synthase activity in the mitochondrial suspension divided by the citrate synthase activity of the whole muscle homogenate as previously described (27, 28). Values for PDP activity in extracted mitochondria were then divided by percent mitochondrial recovery (mitochondrial suspension − whole muscle homogenate citrate synthase activity) (and multiplied by 1,000, as original measures were per mg wet tissue) to determine PDP activity as nanomoles per minute per gram wet tissue weight.

Western blotting. A piece of muscle (~10 mg) was shipped from the frozen biopsy and homogenized on ice in 100 volumes of homogenizing buffer (250 mM sucrose, 100 mM KCl, 2 mM EDTA, pH 6.8), which also contained a protease inhibitor cocktail (Complete Mini EDTA-free, Roche, Laval, QC, Canada). Standard SDS-PAGE electrophoresis was performed, as described previously (17, 19, 20, 26,
27), with proteins being transferred to a polyvinylidene fluoride (Immobilon-P, Millipore, Bedford, MA) (0.45 μm pore size). Membranes were then incubated overnight at 4°C in 10 ml of blocking solution (TBST with 5% skim milk) with a polyclonal antiserum against 53-kDa PDP1 (diluted 1:2,000; a kind gift from Drs. Brian Robinson and Mary Maj, Genetics and Genome Biology, SickKids Hospital, Toronto, ON), or monoclonal antibodies against 42-kDa PDH E1α (diluted 1:5,000; A-21323), PDH E2 (diluted 1:5,000; A-21325), 18-kDa cytochrome c oxidase subunit 4 (COX IV; diluted 1:1,000; A-21348; Molecular Probes), 46-kDa PKD2 (diluted 1:1,000; AP7039b; Abgent) and 42-kDa actin (diluted 1:5,000; 612656; BD Biosciences, Mississauga, ON, Canada). Membranes were washed in TBST and were incubated at 4°C for 2 h in 10 ml blocking solution with either goat anti-mouse IgG (diluted 1:5,000; A4416; peroxidase conjugated, Sigma, Ontario, Canada) or goat anti-rabbit IgG (diluted 1:10,000; sc-2004; Santa Cruz Biotechnology). Membranes were washed in TBST and chemiluminescent substrate (ChemiGlow West, Alpha Innotech) was added for visualization using a Fluorchem 5500 imaging station (Alpha Innotech). All protein values were normalized to actin or Ponceau S (total protein; Sigma, Ontario, Canada) to ensure equal loading within and between blots. (Ponceau S was used for E2 protein content as the molecular weights of actin and E2 are too close to differentiate on the same blots; there was no significant difference in actin content between subjects). Blot density was quantified using ImageJ software (http://rsb.info.nih.gov/ij/) and is expressed in arbitrary densitometry units.

Data analysis and statistics. Values are means ± SE unless otherwise indicated. Differences between males and females were analyzed with independent t-tests, and P < 0.05 was accepted as significant. When no significant differences were found between sexes, data were combined for further analysis. All other comparisons were performed using either simple or multiple linear regression for significant slopes (P < 0.05) and Pearson’s correlation coefficient (r²) using SPSS Software (Chicago, IL). The adjusted slopes (β, in SD units for both variables) are also given. The following questions were addressed: J) How does PDP activity and PDPI expression vary with differences in muscle aerobic capacity (i.e., CS activity)? 2) What is the relationship between muscle aerobic capacity (i.e., CS activity) and PDH-related proteins (E1α, E2, and PDK2)?; and J) Does PDPI protein content and/or E1α content contribute to the variance in PDP activity?

