Mechanism Responsible for Inactivation of Skeletal Muscle Pyruvate Dehydrogenase Complex in Starvation and Diabetes

Pengfei Wu, Krista Inskeep, Melissa M. Bowker-Kinley, Kirill M. Popov, and Robert A. Harris

Regulation of the activity of the pyruvate dehydrogenase complex in skeletal muscle plays an important role in fuel selection and glucose homeostasis. Activation of the complex promotes disposal of glucose, whereas inactivation conserves substrates for hepatic glucose production. Starvation and diabetes induce a stable increase in pyruvate dehydrogenase kinase activity in skeletal muscle mitochondria that promotes phosphorylation and inactivation of the complex. The present study shows that these metabolic conditions induce a large increase in the expression of PDK4, one of four pyruvate dehydrogenase kinase isoenzymes expressed in mammalian tissues, in the mitochondria of gastrocnemius muscle. Refeeding starved rats and insulin treatment of diabetic rats decreased pyruvate dehydrogenase kinase activity and also reversed the increase in PDK4 protein in gastrocnemius muscle mitochondria. Starvation and diabetes also increased the abundance of PDK4 mRNA in gastrocnemius muscle, and refeeding and insulin treatment again reversed the effects of starvation and diabetes. These findings suggest that an increase in amount of this enzyme contributes to hyperphosphorylation and inactivation of the pyruvate dehydrogenase complex in these metabolic conditions. It was further found that feeding rats WY-14,643, a selective agonist for the peroxisome proliferator-activated receptor-α (PPAR-α), also induced large increases in pyruvate dehydrogenase kinase activity, PDK4 protein, and PDK4 mRNA in gastrocnemius muscle. Since long-chain fatty acids activate PPAR-α endogenously, increased levels of these compounds in starvation and diabetes may signal increased expression of PDK4 in skeletal muscle. *Diabetes* 48:1593–1599, 1999

From the Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana.

Address correspondence and reprint requests to Dr. Robert A. Harris, Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, 635 Barnhill Dr., Indianapolis, IN 46202-5122. E-mail: raharris@iupui.edu.

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E1, pyruvate dehydrogenase component of pyruvate dehydrogenase complex; PDK, pyruvate dehydrogenase kinase; PPAR-α, peroxisome proliferator-activated receptor-α.

Regulation of the activity of the pyruvate dehydrogenase complex is of critical importance in all mitochondria-containing cells of higher eukaryotes. The complex has to be active when the complete oxidation of glucose is needed for the generation of energy, whereas its activity has to be severely suppressed when glucose is in short supply, and three carbon compounds are needed for gluconeogenesis. Regulation of the activity of the complex is accomplished in large part by interconversion of its pyruvate dehydrogenase component (E1) between an active nonphosphorylated form and an inactive phosphorylated form. Phosphorylation of E1 is catalyzed by pyruvate dehydrogenase kinase (PDK); dephosphorylation, by pyruvate dehydrogenase phosphatase. Mammalian tissues express four PDK isoenzymes (1) and two pyruvate dehydrogenase phosphatase isoenzymes (2). These isoenzymes have unique regulatory properties and differ in their level of expression in different cell types, thereby establishing a means for tissue-specific control of the pyruvate dehydrogenase complex (1,2). Recent work indicates that expression of two of the isoenzymes, PDK2 and PDK4, is subject to modulation by the nutritional and hormonal state of an animal (3–5). Starvation and chemically induced diabetes increase the amount of PDK2 protein present in rat liver mitochondria (3,4) and the amount of PDK4 protein present in rat heart mitochondria (5). Increased phosphorylation of the pyruvate dehydrogenase complex as a result of increased PDK4 expression and activity has been proposed to be important for suppression of glucose oxidation in the heart in starvation and diabetes (5).

We conducted the present study to determine whether the expression of PDK2 and PDK4 is also subject to regulation in skeletal muscle. Substantial differences exist in the manner in which fuel selection is regulated in skeletal muscle versus cardiac muscle and liver (6–10). For example, glycolysis and flux of carbon through the pyruvate dehydrogenase complex are more sensitive to inhibition by fatty acids in the perfused rat heart than in the perfused rat hindquarter (6,7) or isolated muscle preparations (8,9). Liver and skeletal muscle express different isoforms of carnitine palmitoyl transferase I, the most important regulatory enzyme of fatty acid oxidation, whereas heart expresses both isoforms (10). Another important reason to investigate skeletal muscle is that its large mass relative to the rest of the body makes the capacity of this tissue for glucose oxidation particularly important for blood glucose homeostasis (11).

