J Appl Physiol 96: 2082–2087, 2004.
First published February 13, 2004; 10.1152/japplphysiol.01318.2003.

Pyruvate dehydrogenase activation and kinase expression in human skeletal muscle during fasting

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Submitted 9 December 2003; accepted in final form 5 February 2004

Spriet, Lawrence L., Rebecca J. Tunstall, Matthew J. Watt, Kate A. Mehan, Mark Hargreaves, and David Cameron-Smith. Pyruvate dehydrogenase activation and kinase expression in human skeletal muscle during fasting. *J Appl Physiol* 96: 2082–2087, 2004. First published February 13, 2004; 10.1152/japplphysiol.01318.2003.—Fasting forces adaptive changes in whole body and skeletal muscle metabolism that increase fat oxidation and decrease the oxidation of carbohydrate. We tested the hypothesis that 40 h of fasting would decrease pyruvate dehydrogenase (PDH) activity and increase PDH kinase (PDK) isoform mRNA expression in human skeletal muscle. The putative transcriptional activators of PDK isozymes, peroxisome proliferator-activated receptor-α (PPAR-α) protein, and forkhead homolog in rhabdomyosarcoma (FKHR) mRNA were also measured. Eleven healthy adults fasted after a standard meal (25% fat, 60% carbohydrate, 15% protein) with blood and skeletal muscle samples taken at 3, 15, and 40 h postprandial. Fasting increased plasma free fatty acid, glycerol, and β-hydroxybutyrate concentrations and decreased glucose and insulin concentrations. PDH activity decreased from 0.88 ± 0.11 mmol acetyl-CoA · min^{-1} · kg wet muscle wt^{-1} at 3 h to 0.62 ± 0.10 (P = not significant) and 0.39 ± 0.06 ($P < 0.05$) mmol · min^{-1} · kg wet $mass^{-1}$ after 15 and 40 h of fasting. Although all four PDK isoforms were expressed in human skeletal muscle, PDK-2 and -4 mRNA were the most abundant. PDK-1 and -3 mRNA abundance was ~1 and 15% of the PDK-2 and -4 levels, respectively. The 40-h fast had no effect on PDK-1, -2, and -3 mRNA expression. PDK-4 mRNA was significantly increased ~3-fold after 15 h and ~14-fold after 40 h of fasting. Skeletal muscle PPAR-α protein and FKHR mRNA abundance were unaffected by the fast. The results suggest that decreased PDH activation after 40 h of fasting may have been a function of the large increase in PDK-4 mRNA expression and possible subsequent increase in PDK protein and activity. The changes in PDK-4 expression and PDH activity did not coincide with increases in the transcriptional activators PPAR-α and FKHR.

carbohydrate oxidation; fat oxidation; peroxisome proliferation-activated receptors; pyruvate dehydrogenase kinase isozymes; forkhead homolog in rhabdomyosarcoma; mRNA

FASTING PRECIPITATES THE PROTECTION of carbohydrate stores within the body by decreasing whole body carbohydrate oxidation, decreasing the release of glucose from the liver, and increasing gluconeogenesis (5, 30). Pyruvate dehydrogenase (PDH) activity (PDHa) controls the entry of carbohydrate into the tricarboxylic cycle and is regulated by PDH kinase (PDK), which phosphorylates and inactivates the enzyme, and PDH phosphatase, which dephosphorylates the enzyme to the active form (37). These enzymes are acutely controlled by allosteric regulators and over the longer term by changes in the stable activity of PDK (29–31). Skeletal muscle is a prime target for decreased PDHa and carbohydrate oxidation because it is a large tissue and the major determinant of resting metabolic rate (45). Furthermore, skeletal muscle exhibits marked metabolic flexibility, since diminished rates of carbohydrate oxidation are concomitant with increased adipose tissue-derived free fatty acid (FFA) availability to the muscle and enhanced fat oxidation rates (21, 23, 43).