RESULTS

Subject characteristics. Subjects’ V̇O_{2\text{peak}} ranged from 30.0 to 64.5 ml·min⁻¹·kg⁻¹ (2.10–5.35 l/min) and muscle citrate synthase (CS) measures ranging from 7.0 to 32.3 μmol·min⁻¹·g wet tissue wt⁻¹ (4.65–11.54 μmol·min⁻¹·mg mitochondrial protein⁻¹). In self-reported questionnaires, subjects ranged from 18 to 24.2 yr (4.65–11.54 l/min) and muscle citrate synthase (CS) activity (minimum/maximum: 54 g/182 g), and 31.2 ± 0.68 m·kg⁻¹·min⁻¹ (2.10 –5.35 l/min) and muscle citrate synthase (CS) activity (minimum/maximum: 54 g/182 g), and 31.2 ± 0.68 m·kg⁻¹·min⁻¹.

Table 1. Subject characteristics, aerobic capacity, PDP activity, and PDPI protein content

<table>
<thead>
<tr>
<th>Variable</th>
<th>Males (n = 18)</th>
<th>Females (n = 12)</th>
</tr>
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<tbody>
<tr>
<td>Age, yr</td>
<td>24.2 ± 0.68</td>
<td>22.6 ± 1.24</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.82 ± 0.01</td>
<td>1.68 ± 0.02*</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>81.2 ± 2.18</td>
<td>66.6 ± 2.22*</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25 ± 0.6</td>
<td>23 ± 0.6</td>
</tr>
<tr>
<td>V̇O_{2\text{peak}}, ml·kg⁻¹·min⁻¹</td>
<td>48.0 ± 1.71</td>
<td>40.0 ± 1.46*</td>
</tr>
<tr>
<td>V̇O_{2\text{peak}}, l/min</td>
<td>3.89 ± 0.15</td>
<td>2.67 ± 0.11*</td>
</tr>
<tr>
<td>CS, μmol·min⁻¹·g wet tissue⁻¹</td>
<td>20.1 ± 1.15</td>
<td>16.4 ± 1.54*</td>
</tr>
<tr>
<td>CS, μmol·min⁻¹·mg mitochondrial protein⁻¹</td>
<td>7.94 ± 0.45</td>
<td>6.87 ± 0.49</td>
</tr>
<tr>
<td>PDPI activity, nmol·min⁻¹·mg mitochondrial protein⁻¹</td>
<td>0.526 ± 0.076</td>
<td>0.518 ± 0.107</td>
</tr>
<tr>
<td>PDPI activity, nmol·min⁻¹·g wet tissue wt⁻¹</td>
<td>7.36 ± 1.49</td>
<td>5.74 ± 1.15</td>
</tr>
<tr>
<td>PDPI protein content, arbitrary units</td>
<td>0.643 ± 0.037</td>
<td>0.646 ± 0.079</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index; CS, citrate synthase activity; V̇O_{2\text{peak}} peak oxygen consumption. *Significantly different from males, P < 0.05.

As expected, both muscle measures of CS activity and COXIV content were highly correlated with whole body V̇O_{2\text{peak}} (P < 0.001 and 0.004, respectively), and CS activity and COXIV were highly correlated with each other (P < 0.001). CS activity accounted for ~40% of the variation in V̇O_{2\text{peak}} (r² = 0.393), while COXIV content only accounted for ~27% of the variation in V̇O_{2\text{peak}} (r² = 0.269). Our primary goal was to explore how PDP activity and PDPI and PDH subunit expression varied with muscle aerobic capacity, and therefore for our comparisons we exclusively used CS activity. As dietary composition has been shown to influence PDH activity, a self-reported 3-day dietary analysis was conducted to address the possibility that changes in PDP activity could be influenced by dietary variations between subjects. This revealed that on average subjects consumed 50.1 ± 1.5% carbohydrate (minimum/maximum: 105 g/466 g), 18.0 ± 1.1% protein (minimum/maximum: 54 g/182 g), and 31.2 ± 1.2% fat (minimum/maximum: 33 g/158 g).