Work by others has demonstrated that both starvation and diabetes induce a substantial increase in PDK activity in skeletal muscle (12–14). We show here that these conditions increase the expression of PDK4 in skeletal muscle. We also report the novel observation that the peroxisome proliferator WY-14,643 increases PDK4 expression in skeletal muscle. Based on this finding, the hypothesis is advanced that the increase in long-chain fatty acids caused by starvation and diabetes signals increased PDK4 expression by activation of the peroxisome proliferator-activated receptor-α (PPAR-α).

RESEARCH DESIGN AND METHODS

Materials. Arylamine acetyltransferase was partially purified from pigeon liver by modification of a basic procedure (15) described previously (5). Protamine-zinc insulin for the treatment of diabetic rats was purchased from Anthony Products (Arcadia, CA). [^{125}I]Protein A for Western blot analysis was obtained from ICN Biochemicals (Irvine, CA). The peroxisome proliferator WY-14,643 was from BIOMOL Research Laboratories (Plymouth Meeting, PA). Radioactive nucleotides required for cDNA probes were obtained from NEN Life Science Products (Boston, MA). The random primed DNA labeling kit used to label cDNA probes was from Boehringer Mannheim (Indianapolis, IN). Other chemicals were from Sigma (St. Louis, MO).

Animals. Male Wistar rats obtained from Harlan Industries (Indianapolis, IN) with initial body weights of ~200 g were housed in a temperature- and light-controlled room. Rats were fed Purina rodent laboratory chow (Purina Mills, Richman, IN) ad libitum for 3 days before initiation of experiments. Food was removed from cages of the rats 48 h before they were killed for determination of the effects of starvation. To induce diabetes, overnight fasted rats were injected intravenously with 65 mg streptozotocin/kg body wt. Free access to food and water was then provided until the rats were killed. Half of the diabetic animals were killed 48 h after administration of streptozotocin. The rest of the diabetic animals were started on subcutaneous injections of protamine-zinc insulin (2 U/100 g body wt) 48 h after administration of streptozotocin. Insulin was administered every 12 h until these animals were killed 48 h later. The last insulin injections were given 2 h before the rats were killed.

To study the effects of WY-14,643, rats were fed powdered Purina rodent laboratory chow containing 0.1% WY-14,643 for 3 days.

Enzyme assays. Mitochondria were isolated from gastrocnemius muscle basically by the procedure described by Kerbey et al. (16). Before assay of its activity, the pyruvate dehydrogenase complex was activated completely through the action of endogenous pyruvate dehydrogenase phosphatase by incubating mitochondria with the uncoupler carbonyl cyanide *m*-chlorophenylhydrazone (5,17,18). The mitochondria were then disrupted, and the activity of the complex was assayed spectrophotometrically at 30°C by coupling the generation of acetyl-CoA to the acetylation of P-(P-aminophenylazo)benzene sulfonic acid by arylamine acetyltransferase (5,19). PDK activity was assayed at 30°C in an assay buffer (pH 7.5) of 1 mmol/l $MgCl_2$, 5 U/ml creatine phosphate kinase, 10 mmol/l phosphocreatine, 5 mmol/l KF, and 50 mmol/l potassium phosphate. The reaction was started by adding 0.2–0.4 mg of mitochondrial extract protein followed by 1 mmol/l ATP to 160 µl of the assay buffer. It was terminated by taking 35-µl samples at 15, 30, and 60 s and diluting them with the pyruvate dehydrogenase complex assay buffer given above for immediate assay of residual pyruvate dehydrogenase complex activity by the coupled arylamine acetyltransferase assay. PDK activities are expressed as first-order rate constants for pyruvate dehydrogenase inactivation by ATP, calculated with the single exponential decay option of GraFit version 3.03, Erithacus Software.

Western blot analysis of mitochondrial total protein. Mitochondria previously frozen and stored at –70°C were dissolved in a solution (pH 6.8) of 50 mmol/l Tris-Cl, 2% sodium dodecylsulfate, 0.1 mol/l dithiothreitol, 0.1% bromophenol blue, 10% glycerol, 1 mmol/l benzamidine, 0.1 mg/ml trypsin inhibitor, 1 µg/ml aprotinin, 0.1 mmol/l Nα-p-tosyl-L-lysine chloromethyl ketone, 1 µmol/l leupeptin, and 1 µmol/l pepstatin A. The samples were boiled for 3 min and cooled on ice for 5 min. Western blot analysis was conducted as described in detail previously (20) with polyclonal antisera against recombinant rat PDK2 and PDK4 that were generated in rabbits by a standard protocol (21). Standard curves established by loading four different amounts of each of the recombinant PDK isoenzymes on SDS-PAGE gels were used to quantitate the amounts of the individual PDK isoenzymes as described previously (5). The E1α subunit of the pyruvate dehydrogenase complex was quantitated with rabbit antiserum raised against purified rat pyruvate dehydrogenase complex.

