Muscle fiber type comparison of PDH kinase activity and isoform expression in fed and fasted rats

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after starvation and the induction of diabetes in rat heart and mixed skeletal muscle (32, 33).

However, little work has been done to investigate differences in PDK activity and isoforms in different fiber types. Skeletal muscle is not homogeneous and contains fibers that can be classified into three major types based on contractile and metabolic properties: type I [slow-twitch oxidative (SO)], type IIA [fast-twitch oxidative glycolytic (FOG)], and type IIB [fast-twitch glycolytic (FG)], with the balance made of transition fibers (type IIX or IID) (1, 8). In terms of glucose disposal in skeletal muscle, the fiber types vary greatly with respect to such parameters as GLUT-4 content, insulin sensitivity, hexokinase activity, glycogen synthase protein, and oxidative capacity (2, 6, 13, 25, 26). In general, glucose transport and disposal are higher in oxidative fiber types (13).

The purpose of this study was to comprehensively examine the changes in PDK activity and PDK isoform mRNA and protein changes in all three rat skeletal muscle fiber types in response to a 24-h fast. Our hypothesis was that both oxidative fibers undergo more regulation, with increases in PDK activity and PDK4 isoenzyme expression compared with glycolytic fibers, but that PDK2 and PDK1 would be unaltered between fiber types and in response to fasting. To represent the three major fiber types, we used the soleus (Sol; 84% type I, 7% type IIA, 0% type IIB), red gastrocnemius (RG; 30–51% type I, 35–62% type IIA, 1–8% type IIB), and white gastrocnemius (WG; 0% type I, 0% type IIA, 92% type IIB) muscles to represent SO, FOG, and FG muscles, respectively.

METHODS

Male Sprague-Dawley rats weighing on average 191 ± 1 g were used in the experiments. The animals were housed in a controlled environment with a 12:12-h light-dark cycle and were fed Purina rat chow ad libitum until food withdrawal. This study was approved by the University of Guelph Animal Care Committee.

Study design. One group of rats (fed, n = 4) were used after ad libitum feeding, whereas a second group of rats (fasted, n = 4) had food withdrawn for 24 h before skeletal muscle harvest and mitochondrial extraction. Animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (6 mg/100 g body wt), and the Sol, RG, and WG muscles were excised as quickly as possible (≈90 s). Muscle from one leg was frozen immediately in liquid nitrogen for mRNA and total homogenate citrate synthase (CS) activity analysis. Muscle from the second leg was not frozen and was used to extract mitochondria for Western blotting and PDK activity analysis. Approximately 1–2 ml of blood was drawn through intracardiac puncture with a heparinized syringe after muscle excision. An aliquot (50 μl) of whole blood was deproteinized 1:5 with 6% perchloric acid for analysis of β-hydroxybutyrate, glucose, lactate, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) by radioisotopic measurement as described previously (7, 15, 19). Briefly, mito-
mitochondrial preparation, and CS activity in the total suspension (CS$_{\text{total}}$) was measured after the preparation was frozen and thawed twice to fracture the mitochondria. Triton (0.1%) was included in the cuvette for measurement of CS$_{\text{a}}$ and CS$_{\text{homog}}$.

Recovery of intact mitochondria was calculated as $\%$ fractional recovery = 100 × (CS$_{\text{a}}$ - CS$_{\text{run}}$)/CS$_{\text{homog}}$ where quality of the mitochondrial preparation was calculated as $\%$ intact mitochondria = 100 × (CS$_{\text{a}}$ - CS$_{\text{run}}$)/CS$_{\text{a}}$.

Whole muscle homogenate CS activity was also used as a marker to ensure sampling accuracy between the fed and fasted muscle and between the muscle types.