Correlations of CS activity (marker for muscle aerobic capacity) and PDPI activity and PDPI protein and other PDH complex proteins (E1α, E2, and PDK2). Representative blots for PDPI, E1α, E2, and PDK2 are shown in Fig. 1. CS activity was significantly correlated to PDPI activity (nmol·min⁻¹·g wet tissue wt⁻¹) and PDPI protein content, although the correlation was stronger with PDPI activity (P = 0.001; r² = 0.399 for PDPI activity compared with P = 0.039 and r² = 0.153 for PDPI; Table 2 and Fig. 2, A and B). Even when PDPI activity was normalized to mitochondrial protein (nmol·min⁻¹·mg mitochondrial protein⁻¹), the correlation was still significant (slope = 0.085, adjusted slope = 0.515, r² = 0.265, P = 0.007), indicating that it was not simply the difference in mitochondrial content that would explain the variation in PDPI activity with muscle aerobic capacity.

Since E1α and PDK2 content has been shown to increase with 8 wk endurance training (20), the relationship between muscle CS activity and these measures was also determined. E1α content (but not E2) was significantly correlated with CS activity (P = 0.002), and PDK2 content was also positively correlated with CS activity (P = 0.012; Table 2 and Fig. 2, C–E).
Simple linear regression assessing the relationship between CS activity and PDP1 content, PDP activity, E1 content, and PDK2 content

<table>
<thead>
<tr>
<th>PDP1</th>
<th>PDP Activity</th>
<th>E1 Content</th>
<th>E2 Content</th>
<th>PDK2 Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>b (β)</td>
<td>b (β)</td>
<td>b (β)</td>
<td>b (β)</td>
<td></td>
</tr>
<tr>
<td>With CS activity</td>
<td>0.032* (0.392)</td>
<td>1.909* (0.632)</td>
<td>0.120* (0.557)</td>
<td>0.020 (0.256)</td>
</tr>
<tr>
<td>Slope</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.039</td>
<td>0.001</td>
<td>0.002</td>
<td>0.180</td>
</tr>
<tr>
<td>r²</td>
<td>0.153</td>
<td>0.399</td>
<td>0.310</td>
<td>0.066* (0.478)</td>
</tr>
</tbody>
</table>

b. Slope of the regression line, with adjusted slopes (β, in common SD units for both variables) in parentheses. Based on Pearson’s r² values, CS activity significantly accounts for approximately 15%, 40%, 31%, and 23% of PDP1 content, PDP activity (nmol·min⁻¹·g wet tissue wt⁻¹), E1 content, and PDK2 content, respectively. Correspondingly muscle aerobic capacity as measured through CS activity influences most PDP activity (adjusted slope = 0.632), and followed by E1, PDP1, PDK2 and E2 content. *Significant slopes, P < 0.05.

DISCUSSION

To the best of our knowledge, this is the first study to examine PDP activity in human skeletal muscle, with the PDP activity values obtained in the present study from human skeletal muscle (~0.52 nmol·min⁻¹·mg mitochondrial protein⁻¹) being similar to those previously recorded in rat skeletal muscle (~0.55 nmol·min⁻¹·mg mitochondrial protein⁻¹; 17, 19). The major findings of the present study are: 1) E1α and PDK2 protein correlated with muscle citrate synthase activity as a marker of muscle aerobic capacity, although PDH E2 protein did not (P = 0.066); 2) PDP1 protein content and PDP activity significantly correlated with citrate synthase activity; and 3) PDP1 protein content only accounted for 18% of the variance in PDP activity, while PDH E1α protein contributed to 28% of the variance. Together, E1α and PDP1 accounted for ~38% of the variance in PDP activity.