Northern blot analysis of total RNA. Total cellular RNA was extracted from freeze-clamped gastrocnemius muscle with Tri Reagent (Sigma), following the instructions of the manufacturer. Northern blotting was conducted by the protocol previously described (22). [^{32}P]-Labeled cDNAs of rat PDK2 and PDK4 were used as probes for the hybridization. The oligonucleotide used as probe to detect rat 28S rRNA was synthesized in the Biochemistry Biotechnology Facility of Indiana University according to the sequence published by Chan et al. (23).

Image quantification. Relative densities of bands on films were determined with a U-SCAN-IT GEL automated digitizing system (version 4.1) for Windows from Silk Scientific.

Statistical analysis. Data were analyzed for statistical significance by Student's *t* test for unpaired samples.

TABLE 1
Effect of starvation, diabetes, and WY-14,643 on PDK activity in mitochondria isolated from rat gastrocnemius muscle

Experiment	Treatment of rats	PDK activity (min^{-1})
1	Control	0.53 ± 0.08
	Starved	1.46 ± 0.04*
	Starved/refed	0.63 ± 0.12
2	Control	0.48 ± 0.06
	Diabetic	1.82 ± 0.26*
	Diabetic + insulin	0.93 ± 0.06†
3	Control	0.74 ± 0.05
	WY-14,643	1.55 ± 0.18*

Data are means ± SE and were obtained with four rats in each group. Control animals were untreated in each experiment. Experiment 1: one group of rats was starved for 48 h; another group was starved for 48 h and then refed chow diet for 48 h. Experiment 2: one group of rats was rendered diabetic with streptozotocin and killed after 48 h; another group of 48-h diabetic rats was treated with insulin for 48 h. Experiment 3: rats were fed 0.1% WY-14,643 in a chow diet for 3 days. *$P < 0.01$ vs. control group; †$P < 0.05$ vs. diabetic animals.

RESULTS

Effects of starvation and diabetes on PDK activity in mitochondria isolated from rat gastrocnemius muscle. In agreement with findings reported previously by others (12–14), PDK activities of mitochondria isolated from skeletal muscle (gastrocnemius) were increased nearly threefold by 48-h starvation of rats and nearly fourfold by streptozotocin-induced diabetes (Table 1). Refeeding of starved rats was effective in returning PDK activity to values near those of control animals (Table 1). Insulin treatment of the diabetic animals was likewise effective in reducing PDK activity, although not completely back to the low PDK activity found in control animals (Table 1). The rats treated with streptozotocin were diabetic as determined by their blood glucose levels (mean ± SE 376 ± 16 mg/dl; four rats/group) at the time they were killed. Likewise, treatment of 48-h diabetic rats with long-lasting insulin every 12 h for another 48 h was effective therapy for the diabetes as determined by a lowering of blood glucose levels into the normal range at the time they were killed (106 ± 13 mg/dl; four rats/group).

Effects of starvation and streptozotocin-induced diabetes on the amounts of PDK2 and PDK4 protein in mitochondria isolated from rat gastrocnemius muscle. Western blot analysis was used to determine whether the increased PDK activities caused by starvation and diabetes were due to increased expression of PDK protein. The two PDK isoenzymes that are expressed in significant amounts in rat skeletal muscle (1), PDK2 and PDK4, were examined in detail in this study. PDK1 and PDK3 are most abundantly expressed