Four distinct PDK isozymes exist in human skeletal muscle (14, 36). However, few investigations have examined the in vivo expression of all PDK isoforms in human skeletal muscle at rest or after alterations in physiological demands. Skeletal muscle PDK-4 transcription is increased in humans by high fat feeding (30) and both short and prolonged exercise (33) and is further enhanced by prolonged cycling with low muscle glycogen availability (32). The expression of both PDK-2 and -4 has been measured in response to 3 days of a high fat-low carbohydrate diet (30) and in Pima Indians predisposed to Type 2 diabetes (26). However, to our knowledge, PDK-1 and -3 have not been studied in vivo in human skeletal muscle.

Rodent studies have determined that fasting for 24–48 h increases the expression of PDK-4, increases PDK activity, and decreases PDHa in skeletal muscle (18, 19, 29, 40, 44). In humans, it has recently been demonstrated that PDK-4 transcription is elevated after a 20-h fast in skeletal muscle (34). It is currently unknown what transcriptional activators may be mediating the rapid alterations in PDK-4 mRNA abundance in human skeletal muscle in situations of altered substrate availability. Recently, the mRNA and protein expression of forkhead homolog in rhabdomysarcoma (FKHR), also known as FOXO1, was upregulated after a 24- to 48-h fast in mouse skeletal muscle (12, 24). Furthermore, direct binding of FKHR to the promoter region of PDK-4 upregulated PDK-4 mRNA in C2C12 cells (12). This suggests that FKHR may play an important role in the transcriptional activation of PDK-4 in human skeletal muscle after fasting. An additional possible transcriptional activator of PDK-4 in human skeletal muscle is peroxisomal proliferator-activated receptor (PPAR)-α. PPAR-α agonist treatment markedly activates PDK-4, suggesting that the endogenous rise in FFA with fasting potentiates PPAR-α activity by enhancing PDK-4 gene expression (16, 39, 44).

The present study was designed to examine the time course of a 40-h fast on the activity of PDH, the expression of the four

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PDK isozymes (PDK-1–4), and two putative transcriptional activators of PDK-4 (PPAR-α and FKHR) in human skeletal muscle. We hypothesized that 15 h of fasting ("overnight fast") would decrease PDHa and that 40 h of fasting would result in a further reduction. All four PDK isozymes were expected to be expressed in human skeletal muscle; however, it was hypothesized that PDK-4 mRNA would be increased at 15 h of fasting and that prolonged fasting to 40 h would cause further increases in PDK-4 mRNA and possibly increase the expression of PDK-1–3. It was further hypothesized that an increase in the expression of PPAR-α and FKHR would be evident at 15 h of fasting and further elevated after 40 h of fasting.

MATERIALS AND METHODS

Subjects. Eleven healthy adults (5 women, 6 men) volunteered to participate in the study. The mean (±SE) age, height, weight, and body mass index before the study were 25.5 ± 1.3 yr, 172.9 ± 3.1 cm, 68.0 ± 3.7 kg, and 22.5 ± 0.6 kg/m^2, respectively. All subjects fell into a physical activity range between recreationally active to well trained. Female subjects were premenopausal, and no control was made for the phase of the menstrual cycle. All experimental procedures were formally approved by the Ethics Committees of Deakin University and the University of Guelph. Before participation in the study, each subject was informed of the procedures and potential risks and provided written, informed consent.

Study design. On the day of the experiment, subjects were instructed to eat a normal lunch, which was assessed to be of similar composition between subjects (~50% carbohydrate, 30% fat, 20% protein). Subjects then arrived at the laboratory in late afternoon and consumed a standard meal containing 1,400 kcal that consisted of ~60% carbohydrate, 25% fat, and 15% protein. Subjects then fasted for 40 h, consuming only nonenergy-containing beverages without caffeine. During this period, subjects were instructed to refrain from physical activity and were encouraged to consume water ad libitum.