**Western blotting.** Mitochondria were diluted to a final protein concentration of 1 µg/µl in 50 mM Tris-HCl, pH 6.8, containing 2% SDS, 0.1 M dithiothreitol, 0.1% bromophenol blue, 10% glycerol, 1 mM benzamidine, 0.1 mg/ml trypsin inhibitor, 1 µg/ml aprotinin, 0.1 mM tosyl-lysylphenylmethylketone, 1 µM leupeptin, and 1 µM pepstatin A. Samples were solubilized by boiling for 5 min and then cooled on ice for 5 min. Standard SDS-PAGE was performed with a 4% stacking and 10% separating gel (5 µg of mitochondrial protein per lane). Electrophotoblotting and immunodetection were performed as previously described (33). Polyclonal antisera against recombinant PDK1, PDK2, and PDK4 had been previously tested for cross-reactivity (33). Antibody-antigen complexes were visualized with $^{125}$I-labeled Protein A (ICN Pharmaceuticals, Irvine, CA) followed by autoradiography. Relative densities were quantified using Northern Eclipse from Empix Imaging, and results are expressed as the intensity of the band in arbitrary units. Blots were stripped and reprobed with antisera against the PDH complex, and the subunit E1alpha-band was used to normalize loading.

**Northern blotting.** Total RNA was extracted from frozen muscle using Qiagen RNeasy Mini Kit (Mississauga, ON, Canada). Total RNA (6 µg) was loaded onto 1% denaturing agarose gel and run with 10 mM MOPS, 4 mM sodium acetate, and 0.5 mM EDTA buffer (pH 7.0) at 75 V for 4 h. Gels were visualized under ultraviolet light (the 28S and 18S bands) to ensure even loading and good quality of the RNA. RNA was capillary blotted overnight onto a Nytran supercharged membrane (Schleicher and Schuell, Keene, NH). The gel was revvisualized after blotting to ensure that most of the RNA had transferred and to ensure that it had transferred evenly. cDNAs for PDK1, PDK2, and PDK4 have been described previously (5, 33). The individual cDNAs were labeled using $^{32}$P-labeled ATP and -CTP (Amersham Pharmacia Biotech, Piscataway, NJ) and the random primed DNA labeling kit (Boehringer Mannheim, Montreal, QB, Canada). Stratagene Quickhyb solution and protocol were used for hybridization with the labeled probe, except that hybridization was performed for 2 h at 68°C (Stratagene Cloning Systems, La Jolla, CA). After washing blots according to the method prescribed for Stratagene QuickHyb, we developed autoradiographs on Kodak X-omat film (Rochester, NY). Relative densities were quantified using Northern Eclipse from Empix Imaging, and results are expressed as the intensity of the band in arbitrary units.

**Statistics.** Results for muscle data were analyzed using a two-way ANOVA. A Fisher’s protected least-significant difference post hoc test was used to compare means. Unpaired $t$-tests were used to compare blood analyses. Significance was accepted at $P < 0.05$.

**RESULTS**

**Mitochondrial preparations and CS activity.** The mitochondrial recovery was $21 \pm 2\%$, and the quality of the preparations was high, with $88 \pm 1\%$ of the mitochondria intact. These values were similar to previous work (4, 22). The recovery did not differ between the fiber types and was $24 \pm 4, 17 \pm 3$, and $20 \pm 2\%$ of total mitochondria for WG, Sol, and RG, respectively. The quality was also not different between the fiber types and was $84 \pm 5, 90 \pm 1$, and $88 \pm 2\%$ intact mitochondria for WG, Sol, and RG, respectively.

There were no significant differences between the whole muscle homogenate CS activity in the fed compared with the fasted state in any muscle (Table 1). With the combined data, RG > Sol > WG CS activity, confirming accuracy in muscle sampling.

**Blood results.** Free fatty acid and β-hydroxybutyrate (ketone) levels were elevated after 24 h of starvation. Glycerol levels were not significantly different, and plasma insulin and glucose decreased (Table 2).

**PDK activity.** In fed rats, PDK activity was the lowest in WG, and two- to threefold higher in Sol and RG (Fig. 1). In all muscle fiber types, the activity of PDK was two- to threefold greater compared with the fed state for that muscle. RG and Sol PDK activity in fasted rats was approximately twofold greater than WG.

**PDK isoform protein and mRNA.** There was less PDK4 protein in WG compared with Sol and RG in fed rats. PDK4 protein content was greater in the fasted state compared with the fed in all muscles, increasing approximately twofold in the RG and Sol muscles and approximately fourfold in the WG. In the fasted muscles, differences between the fiber types were maintained as WG contained less PDK4 than Sol and RG (Fig. 2). PDK2 was not different between muscle types in the fed or the fasted rats. There was no effect of fasting on PDK2 protein in any of the muscle fiber types (Fig. 3). PDK1 was four- to fivefold less in WG compared with Sol and RG in both the fasted and the fed rats. PDK1 content was not different between muscle types in any muscle (Fig. 4).