E1α, E2, and PDK2 protein content. In the present study there was a higher E1α protein content with higher muscle aerobic capacity, which supports previous findings from a longitudinal study that the protein content of E1α increases with 8 wk of aerobic exercise training in humans. This increase in E1α is of particular importance, as it is the rate-limiting step in the PDH reaction and has been coupled with an increase in total PDH activity (20). There has been only one other study to document changes in human skeletal muscle PDK2 protein with 8 wk endurance training (20), and our work supports this finding since PDK2 protein was positively correlated with muscle CS activity. This supports the role of PDK2 as the “energy-sensitive” PDK isoform (see 35 for review) and is thought to explain, in part, the increase in total PDH activity observed with endurance training (20). Targeted upregulation of PDK2 makes sense in the context that it has been regarded as the isoform that is most sensitive to changes in cellular energy charge and pyruvate inhibition (3), meaning that during exercise this isoform would detect smaller alterations in these effectors, and “fine-tune” PDH activation appropriately to meet CS activity was combined with E1α content the combination accounts for ~43% of the variance of PDP activity (E1α slope = 6.821, E1α adjusted slope = 0.235, CS slope = 1.484, CS adjusted slope = 0.491, P = 0.001, r² = 0.435). However, it eliminated the individual significance of E1α and PDP1 alone, suggesting that PDP1 and E1α share a portion of the variance in PDP activity with CS activity. Taken together, this suggests that muscle aerobic capacity, as measured by CS activity, partly accounts of the influence of PDP1 and E1α on PDP activity.
a wide range of energy requirements. More importantly, since these data are consistent with data from a longitudinal endurance training study, it suggests that our cross-sectional sample was appropriate to study the variation in the PDH complex proteins and PDP activity with muscle CS activity as a marker for differences in training status.

In our study, E2 protein content was not significantly correlated with muscle CS activity, although there was a strong trend observed ($P = 0.066$). The lack of difference in E2 compared with E1α has been observed before, as previous work looking at the effects of training found that the PDH subunits did not increase in a uniform manner, as E2 and E3BP

Table 3. Correlation between PDP1 and E1α and PDP activity

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>$b$ (β)</td>
<td>$b$ (β)</td>
<td>$b$ (β)</td>
</tr>
<tr>
<td>PDP1</td>
<td>16.022* (0.429)</td>
<td>11.737 (0.314)</td>
<td></td>
</tr>
<tr>
<td>E1α</td>
<td>6.821* (0.529)</td>
<td>6.101* (0.460)</td>
<td></td>
</tr>
<tr>
<td>PDP1 × E1α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P$ value</td>
<td>0.033</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>$r^2$</td>
<td>0.184</td>
<td>0.280</td>
<td>0.383</td>
</tr>
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</table>

Multiple linear regression between PDP1 (model 1) and E1α (model 2) protein content and PDP activity, alone and in combination (model 3). $b$, indicates the slope of the line while $β$ (in parentheses) is the adjusted slope (i.e., the slope calculated in common SD units for both variables). *Significant slope, $P < 0.05$. **Significant slope is significant at $P < 0.001$. **Slope is significant at $P < 0.05$. ***Slope is significant at $P < 0.001$.

Fig. 2. Scatterplots of correlations between muscle citrate synthase activity (marker for muscle aerobic capacity) and PDH complex proteins. A: pyruvate dehydrogenase phosphatase isoform 1 (PDP1) protein. B: pyruvate dehydrogenase phosphatase (PDP) activity ($\text{nmol·min}^{-1}\cdot\text{g wet tissue wt}^{-1}$). C: pyruvate dehydrogenase subunit E1α protein (E1α). D: pyruvate dehydrogenase subunit E2 protein (E2). E: pyruvate dehydrogenase kinase isoform 2 (PDK2). $b$, slope of the regression line; $R^2$, Pearson’s correlation coefficient; *Slope is significant at $P < 0.05$; ***Slope is significant at $P < 0.001$.

Fig. 3. Scatterplot of correlation between both PDP1 (pyruvate dehydrogenase phosphatase isoform 1; ●) and E1α (pyruvate dehydrogenase subunit E1α; ○) protein content and PDP activity ($\text{nmol·min}^{-1}\cdot\text{g wet tissue wt}^{-1}$). Both PDP1 and E1α content contribute to the variance in PDP activity (i.e., significantly correlated with PDP activity). *Slope is significant at $P < 0.05$. **Slope is significant at $P < 0.001$. **Slope is significant at $P < 0.001$. **Slope is significant at $P < 0.001$. **Slope is significant at $P < 0.001$. **Slope is significant at $P < 0.001$. **Slope is significant at $P < 0.001$.
(unlike E1α) did not significantly change following 8 wk of endurance training (20). However, our subjects had maintained a similar training status for at least six mo, and that may explain why there was a strong trend toward E2 correlating with muscle aerobic capacity. This could suggest that E2 does adapt to training, but at a slower rate than E1α, and therefore requires a greater duration of training for changes to reach statistical significance.