Blood sampling and analysis. Venous blood (~6 ml) was obtained from an antecubital vein at 3, 15, and 40 h during the fast. An aliquot of blood was placed in lithium heparin tubes and spun at room temperature, and the plasma was stored at −20°C until analysis. Plasma glucose and lactate were analyzed with an automated glucose analyzer (EML 105, Radiometer, Copenhagen, Denmark). Plasma insulin concentrations were determined by radioimmunoassay (Phadeseph, Pharmacia & Upjohn, Uppsala, Sweden). For analysis of β-hydroxybutyrate and glycerol, plasma was deproteinized with 3 M $HClO_4$ (1:1). The supernatant was directly analyzed for β-hydroxybutyrate with an enzymatic technique (2), and 400 μl of supernatant were added to 100 μl of 6 M KOH for the enzymatic determination of glycerol (7). Blood for the measurement of FFA (~2 ml) was mixed with 30 μl of EGTA and reduced glutathione and spun at 13,000 *g* for 2 min at room temperature, and the plasma was stored at −20°C until analysis with a Wako NEFA C test kit (Wako Chemicals, Richmond, VA).

Muscle sampling. Skeletal muscle samples were obtained at 3, 15, and 40 h of fasting under local anesthesia (1% Xylocaine) from the vastus lateralis using the percutaneous needle biopsy technique (3) modified to include suction (10). An ~15-mg portion of skeletal muscle was chipped from each biopsy under liquid nitrogen and analyzed for PDHa of the active form of the enzyme using the methods described by Putman et al. (35). The remaining frozen muscle tissue from the biopsy was stored at −80°C for subsequent analysis.

Total RNA isolation and reverse transcription. Total RNA from 10–25 mg of muscle was isolated using the FastRNA Kit-Green (BIO 101, Vista) protocol and reagents. Briefly, the samples were placed in 500 μl of chaotropic RNA stabilizing reagent, 500 μl of phenol, and 100 μl of chloroform. The tissue was processed with the FastPrep instrument (BIO 101, Vista) twice for 20 s at a setting of 5.5 separated by 5 min on ice and then centrifuged for 15 min at a rate of 13,000 rpm at 4°C. The aqueous phase was added to 500 μl of chloroform, vortexed for 10 s, and then incubated on ice for 10 min. Subsequently, it was inverted for 10 s and spun at 13,000 rpm for 2 min at 4°C to separate phases. The top aqueous phase was added to 500 μl of DEPC-treated and isopropanol-precipitated solution and 20 μl of NaCl (5 M), mixed, precipitated on ice for 1 h, and spun for 12 min at 13,000 rpm at 4°C. The liquid was removed, and the RNA pellet was washed with 500 μl of salt ethanol wash solution, spun for 5 min at 8,000 rpm, and resuspended in 8 μl of EDTA-treated water. First-strand cDNA was generated from 1 μg of RNA using avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI) as previously described by Wadley et al. (42). The cDNA was stored at −20°C for subsequent analysis.

mRNA quantification. A real-time PCR mix of 10× SYBR green PCR master mix (Applied Biosystems, Foster City, CA), forward and reverse primers (3 μM), and cDNA (12 ng) was run for 40 cycles of PCR in a volume of 25 μl.

Real-time PCR analysis. Primers were designed using Primer Express software package version 1.0 (Applied Biosystems) from gene sequences obtained from GenBank (PDK-1: NM002610; PDK-2: NM002611; PDK-3: NM005391; PDK-4: NM002612; FKHR: NM002015). A BLAST search for each primer confirmed homologous binding to the desired mRNA of human skeletal muscle (1). Primer sequences are shown in Table 1.

Quantification of mRNA expression was performed (in triplicate) by real-time PCR using the ABI PRISM 5700 sequence detection system (Applied Biosystems) as described previously (13). Fluorescent emission data were captured, and mRNA levels were quantitated using the threshhold cycle value.

To compensate for variations in input RNA amounts and efficiency of reverse transcription, β-actin (GenBank accession no. X00351) mRNA was quantified, and results were normalized to these values as described previously (41).