PDK4 mRNA content was not different between muscle types in the fed state. In all muscles, fasting caused large increases (2- to 5-fold) in PDK4 mRNA content, with the greatest change observed in the RG. Fiber type differences were maintained in fasted rat muscle with WG containing less PDK4 mRNA than the

<table>
<thead>
<tr>
<th>Fiber Type</th>
<th>Fed</th>
<th>Fasted</th>
<th>Combined</th>
</tr>
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<tbody>
<tr>
<td>Sol</td>
<td>33.7 ± 1.9</td>
<td>29.8 ± 2.4</td>
<td>31.8 ± 1.3</td>
</tr>
<tr>
<td>RG</td>
<td>37.8 ± 4.2</td>
<td>35.0 ± 3.1</td>
<td>36.4 ± 1.9</td>
</tr>
<tr>
<td>WG</td>
<td>12.9 ± 0.7</td>
<td>15.8 ± 1.4</td>
<td>14.4 ± 0.7</td>
</tr>
</tbody>
</table>

Values are means ± SE in µmol min$^{-1}$ g wet muscle$^{-1}$. There was no significant difference between values for fed vs. fasted rat muscles. For combined data white gastrocnemius (WG) < soleus (Sol) < red gastrocnemius (RG).
RG (Fig. 5). PDK2 mRNA levels were not different between muscle types in the fed rats. With fasting, the PDK2 mRNA was increased in RG but was unaltered in the Sol or WG muscles (Fig. 6). PDK1 mRNA was less in WG compared with both Sol and RG in both fed and fasting muscles. With fasting, PDK1 mRNA content was unchanged in all fiber types compared with fed (Fig. 7).

DISCUSSION

This study describes fiber type variations in the response of PDK activity, protein, and mRNA in all three of the major muscle fiber types represented by WG (FG), RG (FOG), and Sol (SO) muscles after a 24-h fast. It is the first study to compare the expression of all the rat skeletal muscle PDK isoforms (PDK4, 2, and 1) and the specific mRNA level in the three fiber types. The major finding was that the fast-twitch glycolytic muscle differs from the oxidative muscles in total PDK activity and isoform expression. WG displays 50% less PDK activity in fed as well as in fasted rats, and this difference is mirrored in PDK4 protein and mRNA changes. PDK2 protein and mRNA were not different between the fiber types and unaffected by fasting, except for an increase in PDK2 mRNA in fast-twitch oxidative muscle. Another novel finding of this study was that PDK1 expression is much lower in glycolytic compared with oxidative muscles but is unchanged in response to fasting.

Few studies have examined PDK activity or expression in skeletal muscle in response to fasting (11, 28, 29, 32). Most studies examined changes in mixed skeletal muscle, which would exhibit the combined proper-

| Table 2. Plasma insulin and FFA concentrations and whole blood β-HB, glycerol, and glucose concentrations |
|-----------------|-----------------|
| Fed             | Fasted          |
| Insulin, mIU/L  | 29.9 ± 8.5      | 8.6 ± 2.6*     |
| FFA, mM         | 0.40 ± 0.11     | 0.65 ± 0.12*   |
| β-HB, mM        | 0.18 ± 0.01     | 1.17 ± 0.04*   |
| Glycerol, mM    | 0.09 ± 0.04     | 0.12 ± 0.02*   |
| Glucose, mM     | 6.4 ± 0.4       | 4.9 ± 0.3*     |

Values are means ± SE. FFA, free fatty acids; β-HB, β-hydroxybutyrate. *Significantly different from fed rats.
ties of all the fiber types (11, 32), or have limited the investigation to type I fibers (28). Recently Sugden et al. (29) compared the effect of a 48-h fast on PDK activity and PDK4 and 2 protein content in slow-twitch (Sol and adductor longus) and fast-twitch muscle (tibialis anterior). Their results demonstrated that PDK activity and PDK4 protein levels were increased in both slow- and fast-twitch muscle. However, the tibialis anterior is a mixture of FOG (24%), FG (36%), and "transition" fibers (type IIX or IID; 31%) (8). We chose the WG (92% FG) muscle to examine whether type IIB fibers behaved in a different manner than the type IIA fibers, which are abundant in the RG. The RG is almost devoid of type IIB fibers (1%) (8).

