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Pyruvate dehydrogenase kinase-4 contributes to the recirculation of gluconeogenic precursors during postexercise glycogen recovery

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Since the discovery of enhanced muscle glycogen synthesis following exercise almost 50 years ago (1), multiple mechanisms have been proposed and investigated to characterize this phenomenon. Although the events upstream of glycogen formation (glucose uptake) (7, 26, 27), as well as the fates of gluconeogenic precursors (e.g., lactate and alanine) (2, 3, 29) have been extensively studied, a mechanism for promoting the shift to fat oxidation and recirculation of glucose-derived substrates following exercise has received less attention. As pyruvate is the dominant precursor for lactate and alanine production, as well as carbohydrate oxidation, the chosen fate of this metabolite following exercise could have a heavy influence on whole body carbohydrate metabolism. Pyruvate oxidation is regulated by the pyruvate dehydrogenase (PDH) complex, which may also play a role in enhancing muscle glycogen resynthesis postexercise. Studies examining changes in fuel selection after exercise in human skeletal muscle have observed decreased PDH activation (PDHα activity) (15, 19) along with increased reliance on fatty acids and concomitant increases in glycogen storage (15). As the activity of the PDH complex is regulated, being stimulated by two PDH phosphatases and depressed by four pyruvate dehydrogenase kinases (PDK1–4), observed reductions in PDHα activity during exercise recovery may be mediated by one of the PDK isoforms.

Although changes in the activity of PDHα during and after exercise have been previously examined in muscle (14, 15, 19, 23–25), the roles of each kinase during and after exercise have only been marginally elucidated because of the challenge of isolating the roles of the individual PDKs. Multiple studies have demonstrated increased PDK4 transcription and mRNA production with exercise (10, 19, 21, 22), suggesting that changes in PDK4 activity may reduce flux through PDH during both exercise and recovery. Investigating this, a recent study by our group observed impaired PDH regulation in PDK4-knockout (PDK4-KO) mice with acute muscle contraction ex vivo, supporting the involvement of PDK4 during exercise (9). As PDK4 transcription is markedly elevated postexercise, it is likely that PDK4 may also regulate the PDH complex in the period following exhaustive exercise and that this may enhance glycogen resynthesis. Therefore, the purpose of this study was to examine the role of PDK4 in promoting glycogen resynthesis during recovery from exhaustive exercise in skeletal muscle of wild-type (WT) and PDK4-KO mice. Considering that both...
Postexercise nutritional intake

Table 1. Postexercise nutritional intake

<table>
<thead>
<tr>
<th></th>
<th>H₂O, ml</th>
<th>Food, g</th>
<th>Total kcal</th>
<th>Carb, kcal</th>
<th>Fat, kcal</th>
<th>Pro, kcal</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>2.44 ± 0.36</td>
<td>0.55 ± 0.09</td>
<td>2.57 ± 0.41</td>
<td>0.30 ± 0.05</td>
<td>0.14 ± 0.02</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>PDK4-KO</td>
<td>3.47 ± 0.68</td>
<td>1.01 ± 0.10*</td>
<td>4.71 ± 0.48*</td>
<td>0.55 ± 0.06*</td>
<td>0.25 ± 0.03*</td>
<td>0.20 ± 0.02*</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. Feeding and nutritional composition during exercise recovery in wild-type (WT) and PDK4-knockout (PDK4-KO) mice (n = 8). Pair-fed (PF) values are equal to those of WT animals and were fed 0.55 g of chow. *Significantly different from WT, P < 0.05.
Glycogen concentrations. Muscle glycogen decreased at Exh to 42% of resting concentrations in WT mice (P < 0.001) and 51% in PDK4-KO mice (P < 0.001), with no significant differences between genotypes. At Rec, muscle glycogen restored to resting levels in WT mice and PF PDK4-KO mice, and supercompensated in PDK4-KO mice an additional 19% over resting levels (P < 0.05) (Fig. 3). Liver glycogen concentrations at Exh were decreased to 16% of initial resting values in WT mice (P < 0.001) and 24% in PDK4-KO mice (P < 0.001), with no differences between genotypes. Liver glycogen was replenished at Rec to resting levels in WT and PDK4-KO mice, yet remained at only 49% of resting levels in PF PDK4-KO mice (P < 0.001) (Fig. 3).

Metabolite concentrations. Blood glucose was significantly lower in WT mice at Exh only, with no differences among groups at Rec (Fig. 4). Blood lactate at Rec was significantly lower in PF PDK4-KO mice than WT and PDK4-KO mice, with no differences between genotypes at other time points (Fig. 4). No differences were detected in muscle glucose concentrations between groups; however, lactate and alanine concentrations in muscle were significantly lower in the PF PDK4-KO group compared with Rec WT and PDK4-KO mice (Table 2). In the liver, glucose concentrations decreased as a result of exercise in WT (P < 0.001) and PDK4-KO mice (P = 0.043), while after recovery, glucose returned to resting values in PDK4-KO mice, recovered to ~65% of rest in WT mice, and remained low in PF PDK4-KO mice (P < 0.001) (Table 3).