PPAR-α protein quantification. Samples were thawed in ice-cold lysis buffer (40 μl/mg muscle) containing 1% SDS, 1% IGEPAL (Sigma Chemical, St. Louis, MO) and complete protease inhibitor cocktail (Sigma Chemical) and then homogenized with a Polytron PT 1200 (Kinematica). Homogenates were spun at 10,000 rpm (4°C, 15 min), and the supernatants were removed and analyzed for total protein (BCA protein assay kit; Pierce, Rockford, IL). Denatured total

Table 1. *Gene primer and probe sequences*

Gene	Sense Primer (5′-3′)	Antisense Primer (5′-3′)
β-actin	GACAGGATGCAGAAGGAGATTACT	TGATCCACATCTGCTGGAAGGT
PDK-1	CCGCTCTCCATGAAGCAGTT	TTGCCGCAGAAACATAAATGAG
PDK-2	CCGCTGTCCATGAAGCAGTT	TGCCTGAGGAAGGTGAAGGA
PDK-3	CAAGCAGATCGAGCGCTACTC	CGAAGTCCAGGAATTGTTTGATG
PDK-4	CCCGAGAGGTGGAGCATTT	GCATTTTCTGAACCAAAGTCCAGTA
FKHR	TCATGGATGGAGATACAT	TCCTGCTGTCAGACAATCTGAAG

PDK, pyruvate dehydrogenase isoform; FKHR, forkhead homolog in rhabdomyosarcoma.

proteins (120 μg) from each sample were separated by electrophoresis on a 10% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane for 2 h at 50 mA/membrane (Muliphor II Nova Blot, Pharmacia Biotech). Membranes were blocked for 1 h with 5% skim milk in Tris-buffered saline (50 mM Tris·HCl, 750 mM NaCl, 0.25% Tween) and were incubated overnight at 4°C with a polyclonal anti-PPAR-α antibody (1:200 dilution) (Santa Cruz Biotechnology, Santa Cruz, CA). Membranes were washed (4 × 5 min), followed by a 60-min incubation with anti-rabbit IgG conjugated to horseradish peroxidase (1:8,000 dilution) (Santa Cruz Biotechnology) and then washed again. Immunoreactive bands were detected with enhanced chemiluminescence (SuperSignal West Pico chemiluminescence substrate, Pierce). An internal control of previously extracted human muscle was used in each gel to normalize for variation in signal observed across the membranes. The membranes were exposed to Biomax Light Film (Eastman Kodak, Rochester, NY) and developed with Fuji FPM 100A (Fuji Film, Elmsfors, NY). Protein bands were quantified using the Electrophoresis documentation and analysis system 120 (Kodak Digital, Rochester, NY).

Statistical analysis. All data are presented as means ± SE. Data were analyzed with one-way ANOVA with repeated measures. Post hoc analysis was performed to determine differences using Tukey's test where appropriate. Significance was set at $P < 0.05$.

RESULTS

Plasma metabolites. Plasma glucose and insulin concentrations fell significantly after 15 and 40 h of fasting, compared with 3 h postprandial (Table 2). Plasma FFA concentration was significantly elevated after 15 h of fasting and to a new higher level at 40 h, whereas β-hydroxybutyrate and glycerol were significantly elevated only after 40 h (Table 2). Plasma lactate concentrations remained unchanged after 40 h of fasting (Table 2).

PDHa. PDHa decreased over time during fasting and was significantly lower at 40 h (Fig. 1).

PDK mRNA expression. PDK-2 before fasting was the most abundant PDK isoform in human skeletal muscle, but its expression was unaffected by 40 h of fasting (Fig. 2). PDK-4 abundance at 3 h was ~15% of the PDK-2 isoform before fasting, but fasting increased PDK-4 expression by ~3-fold at 15 h and ~14-fold after 40 h (Fig. 2). The PDK-4 abundance after 40 h fasting was approximately twofold higher than the PDK-2 isoform. The presence of PDK-1 and -3 was detectable in human skeletal muscle, although the abundance was only ~1% of the PDK-2 isoform at 3 h (Fig. 2). Both PDK-1 and -3 expression were unaffected by 40 h of fasting.