Fig. 4. PDK1 protein content of WG, Sol, and RG muscles from fed (open bars) and 24-h fasted (solid bars) rats. Standard SDS-PAGE and electrophotography was performed (5 μg of mitochondrial protein per lane). Immunodetection was performed using polyclonal antisera directed against PDK1 followed by 125I-labeled Protein A. Blots were exposed to films overnight. Relative density was quantified, and results are expressed as the intensity of the band in arbitrary units. Blots were stripped and reprobed with antisera against the pyruvate dehydrogenase complex, and the E1α-subunit band was used to normalize loading. Fast-twitch glycolytic (type IIB), slow-twitch oxidative (type I), and fast-twitch oxidative (type IIA) fibers are represented by WG, Sol, and RG muscles, respectively. Bars with the same letters are not significantly different.

Fig. 5. PDK4 mRNA content of WG, Sol, and RG muscles from fed (open bars) and 24-h fasted (solid bars) rats. Fed WG, lanes 1, 2; fasted WG, lanes 3, 4; fed Sol, lanes 5, 6; fasted Sol, lanes 7, 8; fed RG, lanes 9, 10; fasted RG, lanes 11, 12. Total RNA (6 μg per lane) was loaded onto 1% denaturing agarose gels and run with MOPS-acetate-EDTA buffer (pH 7.4) at 75 V for 4 h and capillary blotted onto nylon membrane. PDK4 cDNA was 32P-labeled and hybridized to blot as described in METHODS. Films were exposed for 20–24 h. Relative density was quantified, and results are expressed as the intensity of the band in arbitrary units. Fast-twitch glycolytic (type IIB), slow-twitch oxidative (type I), and fast-twitch oxidative (type IIA) fibers are represented by WG, Sol, and RG muscles, respectively. Bars with the same letters are not significantly different.

Fig. 6. PDK2 mRNA content of WG, Sol, and RG muscles from fed (open bars) and 24-h fasted (solid bars) rats. Fed WG, lanes 1, 2; fasted WG, lanes 3, 4; fed Sol, lanes 5, 6; fasted Sol, lanes 7, 8; fed RG, lanes 9, 10; fasted RG, lanes 11, 12. Total RNA (6 μg per lane) was loaded onto 1% denaturing agarose gels and run with MOPS-acetate-EDTA buffer (pH 7.4) at 75 V for 4 h and capillary blotted onto nylon membrane. PDK2 cDNA was 32P-labeled and hybridized to blot as described in METHODS. Films were exposed for 20–24 h. Relative density was quantified, and results are expressed as the intensity of the band in arbitrary units. Fast-twitch glycolytic (type IIB), slow-twitch oxidative (type I), and fast-twitch oxidative (type IIA) fibers are represented by WG, Sol, and RG muscles, respectively. Bars with the same letters are not significantly different.

Fig. 7. PDK1 mRNA content of WG, Sol, and RG muscles from fed (open bars) and 24-h fasted (solid bars) rats. Fed WG, lanes 1, 2; fasted WG, lanes 3, 4; fed Sol, lanes 5, 6; fasted Sol, lanes 7, 8; fed RG, lanes 9, 10; fasted RG, lanes 11, 12. Total RNA (6 μg per lane) was loaded onto 1% denaturing agarose gels and run with MOPS-acetate-EDTA buffer (pH 7.4) at 75 V for 4 h and capillary blotted onto nylon membrane. PDK1 cDNA was 32P-labeled and hybridized to blot as described in METHODS. Films were exposed for 48 h. Relative density was quantified, and results are expressed as the intensity of the band in arbitrary units. Fast-twitch glycolytic (type IIB), slow-twitch oxidative (type I), and fast-twitch oxidative (type IIA) fibers are represented by WG, Sol, and RG muscles, respectively. Bars with the same letters are not significantly different.
Measurement of muscle CS activity confirmed that the RG was the most oxidative muscle, followed closely by Sol, as previously reported. These oxidative muscles have much higher CS activities than the WG muscle, containing mainly type IIB glycolytic fibers.