**PDPI protein content and PDP activity.** PDPI protein content was greater in individuals with higher muscle citrate synthase activity along with E1α protein content, suggesting that any increment in E1α content would sustain a proportional increase in the ability to activate the subunit. Specifically, a higher level of the calcium-sensitive isoform, PDPI, would increase activation of PDH during higher-intensity exercise when there is an increased requirement for carbohydrate oxidation to meet the energy demand.

The positive correlation of PDP activity with muscle citrate synthase activity suggests that the PDH complex is more readily activated to permit increased energy turnover in those who have the capacity to work at higher levels of exercise (i.e., trained individuals). In addition to this, even when PDP activity is expressed relative to mitochondrial protein content, there is still a correlation with muscle aerobic capacity, indicating that the higher PDP activity is not simply due to a higher mitochondrial protein content. This observation that PDP activity is higher in individuals that have higher aerobic capacity is further supported by the findings of the present study that PDP activity levels are positively correlated with E1α protein content (which is also known to increase with aerobic training; 20). Previous work has demonstrated that PDK activity is increased in human skeletal muscle following 8 wk of aerobic exercise training, and therefore the higher PDP activity would be sufficient to offset this increase in PDK activity and facilitate reactivation of the PDH complex at higher rates of ATP turnover (18). An increase in PDP activity is an important adaptation, as allosteric inhibition of PDH activity would perhaps be insufficient to reactivate the PDH complex when needed for higher rates of ATP production (e.g., during a sprint). An increase in PDP activity and PDPI protein content has also been observed in obese Zucker rats, but not in lean rats, following 8 wk of endurance training (19). Although none of the subjects in the present study could be classified as obese according to body mass index (<30 kg/m²; see Table 1), closer inspection of the data from lean rats indicates that there was a trend toward increased PDP activity in the previous work (19). Therefore, although it is possible that there is a species difference, it may be that a longer duration of training is required to observe a significant change, as the subjects in the present study had maintained a consistent level of training for at least 6 mo and often much longer, compared with only 8 wk of training in previously sedentary rats.

**PDP activity vs. PDPI protein content.** The positive correlation seen between PDPI protein content and PDP activity at the level of the whole muscle suggests that the higher PDPI protein content seen with higher muscle aerobic capacity is at least partly responsible for the higher maximal PDP activity observed in this tissue. This is a similar relationship to what has been previously documented in human skeletal muscle on the kinase side of the complex, with the finding that total PDK activity increased with PDK2 protein content following endurance training (20). We also found that E1α protein content accounted for ~28% of the variance in PDP activity, underscoring the relationship between the capacity for carbohydrate oxidation through the rate-limiting E1α reaction and the capacity to activate the complex through PDP activity. In addition, as PDPI has been found to be the predominant isoform in mammalian skeletal muscle (11, 17, 19), it would appear that there is a coordinated expression between the protein content of the predominant PDH regulatory proteins in skeletal muscle, PDK2 and PDPI, and the maximal activity levels of the kinase and phosphatase, respectively, in this tissue. However, PDPI protein content only accounted for ~18% of the variation in PDP activity, indicating that there may be other factors influencing maximal PDP activity. This discrepancy may be explained by possible posttranslational modifications of PDPI that increase activity. For example, PDP activity may be increased above that of protein expression via phosphorylation, as recent findings have demonstrated that PDP itself is capable of being phosphorylated to become active (4). Thus it is possible that changes in PDP activity may be occurring due to PDP phosphorylation without as great of a change in protein content.