PPAR-α and FKHR expression. The protein expression of the transcription factor PPAR-α was unaffected by 15 and 40 h of fasting (Fig. 3). The mRNA expression of FKHR was unchanged after 15 and 40 h of fasting (Fig. 4).

Table 2. *Venous plasma metabolite, fuel, and hormone concentrations during 40 h of fasting*

	3 h	15 h	40 h
Lactate, mM	1.5±0.2	1.0±0.2	1.3±0.2
Glucose, mM	5.3±0.2	4.7±0.2*	4.3±0.1*
Glycerol, μM	27±9	64±12	134±29*†
Insulin, pM	124.1±18.0	53.4±7.4*	43.5±8.2*
Free fatty acids, mM	0.09±0.1	0.37±0.07*	0.83±0.11*†
β-Hydroxybutyrate, mM	0.018±0.004	0.032±0.007	0.88±0.245*†

Values are means ± SE; $n = 11$ subjects. *Significantly different from 3 h ($P < 0.05$). †Significantly different from 15 h ($P < 0.05$).

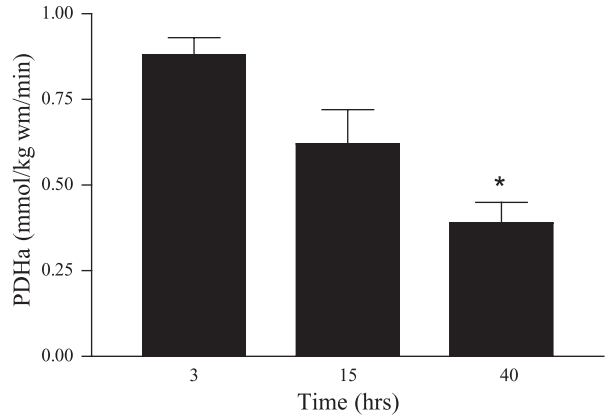

Fig. 1. Pyruvate dehydrogenase activation (PDHa) in human skeletal muscle during 40 h of fasting. wm, Wet muscle. *Significantly different from 3 h ($P < 0.05$).

DISCUSSION

This study examined the effects of a 40-h fast on resting PDHa and the expression of the four PDK isoforms in human skeletal muscle. The results confirm and extend previous findings in humans (34) to demonstrate that PDK-4 mRNA expression is increased by ~3-fold after 15 h and by ~14-fold after 40 h of fasting, with no alteration in the expression of PDK-1–3. To our knowledge, this is the first study to directly