**PDK activity.** In both fed and the fasted rat muscles, PDK activity in the glycolytic (WG) muscle was less than in the oxidative muscles. This distinction was unrelated to the contractile properties of the muscle, because both RG and WG are fast-twitch muscles, which differ primarily in their oxidative capacity (8). This difference was reflected in many other processes responsible for oxidative glucose disposal in oxidative vs. glycolytic fibers. FG fibers have the lowest GLUT-4 content, glucose uptake, and insulin sensitivity compared with SO and FOG fibers (13, 26).

PDK activity increased markedly in all muscle types in response to starvation, but to a lower total activity in the WG. Previously, the effects of starvation were studied in mixed hindlimb muscle (11), mixed fast-twitch muscle (29), and in SO muscle (28), but this is the first study to document this increase in FG muscle alone. It is interesting to note that although both high-fat diet and starvation increase PDK activity in SO muscle, only starvation induced an increase in activity in mixed fast-twitch muscle (29), whereas no increase in activity was observed with a high-fat diet (10). In general, the high-fat diet is not as powerful a stimulus as starvation in rats, requiring 28 days to increase PDK activity in rat hearts and skeletal muscle compared with only 48 h of starvation (10, 21). It may be that the decreased PDK activity of the FG fibers in the mixed fast-twitch fibers contributes to the attenuated increase with the diet perturbation. Further work is needed to determine whether this differential response to the high-fat diet may be attributed to the FG or the FOG fibers.

Earlier studies examined the effect of 48 h of starvation on skeletal muscle PDK activity (10, 11, 21, 28, 29, 32). This work extends this time course information by documenting an increase in PDK activity at 24 h. It has also been observed that PDHa activity increased in as little as 15 h of fasting, but PDK measurements were not made (14).

**PDK4.** PDK4 is found primarily in rat heart and skeletal muscle and has been identified as the isoform that changes in parallel with observed PDK activity increases in heart and mixed skeletal muscle (32, 33). It has a high maximal activity (8-fold higher than PDK2, but ~62% of PDK1) and is relatively insensitive to inhibition by a synthetic pyruvate analog (dichloroacetate) compared with PDK2. The in vitro inhibitory constants for dichloroacetate in PDK2, 4, and 1 are 0.2, 0.5, and 1.0 mM, respectively (5). Therefore it has been suggested that the increase in PDK4 renders rat muscles fasted for 48 h less sensitive to pyruvate, requiring higher concentrations to inhibit PDK and promote PDH activation and increased glucose uptake (29).

Absolute PDK activity and PDK4 protein content were lowest in the FG fibers in both fed and fasted rats compared with the oxidative muscles. However, the increase in PDK activity after the fast (3-fold for WG and 2-fold for Sol and RG) correlated well with the increase in PDK4 protein (4-fold for WG and 2-fold for Sol and RG). The induction was higher in the FG fibers, and this observation is in agreement with Sugden et al. (29), who observed a 2.3-fold increase in PDK activity in the Sol and a 3-fold increase in the tibialis anterior (mixed fast-twitch) after 48 h of starvation. The greater relative increase in PDK activity and PDK4 protein in the WG and tibialis anterior muscles may be related to the pyruvate insensitivity of this isoform. Enhanced expression of this isoform would increase nonoxidative disposal of carbohydrate (lactate and alanine production) in muscle and recycling of the three carbon intermediates through gluconeogenesis in the liver.

It is interesting to note that the increases in PDK4 mRNA did not correlate with the increases in PDK4 protein or total activity across the fiber types. Although the mRNA increased in all fiber types, the relatively higher increase in WG protein and activity was not observed in mRNA. This suggests a differential regulation in FG fibers that may be posttranscriptional.

Lastly, decreased insulin concentration (or sensitivity) and increased fatty acid oxidation have been implicated in the upregulation of PDK activity and PDK4 mRNA and protein in response to starvation and diabetes (18, 21). The results of the present study are consistent with this suggestion, as PDK activity and PDK4 mRNA and protein increased within 24 h of fasting in all three fiber types. Despite the fact that rat FG fibers have markedly decreased insulin sensitivity and postreceptor signaling capacity compared with SO or FOG fibers (26), the fasting situation produced the largest relative increases in PDK activity and PDK4 protein in the FG fibers. This suggests that insulin sensitivity is not the only factor determining these changes.