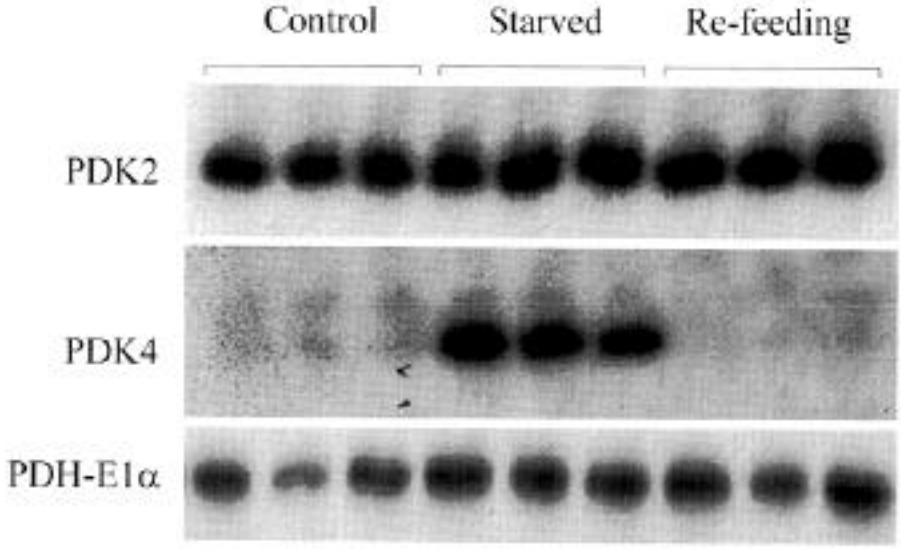

FIG. 1. Effects of fasting and refeeding on the amounts of PDK2 and PDK4 protein in rat gastrocnemius muscle mitochondria. Rabbit antisera raised against PDK2 and PDK4 were used to detect these proteins by Western blot analysis. Lanes correspond to 5 µg of mitochondrial protein isolated from the gastrocnemius muscle of separate rats. There were four rats in each group of control fed, 48-h starved, and 48-h starved/48-h refed rats. Data for three rats from each group are shown; the same result was obtained for the fourth animal in each group on a separate Western blot. The findings were confirmed in a separate experiment with different rats. Rabbit antiserum raised against the pyruvate dehydrogenase complex was used to detect the E1α subunit as a loading control.

in the heart and testis, respectively, and are not readily quantitated at the low levels present in gastrocnemius muscle mitochondria. Starvation had no effect and diabetes caused only a slight increase in the amount of PDK2 protein present in gastrocnemius muscle mitochondria (Figs. 1 and 2). In contrast, starvation and diabetes caused 3.5- and 5.2-fold increases, respectively, in the amounts of PDK4 protein expressed in these mitochondria (Figs. 1 and 2). Quantification of the amounts of PDK2 and PDK4 relative to each other revealed that starvation and diabetes induced PDK4 to be the more abundant of the two proteins expressed in gastrocnemius muscle. The percentage of PDK4 relative to the total PDK2 plus PDK4 increased from 22 ± 2% in the control animals to 57 ± 3% in the starved animals and 61 ± 4% in the diabetic animals (four rats/group). Refeeding was effective in reversing the increases in PDK4 protein caused by starvation (Fig. 1). Insulin treatment was likewise effective in lowering PDK4 protein present in the mitochondria of diabetic rats (Fig. 2), but PDK4 protein was not decreased completely back to the low level of control animals by insulin treatment, consistent with the lack of complete reduction of PDK activity of diabetic skeletal muscle back to that of control animals by this treatment (Table 1).

Effects of starvation and streptozotocin-induced diabetes on the abundance of PDK2 and PDK4 mRNAs in rat gastrocnemius muscle. The treatment of animals in this study was not found to have significant effects on the relative abundance of the mRNA encoding PDK2 in gastrocnemius muscle (Figs. 3 and 4). However, 3.8- and 7.5-fold increases in abundance of the mRNA encoding PDK4 were induced by starvation and diabetes, respectively (Figs. 3 and 4). Refeeding the starved rats for 48 h lowered PDK4 message to a level comparable to that observed in control animals (Fig. 3). Insulin treatment of the diabetic animals was likewise effective in lowering the PDK4 message level, although it was not lowered completely back to the level of the control animals (Fig. 4). The latter finding again follows from the lack of complete effectiveness of insulin treatment in restoring normal PDK activity (Table 1) and PDK4 protein amounts (Fig. 2) in diabetic rats, most likely because the insulin treatment