It should be noted that the measurements of enzyme activity in this study are maximal in nature; therefore it is possible that there is a disconnect between maximal activity and that which is observed acutely in vivo as this study did not control for mitochondrial effectors. Also, the in vitro method to measure PDP activity uses a specific synthetic peptide substrate instead of the native PDH complex. Therefore, we cannot ascertain the extent to which PDP may be active in vivo, as activity varies with mitochondrial effector concentrations and the true substrate. In addition, in its natural state, PDP activity is affected by its association with the lipoyl domain on the PDH complex (allowing it to colocalize with phosphorylated E1α; 14), and this cannot be fully reproduced with the synthetic peptide assay.

It is also possible that the differences observed in PDP activity are a result of intersubject variability in insulin sensitivity, as insulin sensitivity is known to increase with aerobic exercise training (see 38 for review). Due to the nature of the assay used in the present study we were unable to differentiate between the activities of the two PDPI isoforms. Insulin sensitivity increases with exercise training, and since the PDPI ii isoform is believed to be stimulated by insulin, this increased insulin sensitivity may cause PDPI to become more active and contribute to the higher PDP activity observed with aerobic capacity. However, due to the very low concentrations of PDPI believed to be present in mammalian skeletal muscle (11, 12, 17), and the fact that under our experimental conditions the subjects would have basal insulin concentrations, this is unlikely to have significantly influenced the results.

The proportion of type I and IIa fibers in the biopsies taken may also have contributed to the discrepancy between PDPI content and PDP activity. Previous studies in rat skeletal muscle have shown that PDPI protein content is significantly greater in the red gastrocnemius (RG; predominantly type IIa fibers, with some type I) than white gastrocnemius (WG; predominantly type IIb) and soleus (predominantly type I; 17, 19). In addition to this, it has been established that PDPI protein is only expressed in red gastrocnemius of rats, with no detectable levels in other fiber types. PDP activity values have
also revealed a similar trend, as it has been shown that activity is higher in RG than WG and soleus (17). Therefore, it is possible that fiber type differences in subjects’ biopsies influenced the PDP activity and protein content findings in this study. However, more research is required, since previous studies were exclusive to rat skeletal muscle, and it cannot be said for certain that PDP activity and protein content would be similarly expressed in the human fiber types. It is known that the metabolic differences between fiber types in humans are generally much less than those observed in rats (9).

Summary and Perspectives

This study is the first to examine PDP activity and PDP1 protein content in human skeletal muscle and demonstrate that they positively correlate with muscle citrate synthase activity as a marker for muscle aerobic capacity. This study also represents only the second study to investigate how PDK2 and other PDH subunits (E1α and E2) vary with muscle citrate synthase activity, thereby providing important confirmatory measures. These data indicate that the cross-sectional variations seen in PDP activity and PDP1 protein content between subjects are likely the result of differences in muscle aerobic capacity relative to training status. The variance in PDP activity across a range of individuals with differing aerobic capacity is only partially explained by PDP1 (the most abundant isoform) content, suggesting that there may be alternate regulation of PDP activity. However, PDP activity is strongly correlated with E1α content, indicating that as the capacity for carbohydrate oxidation increases, the capacity to activate the complex also increases.

In a practical application of these results, research has shown that endurance training causes VO₂ on-kinetics to speed-up at the onset of exercise, allowing the body to reach steady state faster (8). Although several factors are suggested to be a cause for this, one such factor is thought to be that of metabolic inertia with the PDH complex suspected of being a key component in this limitation (7). Therefore, the results of this study may help in this explanation, since it appears that it would be reasonable to expect that with increasing training status and higher muscle aerobic capacity, one would have both an increased potential for carbohydrate oxidation as well as higher PDP activity and the potential to activate the complex. This could play a role in this “speeding,” by more quickly activating the PDH complex, leading to a quicker supply of aerobic ATP production at the onset of exercise.

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DISCLOSURES

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