**PDK2.** PDK2 is a more ubiquitous isoform of the kinase and is relatively resistant to change with dietary perturbation (5, 32). It appears to be more sensitive to redox potential in vitro. With decreasing NAD+/NADH ratio in the presence of acetyl-CoA, a threefold increase in PDK2 activity was observed (compared with 1.5- to 1.8-fold increases in PDK4 and 1) (5). PDK2 is more sensitive to dichloroacetate compared with PDK4, and it was believed that increasing the population of PDK2 relative to PDK4 would confer increased pyruvate sensitivity in intact mitochondria in mixed fast-twitch muscle from fed or fasted rats (29).

Results from the current study demonstrate that the concentrations of both PDK2 and 4 were similar between Sol and RG muscles in the fed state, and therefore one would not expect to see differences in pyruvate sensitivity between these muscles. The WG also had the same PDK2 content as Sol and RG but contained ~50% less PDK4 content in muscles from fed rats. This suggests that pyruvate sensitivity in FG fibers would be higher than FOG fibers (higher relative PDK2 content) and that the increased pyruvate sensitivity in mixed fast-twitch muscle from fed rats (29) is due to the FG fiber content.
Although an increase in PDK2 protein in mixed fast-twitch muscle was observed after a more prolonged (48 h) fast (29), we found no change in PDK2 protein in the three fiber types after a 24-h fast. However, PDK2 mRNA was increased in the RG. Together, these studies suggest that the time course of PDK2 upregulation is slower, with the increase in mRNA preceding a protein increase by 24 h in FOG fibers. This also suggests a differential activation between the oxidative and glycolytic fibers, with oxidative fast-twitch fibers responsible for the increase in PDK2 in mixed fast-twitch muscle (29). Further work on the time course of PDK2 induction in response to fasting is needed to fully explore this possibility.

**PDK1.** PDK1 is the least studied isoform in rat skeletal muscle. Although it is predominantly found in heart muscle (5), we demonstrated its presence in rat skeletal muscle in low amounts. It has a 13-fold higher maximal activity than PDK2 and hence a more profound effect at lower protein concentrations. It is similar to PDK4 in maximal activity but has demonstrated a two- to fivefold lower inhibition by dichloroacetate in vitro compared with PDK2 and 4 (5).

Our results indicate that PDK1 was expressed primarily in oxidative fibers, with almost none detected in FG fibers. Both protein and mRNA data indicate that PDK1 expression was not altered by fasting for 24 h. The physiological significance of this isoform is uncertain, because it is differentially regulated compared with PDK2 or 4. It is possible that PDK1 has the greatest contribution to overall PDK activity in oxidative muscles from fed rats, because PDK4 abundance is relatively low and PDK2 has a lower maximal activity.

In summary, the muscle fiber type distribution as it relates to the kinetic properties of the PDK isoforms is an important link in understanding the regulation of carbohydrate oxidation and its contribution to skeletal muscle glucose disposal. This study describes total PDK activity and the fiber type distribution of the three rat skeletal muscle PDK isoforms from fed and 24-h fasted rats. Total PDK activity was higher in both FG and SO muscles compared with FG muscle in both fed and fasted rats. In response to fasting, total activity increased two- to threefold in oxidative and glycolytic fiber types, respectively. These changes were reflected in PDK4 protein content and to a lesser extent in PDK4 mRNA. PDK2 protein levels were not different between the fiber types and unchanged in response to fasting. However, PDK2 mRNA increased in the FOG muscle with fasting, and further work is necessary to determine whether the time course of PDK2 induction plays a role in the increase in PDK activity. PDK1 mRNA and protein were unaltered by starvation. However, its distribution varied greatly, with almost no PDK1 in FG muscle, and larger amounts of mRNA and protein in the oxidative muscles. The functional significance of this is unknown.

The changes in mRNA in this study reflect only total content, with no information on whether this reflects an increase in transcriptional rate and/or an increase in specific mRNA stability. Insulin has been implicated in decreasing mRNA stability for other proteins (e.g., GLUT-4, glycogen synthase, and phosphoenolpyruvate carboxykinase) (20), and the starvation-induced decrease in insulin correlated with the increase in total PDK4 mRNA in all muscle types and PDK2 mRNA in the FOG muscle. Direct measurements of transcriptional rate, such as nuclear run-on, will be needed to further work in this area.

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