DISCUSSION

The present study investigated the involvement of PDK4 in regulating the PDH complex during postexercise glycogen resynthesis and found that 1) PDK4 plays a role during recovery from exhaustive exercise; however, 2) contrary to our hypothesis, the absence of PDK4 does not compromise glycogen resynthesis in skeletal muscle, but 3) causes increased
feeding in PDK4-KO mice during recovery, and 4) leads to a decreased availability of gluconeogenic substrates and impaired hepatic glycogen resynthesis in PDK4-KO mice when pair-fed (PF) with WT mice.

In this study, glycogen concentrations in skeletal muscle and liver were successfully reduced at exhaustion and resynthesized during recovery in both genotypes fed ad libitum. This observation was contrary to the hypothesis that muscle glycogen utilization would be increased and glycogen synthesis would be impaired in PDK4-KO mice due to increased loss of carbohydrate through PDH. We have previously observed increased PDHα activation during muscle contraction in isolated extensor digitorum longus muscle, and this increased activation would be expected to increase oxidative loss of carbohydrate (9). In this study, however, we also observed a twofold greater caloric intake in PDK4-KO muscle feeding ad libitum during recovery, and although the mechanism for this is unknown, this potentially indicates compensation for an oxidative loss of carbohydrate. As a result of this increased feeding, muscle glycogen unexpectedly supercompensated by an additional ~20% over resting concentrations. To further investigate this discrepancy, a group of PDK4-KO mice was pair-fed (PF PDK4-KO group) 0.55 g of chow as consumed by WT mice during recovery, and in these mice, skeletal muscle glycogen recovered to resting levels. These observations strongly support that PDK4 is not required for postexercise glycogen resynthesis in skeletal muscle.

Although liver glycogen resynthesized to the same degree in recovered WT and PDK4-KO mice, PF PDK4-KO mice demonstrated impaired liver glycogen resynthesis in spite of fully restored muscle glycogen levels. This observation suggests preferential resynthesis of muscle glycogen when caloric availability was limited to levels consumed by WT controls during recovery and is supported by previous studies showing complete muscle glycogen resynthesis but impaired hepatic glycogen levels in both rodents and humans with fasting following exercise (5, 16). Although blood glucose concentrations were maintained in all groups during recovery, intracellular liver glucose concentrations were significantly lower in PF PDK4-KO compared with recovered WT and PDK4-KO mice. This supports that hepatic glucose output is sufficient for maintenance of blood glucose homeostasis, but also that the abundance of available gluconeogenic substrates were not significant enough to promote both hepatic glucose output and repletion of glycogen stores. The liver has a substantially increased reliance on muscle-released lactate and alanine to support gluconeogenesis during recovery (2, 3, 29, 32, 33), and in this study, the concentrations of both of these substrates were reduced in PF PDK4-KO muscle with concomitant decreases in circulating blood lactate concentrations and their conversion to glucose in the liver. As WT and PF PDK4-KO mice differ only in their PDK4 content, these data suggest that the absence of PDK4 during recovery may result in a loss of pyruvate to oxidation in muscle, decreasing the formation of lactate and alanine, and impairing the recirculation of these carbohydrate-derived substrates for gluconeogenesis. The role of PDK4 in promoting recycling of gluconeogenic substrates has been suggested previously (31), and our conclusion is supported by a previous study in which the indiscriminate PDK inhibitor, dichloroacetate, was infused into rodents recovering from exercise, impairing the activity of all PDH isofoms, reducing the concentrations of gluconeogenic precursors in the blood, and impairing hepatic glycogen resynthesis (4).

Finally, although mice in this study were bred in separate facilities, we have demonstrated previously (9) that under resting conditions, mice from both facilities show no differences in body weight, resting metabolite concentrations, or resting PDHα activity in vitro. In addition, WT and PDK4-KO mice in the present study show no differences in age or body weight, exercise time to exhaustion, or in key variables such as

### Table 2. Muscle metabolites

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Rest</th>
<th>PDK4-KO</th>
<th>Exh</th>
<th>PDK4-KO</th>
<th>Rec</th>
<th>PDK4-KO</th>
<th>PF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>4.3 ± 1.5</td>
<td>4.7 ± 0.6</td>
<td>2.5 ± 0.8</td>
<td>3.2 ± 0.8</td>
<td>4.6 ± 0.5</td>
<td>3.4 ± 0.5</td>
<td>3.8 ± 0.2</td>
</tr>
<tr>
<td>G-6-P</td>
<td>8.1 ± 3.0</td>
<td>8.2 ± 2.7</td>
<td>1.5 ± 0.7</td>
<td>4.5 ± 1.1</td>
<td>8.5 ± 2.7</td>
<td>7.9 ± 1.7</td>
<td>2.9 ± 0.4</td>
</tr>
<tr>
<td>Lactate</td>
<td>23.0 ± 8.6</td>
<td>9.8 ± 4.5</td>
<td>8.0 ± 2.2</td>
<td>8.5 ± 1.1</td>
<td>16.8 ± 5.3</td>
<td>14.7 ± 3.7</td>
<td>3.7 ± 1.5*#</td>
</tr>
<tr>
<td>Alanine</td>
<td>5.4 ± 2.0</td>
<td>4.5 ± 1.6</td>
<td>6.0 ± 1.5</td>
<td>5.0 ± 2.0</td>
<td>4.8 ± 1.1</td>
<td>7.0 ± 1.3</td>
<td>3.0 ± 0.8*#</td>
</tr>
</tbody>
</table>