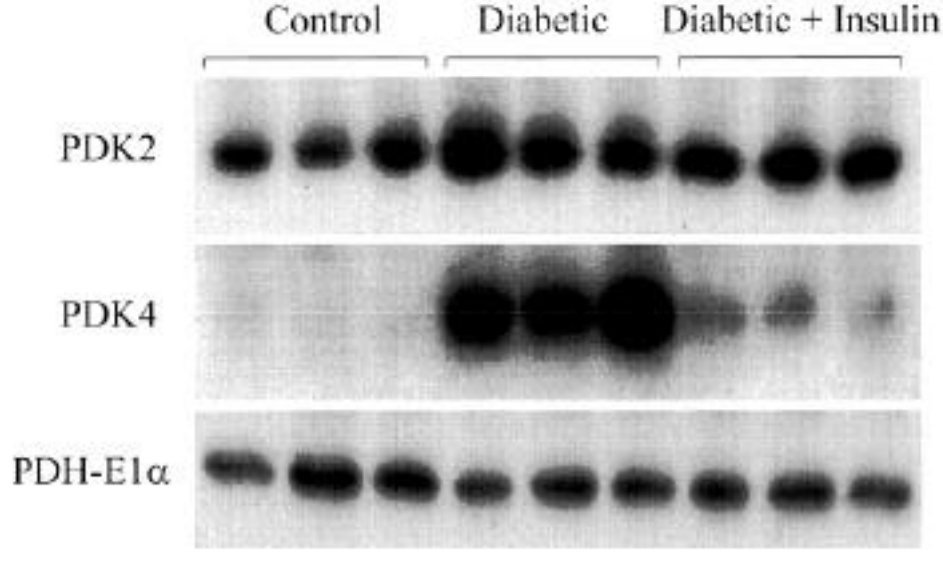

FIG. 2. Effects of experimental diabetes and insulin treatment on the amounts of PDK2 and PDK4 protein in rat gastrocnemius muscle mitochondria. The procedure used for Western blot analysis is described in the legend for Fig. 1. Each lane corresponds to a different animal. There were four rats in each group of control, 48-h diabetic, and 48-h insulin-treated diabetic rats. Data for three rats from each group are shown; the same result was obtained for the fourth animal in each group on a separate Western blot.

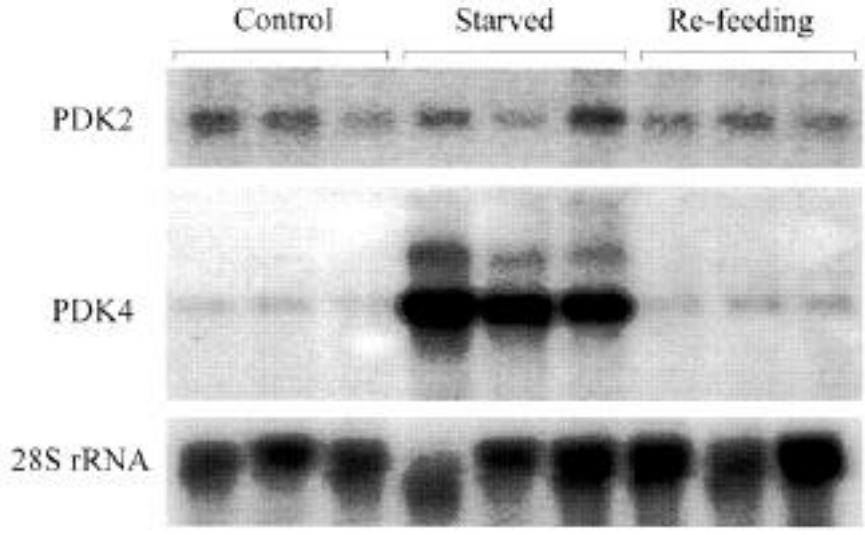

FIG. 3. Effects of fasting and refeeding on the abundance of the mRNAs encoding PDK2 and PDK4 in rat gastrocnemius muscle. [^{32}P]-labeled cDNAs of PDK2 and PDK4 were used to detect the corresponding mRNAs by Northern blot analysis. Lanes correspond to 10 µg of total RNA isolated from the skeletal muscle of different rats. There were four rats in each group of fed, 48-h starved, and 48-h starved/48-h refed rats. Data for three rats from each group are shown; the same result was obtained for the fourth animal in each group with a separate Northern blot. Comparable results were obtained in a separate experiment with different animals. Messenger RNA sizes were 2.4 kb for PDK2 and 3.9 kb for PDK4. Ribosomal RNA (loading control) was detected with [^{32}P]-labeled antisense 28S rRNA.

protocol used was not sufficient to completely control diabetes in these animals.

Effect of supplementing the diet with WY-14,643 on PDK activity and PDK4 expression in gastrocnemius muscle. Maintaining rats on a diet containing 0.1% WY-14,643 for 3 days caused a twofold increase in PDK activity of mitochondria isolated from gastrocnemius muscle (Table 1). This treatment likewise caused a 3.9-fold increase in PDK4 protein present in the mitochondria (Fig. 5*A*) and a 7.7-fold increase in abundance of PDK4 mRNA in this tissue (Fig. 5*B*).