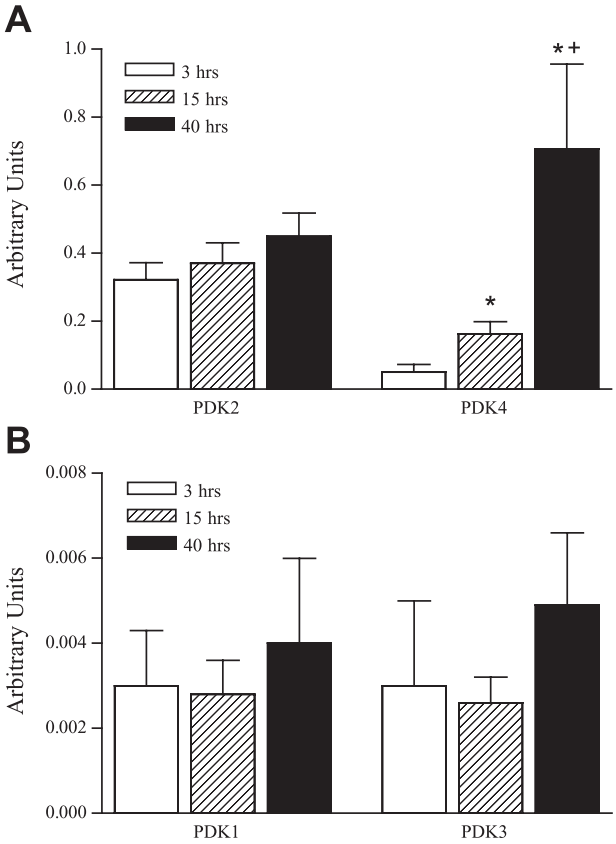

Fig. 2. Pyruvate dehydrogenase kinase (PDK) isozyme-1, -2, -3, and -4 mRNA expression in human skeletal muscle during 40 h of fasting. *Significantly different from 3 h ($P < 0.05$). +Significantly different from 15 h ($P < 0.05$).

examine the in vivo expression of PDK-1 and -3 isoforms in human skeletal muscle. PDHa was markedly lower after 40 h of fasting, which suggests that the transcriptional regulation of PDK-4 after fasting may have a significant impact on the synthesis of the translated functional protein to increase PDK protein and activity in resting skeletal muscle. Importantly, the present study is the first to demonstrate that, unlike findings in rodents, the fasting-induced upregulation of PDK-4 expression was not associated with an increase in the mRNA expression of FKHR or protein abundance of PPAR-α.

Expression of PDK isoforms in human skeletal muscle. Until recently, little was known regarding the expression of the PDK isoforms in human skeletal muscle. Majer et al. (26) reported that PDK-2 expression was more abundant than PDK-4 (~26% of PDK-2) in the Pima Indians. The results of the present study are in reasonable agreement because, 3 h after a meal, PDK-2 mRNA expression was the most abundant and PDK-4 expression was ~15% of PDK-2 mRNA. The subjects in the Majer et al. (26) study were overnight fasted, whereas the subjects in this study were 3 h postprandial. Because the present results demonstrated that an overnight fast increased PDK-4 expression, postprandial sampling of muscle in the Majer et al. (26) study would have likely revealed lower PDK-4 expression and decreased the relative expression closer to the 15% reported in the present study.

It has been reported that the human PDK-3 isoform is only present in heart and skeletal muscle (14), is not present in rat skeletal muscle (4), and has the highest specific activity of the four isoforms (~25-fold higher than PDK-2). This suggests that PDK-3 may play a unique role in the regulation of PDK activity, PDH activation, and carbohydrate oxidation in human skeletal muscle (14). However, the in vivo expression of PDK-3 in the present study was only ~1% of the PDK-2 abundance and was not responsive to a 40-h fast. The PDK-1 isoform has also been identified in human skeletal muscle and has a specific activity ~13-fold higher than PDK-2. However, the in vivo abundance measured in this study was low and unresponsive to fasting. From these data, it appears that PDK-2 and -4 are the dominant isoforms in human skeletal muscle. Despite the lower abundance of PDK-4 relative to PDK-2 (~15%), the specific activity of PDK-4 is ~10-fold higher than PDK-2 (4). This suggests that the two isoforms may contribute about equally to postprandial PDK activity in human skeletal muscle. Although PDK-1 and -3 have high specific activity, they do not appear to contribute significantly to basal PDK activity or to the upregulation in response to fasting in human skeletal muscle due to their very low abundance.

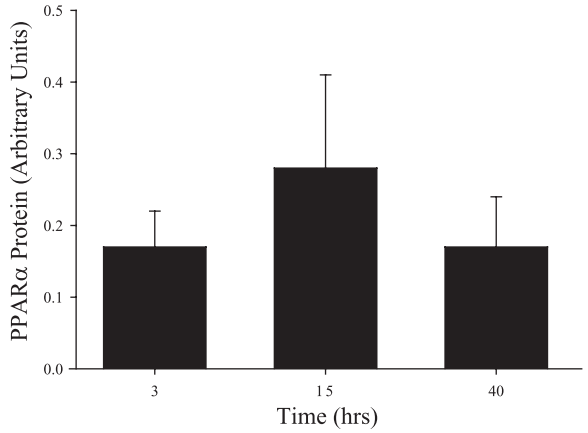

Fig. 3. Peroxisome proliferator-induced receptor-α (PPAR-α) protein expression in human skeletal muscle during 40 h of fasting.