Values are expressed as μmol/g dry weight ± SE. Muscle metabolite concentrations at rest, (n = 6) exhaustion (Exh) (n = 6), and recovery (Rec) (n = 8) (exceptions: PF lactate, n = 5, and PF G-6-P, n = 6). WT, wild-type; PDK4-KO, PDK4-knockout; PF, pair fed PDK4-KO. †Different than rest within a genotype, P < 0.05. *Significantly different than WT for a given time point, P < 0.05. #Significantly different than Rec PDK4-KO, P < 0.05.

### Table 3. Liver metabolites

<table>
<thead>
<tr>
<th>Liver</th>
<th>Rest</th>
<th>PDK4-KO</th>
<th>Exh</th>
<th>PDK4-KO</th>
<th>Rec</th>
<th>PDK4-KO</th>
<th>PF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>86.5 ± 8.8</td>
<td>65.3 ± 9.6</td>
<td>17.3 ± 0.1†</td>
<td>34.4 ± 9.2†</td>
<td>56.0 ± 7.8†</td>
<td>66.6 ± 7.8</td>
<td>27.1 ± 5.1*#</td>
</tr>
<tr>
<td>G-6-P</td>
<td>1.8 ± 0.4</td>
<td>2.2 ± 0.4</td>
<td>0.7 ± 0.1</td>
<td>1.01 ± 0.1 ±</td>
<td>1.4 ± 0.4</td>
<td>2.7 ± 1.1</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>Lactate</td>
<td>31.0 ± 5.0</td>
<td>29.9 ± 7.8</td>
<td>6.7 ± 3.4†</td>
<td>8.2 ± 2.8†</td>
<td>22.1 ± 3.7</td>
<td>29.2 ± 3.1</td>
<td>19.8 ± 7.9</td>
</tr>
<tr>
<td>Alanine</td>
<td>8.8 ± 3.3</td>
<td>6.5 ± 2.4</td>
<td>3.6 ± 0.7</td>
<td>11.0 ± 1.5</td>
<td>14.9 ± 3.3</td>
<td>15.8 ± 8.6</td>
<td>12.4 ± 3.7</td>
</tr>
</tbody>
</table>

Values are expressed as μmol/g dry weight ± SE. Liver metabolite concentrations at rest (n = 6), exhaustion (Exh) (n = 8), and recovery (Rec) (n = 8). WT, wild-type; PDK4-KO, PDK4-knockout; PF, pair-fed PDK4-KO. †Different than rest within a genotype, P < 0.05. *Different than WT for a given time-point, P < 0.05. #Different than Rec PDK4-KO, P < 0.05.
glycogen or metabolite concentrations at rest. Although this may not account for all possible variables in genetic drift, the most novel findings of this article are seen when comparing the PDK4-KO mice, pair-fed vs. ad libitum feeding postexercise, demonstrating that our observed phenomenon is due to impaired glucose handling in PDK4-KO mice postexercise as opposed to differences between mice from separate facilities.

**Perspectives and Significance**

Although the mechanisms regulating the shift from carbohydrate oxidation for use in glycogen resynthesis are unknown, they likely involve altering the activity of the PDH complex. Therefore, we examined the role of PDK4 in regulating the PDH complex during glycogen resynthesis after exhaustive exercise in mice. This study demonstrates that PDK4 is not necessary for resynthesis of skeletal muscle glycogen stores. However, the absence of PDK4 results in potentially impaired oxidative handling of pyruvate in muscle, leading to reduced lactate and alanine production and recirculation when caloric availability is limited, impairing hepatic glucose availability and glycogen resynthesis. These data suggest that the role for PDK4 postexercise is not to aid in enhancing muscle glycogen resynthesis, but to promote the recirculation of gluconeogenic precursors.

It has been previously suggested that inhibition of select PDK isoforms could be a potential drug target for the treatment of diabetes (17, 18, 28, 30), and, indeed, the removal of PDK4 has been shown to increase glucose sensitivity (13) and aid in resistance to the effects of high-fat feeding (11, 12). As drug treatments would ideally act as a transient stepping stones to resistance to the effects of high-fat feeding (11, 12). As drug.

**REFERENCES**


**AUTHOR CONTRIBUTIONS**