DISCUSSION

The oxidation of glucose and lactate by skeletal muscle is markedly inhibited by starvation (6), a result in part of marked inhibition of the activity of the pyruvate dehydrogenase complex (7,12,24–27). Inhibition of the complex can in turn be explained by an increase in the extent to which its E1 component is phosphorylated—a consequence of a change in the balance between the relative enzymic capacities of its phosphatases and kinases. The possibility of long-term regulation at the level of pyruvate dehydrogenase phosphatase deserves more study, particularly now that it is realized that more than one isoenzyme of this phosphatase is expressed in tissues (2). Thus far, however, it has not been possible to demonstrate a significant effect of different metabolic states on the activity of this phosphatase in various tissues (16,28,29). In contrast, there is considerable evidence that starvation markedly increases PDK activity in many tissues, including skeletal muscle. Part of this activation of PDK in skeletal muscle mitochondria is likely the result of increased availability of fatty acids and ketone bodies in the blood of starved animals. Oxidation of these compounds produces increased mitochondrial levels of NADH and acetyl-CoA (8,12,30), which are well established to cause increased PDK activity via reduction and acetylation of the lipoyl residues of the dihydrolipoamide transacetylase component of the complex (31). Supplementing this allosteric mechanism is another means for regulation of PDK activity, one that requires several hours of starvation to produce a "stable" increase in PDK activity. This long-term mechanism was ini-

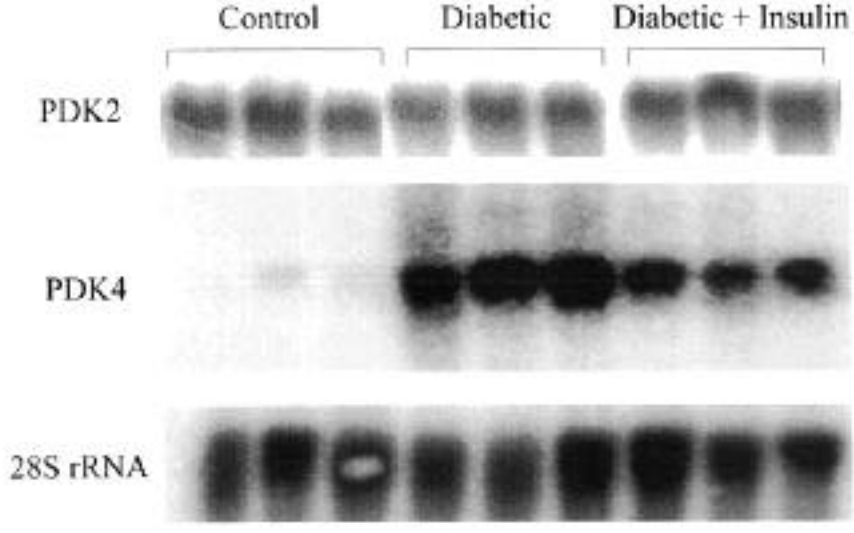

FIG. 4. Effects of experimental diabetes and insulin treatment on the abundance of the mRNAs encoding PDK2 and PDK4 in rat gastrocnemius muscle. The procedure used for Northern blot analysis is described in the legend for Fig. 3. There were four rats in each group of control, diabetic, and insulin-treated diabetic rats. Data for three rats from each group are shown; the same result was obtained for the fourth animal of each group on a separate Northern blot.