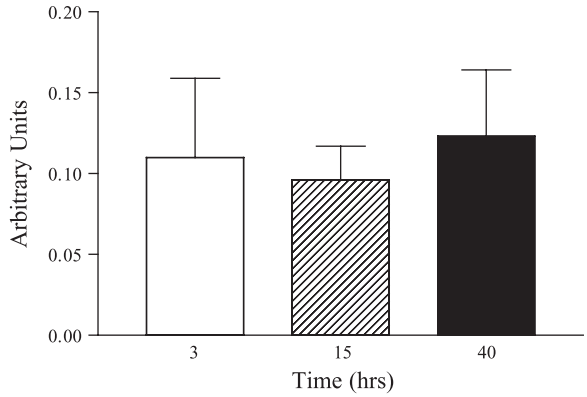

Fig. 4. Forkhead homolog in rhabdomysarcoma mRNA expression in human skeletal muscle during 40 h of fasting.

Time course of increased PDK-4 expression and reduced PDHa. The expression of PDK-4 increased approximately threefold after 15 h of fasting, whereas PDHa was not significantly decreased. By 40 h of fasting, PDK-4 expression had further increased ~14-fold above the postprandial level, and PDHa was significantly decreased by 55% from postprandial activation. A 29% decrease in PDHa occurred at 15 h, and a decrease was observed in 8 of 11 subjects. However, this interindividual variability precluded this decrease from reaching statistical significance. By 40 h of fasting, all subjects demonstrated a large decrease in PDHa. Although PDK-4 protein and PDK activity were not measured in the present study, they were increased in concert with increased PDK-4 mRNA in human skeletal muscle within 24 h of exposure to a high-fat diet (30). The present data suggest that the increase in PDK-4 mRNA and a possible subsequent increase in protein content may be responsible for increasing total PDK activity, such that PDH shifted away from the active dephosphorylated form toward the inactive phosphorylated form. The decreased PDHa would contribute to decreasing carbohydrate oxidation during fasting.

The PDK-4 isozyme is predominantly expressed in heart and skeletal muscle in both rats and humans (4). The present study confirms and extends previous findings of fasting experiments in humans (34) and rats examining PDK-4 expression and PDHa. Early work reported that fasting decreased PDHa (6, 11, 15) and increased PDK activity (11, 38) in mixed rat skeletal muscle. A 48-h fast decreased PDHa by ~50% in the predominantly slow-twitch soleus muscle and between ~65 and 90% in muscles that contained mainly fast-twitch fibers (19). Refeeding for 2 h restored PDHa by 45–75%, and by 6 h PDHa was completely restored. Others have reported increased PDK-4 mRNA and protein and increased PDK activity after 24–48 h of fasting in rat slow-twitch oxidative, fast-twitch oxidative-glycolytic, and fast-twitch glycolytic skeletal muscle (29, 40, 44). PDK activity was also less sensitive to inhibition by pyruvate after fasting, because the PDK-4 isozyme is less sensitive to pyruvate than PDK-2 (37).

In general, fasting did not cause large changes in PDK-1 and -2 expression in rat skeletal muscle (29, 40). However, PDK-2

mRNA was increased by 24 h of fasting in fast-twitch oxidative muscle (29), and PDK-2 protein was increased after 48 h of fasting in mixed fast-twitch muscle (40). In contrast, in the present study, 40 h of fasting caused no changes in PDK-1 and -2 mRNA in human skeletal muscle.

Putative mediators of the fasting-induced upregulation of PDK-4 expression. There has been considerable discussion as to the mechanism responsible for the increased expression of skeletal muscle PDK-4 in response to fasting. Human skeletal muscle PDK-4 expression increases in situations where carbohydrate availability and insulin levels are decreased and FFAs are increased, including fasting, high fat-low carbohydrate diets, and exercise (present study, Refs. 30, 33, 34).