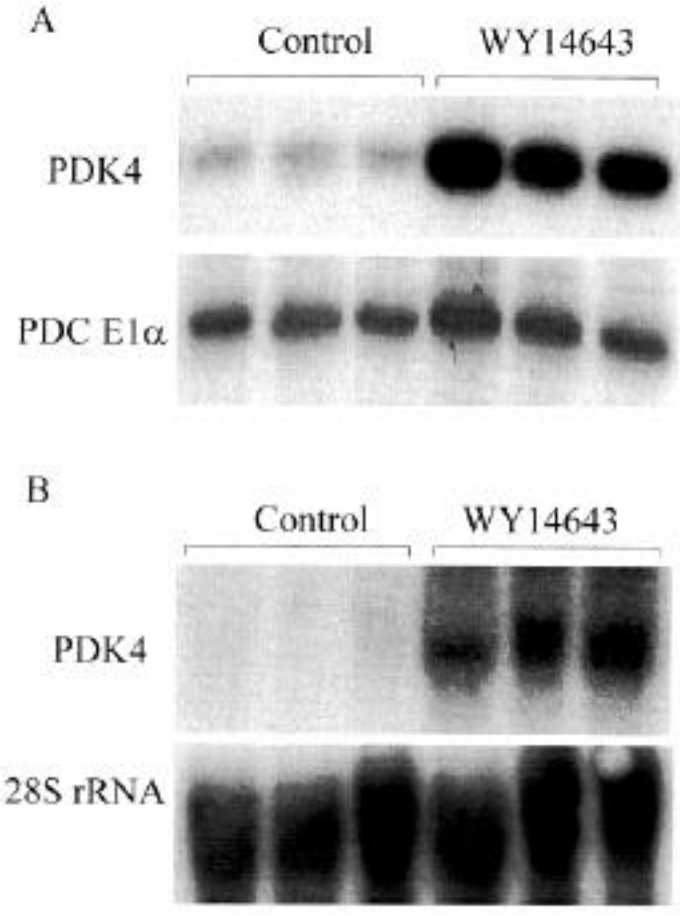

FIG. 5. Effect of feeding WY-14,643 on the amount of PDK4 protein in rat gastrocnemius muscle mitochondria and the abundance of the mRNA encoding PDK4 in rat gastrocnemius muscle. The procedures used for Western (*A*) and Northern (*B*) blot analyses are described in the legends for Figs. 1 and 3, respectively. There were four rats in the control and WY-14,643-treated groups. Data for three rats from each group are shown; the same result was obtained for the fourth animal in each group on separate Western and Northern blots.

tially proposed to exist because starvation was observed to have a greater inhibitory effect on pyruvate dehydrogenase activity than can be explained on the basis of changes in NADH and acetyl-CoA levels (17,32). A relatively long latency period also occurs during starvation before maximum inactivation of the complex is produced (12,17,32), consistent with significant time being required for the synthesis of a component involved in the inactivation mechanism. Finally and most importantly, starvation has been shown to induce a "stable" increase in PDK activity that cannot be explained by small molecule activators of the enzyme (12–14). The present study documents that starvation induces a large increase in the amount of PDK4 protein present in mitochondria isolated from rat gastrocnemius muscle. This provides a molecular explanation for the increase in skeletal muscle PDK activity as well as the latency in starvation-induced inactivation of the pyruvate dehydrogenase complex. Thus, increased PDK activity due to increased PDK4 protein expression is likely to be an important contributing factor to the low activity of the pyruvate dehydrogenase complex occurring in skeletal muscle in the starved state. The physiologic advantage for the animal relates to the importance of inhibiting the oxidation of glucose by skeletal muscle in the starved state to conserve glucose for the brain (33). Increased expression of PDK4 in skeletal muscle is probably an important component of the mechanism by which euglycemia is normally maintained during starvation. Furthermore, inactivation of the muscle pyruvate dehydrogenase complex by phosphorylation as a consequence of increased PDK4 activity serves to conserve three carbon compounds needed by the liver for gluconeogenesis in the starved state. Muscle mass is also conserved when the pyruvate dehydrogenase complex is inactivated, since less muscle protein has to be broken down to satisfy the need for compounds that can be converted to glucose (33).

Experimentally induced diabetes also causes the pyruvate dehydrogenase complex to become hyperphosphorylated and therefore inactivated in skeletal muscle of rats (7,12,13,24,34). One consequence is increased lactate production by skeletal muscle (34), which probably promotes overproduction of glucose from lactate by the liver of diabetic animals. Diabetes induced in this manner is also known to cause a stable increase in PDK activity in skeletal muscle (12–14), and we further found in the present study that diabetes induces a large increase in PDK4 protein in this tissue. It is likely, therefore, that increased expression of PDK4 is an important factor in producing hyperphosphorylation and low activity of skeletal muscle pyruvate dehydrogenase in the diabetic state. Although increasing PDK activity is critically important during starvation to conserve glucose, it is counterproductive in the diabetic state, because it spares gluconeogenic precursors in the face of abundant levels of glucose and other fuels in the blood. New therapies for diabetes could result from creating a compound that specifically inhibits the activity of PDK4 or, alternatively, finding a means to prevent overexpression of PDK4.

Based simply on blood glucose determinations, the insulin dose rate used in the present study to treat the diabetic rats appeared adequate. However, PDK activity of the diabetic rats treated with insulin was not restored completely back to that of control animals. Likewise, the amount of PDK4 protein and the abundance of its mRNA remained higher than those of control animals, as would be expected if altered PDK4 expression is responsible for changes in PDK activity. Since blood glucose was determined 2 h after the final administration of insulin, the simplest explanation for this finding is that the diabetes was not completely controlled over the 12-h intervals between insulin injections. Alternatively, a long latency period may exist before insulin treatment is completely effective in downregulating PDK4 expression. This would allow continued sparing of carbohydrate for the synthesis of glycogen, as previously suggested for the refeeding transition of starved animals by Sugden et al. (35).

Increased expression of PDK4 was shown previously (5) to be responsible for the activating effects that starvation and diabetes have on PDK activity in rat heart (36,37). The present study demonstrates that increased expression of PDK4 also occurs in skeletal muscle in response to these metabolic conditions. In spite of the finding that the same mechanism operates in both tissues, it was critically important to determine that this really was the case with skeletal muscle. The regulatory mechanisms for glucose catabolism differ considerably in the two tissues (6,8,9,24), and skeletal mus-

cle is the more important for glucose homeostasis because of its large mass relative to other tissues of the body.

Starvation and diabetes were found to have little or no effect on the level of PDK2 protein in rat gastrocnemius muscle. We previously found that these conditions did not affect the amount of PDK2 protein expressed in rat heart (5). Thus, long-term control of PDK activity appears to specifically involve PDK4 expression in both skeletal and cardiac muscle tissues. This is not the case for all tissues, since starvation has significant effects on expression of PDK2 in rat liver (3,4). Preliminary studies indicate that PDK4 is also induced by starvation in rat liver but not in brain and adipose tissue (P.W., K.M.P., R.A.H., unpublished observations). Thus, tissue-specific regulation of expression of individual PDK isoenzymes is emerging as an important mechanism for control of the pyruvate dehydrogenase complex in different metabolic states.

We found that feeding rats the peroxisome proliferator WY-14,643 mimics the effect of starvation and diabetes on PDK4 expression in gastrocnemius muscle. WY-14,643 is a very potent and selective agonist for PPAR-α (38,39). Binding of WY-14,643 to PPAR-α (39,40) results in *trans*-activation of the transcription of several genes that encode key enzymes of lipid metabolism, including peroxisomal fatty acyl-CoA oxidase (41), mitochondrial medium-chain acyl-CoA dehydrogenase (42), and mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (43). The best studied target tissue for the effects of WY-14,643 is liver, the tissue that expresses PPAR-α most abundantly (44). To our knowledge, this is the first report that WY-14,643 induces the expression of an important mitochondrial regulatory enzyme in skeletal muscle. Although it is possible that WY-14,643 exerts its effects on PDK4 expression in skeletal muscle by some indirect mechanism not involving PPAR-α activation, this form of the receptor is expressed in skeletal muscle cells (44), and most of the effects of WY-14,643 in animals appear traceable to PPAR-α activation (45). Apparent induction of PDK4 by this mechanism is of considerable interest because of the growing evidence that long-chain fatty acids or their metabolites function as the naturally occurring activators for PPAR-α (39,46–49). Thus, the dramatic increase in free fatty acids occurring in starvation and diabetes may signal increased expression of PDK4 in skeletal muscle by way of PPAR-α activation. Liver is already known to respond to starvation and diabetes with increased expression of enzymes induced by PPAR-α activation (50,51), presumably because the high levels of free fatty acids in these metabolic states act as endogenous peroxisome proliferators (51). Operation of this novel mechanism in skeletal muscle may contribute to the remarkable effectiveness of fatty acids in depressing glucose utilization in long-term starvation and diabetes. It may also account for the dramatic increase in PDK4 expression in the heart during starvation and diabetes, and may explain why PDK activity is increased in several tissues when rats are treated in other ways that increase free fatty acid levels, such as high-fat feeding (52). Finally, these findings raise the question of whether alterations in free fatty acid levels in other health risk conditions for humans, such as obesity, type 2 diabetes, hyperthyroidism, and hypertension, cause overexpression of PDK4 and inactivation of the pyruvate dehydrogenase complex. The defective glucose metabolism and insulin resistance characteristic of these conditions may be explained in part by such a mechanism.

Many factors other than fatty acids are likely involved in regulation of PDK4 gene expression. Indeed, the work of others suggests some other signaling molecules that may contribute to the regulation. For example, dibutyryl cyclic AMP causes a stable increase in PDK activity in soleus muscle strips (14) and cardiac myocytes in culture (53), and insulin prevents these effects in the latter model system (53). Insulin likewise reverses the effect of diabetes on skeletal muscle PDK activity (13) and PDK4 protein, as indicated in this study, making it a good candidate for negative regulation of PDK4 expression. Positive effects may be exerted by other factors that increase during starvation and in the diabetic state, such as glucocorticoids, catecholamines, and ketone bodies. Studies are currently being conducted with cultured cell lines in an effort to further define the mechanisms responsible for regulation of PDK4 expression.

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