FKHR. FKHR, a member of the subfamily of forkhead-type transcription factors, has been demonstrated to be upregulated after a 24- to 48-h fast in mouse skeletal muscle (12, 24) and rat liver (22). Indeed, the increased FKHR mRNA and protein were closely matched to the induction of the PDK-4 gene (12). These researchers further demonstrated that FKHR bound directly to the promoter region and activated PDK-4 transcription in C2C12 cells (12). Thus increased FKHR gene expression and protein synthesis enable greater PDK-4 transcription. FKHR localizes to the nucleus and is regulated by phosphorylation on the serine 256 residue, leading to its nuclear export (8). However, serine 256 phosphorylation was not increased by fasting in mouse skeletal muscle (12). In the present study, no increase in FKHR in human skeletal muscle was measured after 40 h of fasting. Therefore, it is unlikely that FKHR is mediating the observed elevation in PDK-4 mRNA abundance in the fasted state in human skeletal muscle. However, FKHR has also been shown to be upregulated in mice after treadmill running and streptozotocin-induced diabetes (24). Both exercise and diabetes result in elevated PDK-4 mRNA expression in skeletal muscle (33, 44). Therefore, further studies are required in humans to elucidate the possible role of FKHR in mediating PDK-4 expression in these physiological conditions.

PPAR-α. As reviewed for rat skeletal muscle (16, 39), the majority, but not all (9), of the evidence favors a major role for FFA activation of PPAR-α as the mechanism that upregulates PDK-4 mRNA expression. PPAR-α has been identified as having a critical transcriptional regulatory role in the response to fasting, with PPAR-α required for the upregulated expression of genes involved in mitochondrial fatty acid oxidation (25). PPAR-α activation is also significant in the regulation of lipid oxidative genes, including PDK-4 in human skeletal muscle (28). The present study demonstrated no upregulation of PPAR-α protein content during the 40-h fast, despite large increases in PDK-4 mRNA. Although these results are contrary to the majority of findings in rat skeletal muscle, the present study does concur with recent findings in mice that demonstrated no upregulation of PPAR-α in skeletal muscle after a 24- to 48-h fast (20). In addition, Holness et al. (17) demonstrated that fatty acid-induced PDK-4 protein expression in oxidative skeletal muscle does not involve the obligatory participation of PPAR-α. Furthermore, in PPAR-α knockout mice, the fasting-induced upregulation of PDK-4 was unaffected in skeletal muscle compared with wild-type controls, whereas PPAR-δ (also referred to as PPAR-β) was several times more abundant than PPAR-α and -γ in these PPAR-α knockout mice (27). This suggests that PPAR-δ may compensate for the lack of PPAR-α in the knockout mice. PPAR-δ was increased two- to threefold in skeletal muscle of fasted mice (20), which is suggestive of a role for PPAR-δ in mediating the fasting-induced upregulation of PDK-4. It therefore seems possible that increased PPAR-δ may be responsible for the fasting-induced upregulation of PDK-4 expression in human skeletal muscle, although this needs to be tested.

In summary, this study examined the effects of a 40-h fast on resting PDHa and PDK isoform expression in human skeletal muscle. A major finding of the study was the rapid increase in PDK-4 mRNA expression by ~3-fold after 15 h and ~14-fold after 40 h of fasting. PDH activity was significantly lower after 40 h of fasting, suggesting a PDK-4-mediated increase in PDK protein and activity and a decrease in carbohydrate oxidation in resting skeletal muscle. Contrary to findings in rat skeletal muscle, the fasting-induced upregulation of PDK-4 expression was not the result of an induction of FKHR mRNA abundance or PPAR-α protein content.

ACKNOWLEDGMENTS

We thank Dr. Andrew Garnham for excellent medical skills and the volunteers who participated in the study.

GRANTS

This study was supported by operating grants from Deakin University (Priority Area Funding) and the Natural Science and Engineering Research Council of Canada.

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