Original Article

The STAT5A-Mediated Induction of Pyruvate Dehydrogenase Kinase 4 Expression by Prolactin or Growth Hormone in Adipocytes

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The purpose of this study was to determine whether pyruvate dehydrogenase kinase (PDK)4 was expressed in adipocytes and whether PDK4 expression was hormonally regulated in fat cells. Both Northern blot and Western blot analyses were conducted on samples isolated from 3T3-L1 adipocytes after various treatments with prolactin (PRL), growth hormone (GH), and/or insulin. Transfection of PDK4 promoter reporter constructs was performed. In addition, glucose uptake measurements were conducted. Our studies demonstrate that PRL and porcine GH can induce the expression of PDK4 in 3T3-L1 adipocytes. Our studies also show that insulin pretreatment can attenuate the ability of these hormones to induce PDK4 mRNA expression. In addition, we identified a hormone-responsive region in the murine PDK4 promoter and characterized a STAT5 binding site in this region that mediates the PRL (sheep) and GH (porcine) induction in PDK4 expression in 3T3-L1 adipocytes. PDK4 is a STAT5A target gene. PRL is a potent inducer of PDK4 protein levels, results in an inhibition of insulin-stimulated glucose transport in fat cells, and likely contributes to PRL-induced insulin resistance.
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It is well known that growth hormone (GH) and prolactin (PRL) induce signaling via the JAK-STAT pathway. In particular, STAT5 proteins are potently activated by these hormones (rev. in 1). GH is known to have profound effects on lipid metabolism (rev. in 2). The effects of PRL have been well characterized in mammary tissues, yet there is also evidence demonstrating that this hormone can affect adipose tissue in mice and humans (3,4). Yet, few molecular targets for the STAT5-mediated actions of GH and PRL on adipocytes have been identified. Although multiple lines of recent evidence suggest that STAT5 proteins can modulate adipocyte function (5–11), very few studies have identified direct STAT5 target genes in adipocytes. We recently observed that the GH and PRL inhibition of fatty acid synthase (FAS) transcription was mediated by a STAT5A binding site in the rat FAS promoter (12). Hence, our current efforts have been to identify other genes associated with glucose or lipid metabolism that are directly modulated by STAT5 proteins in adipocytes. In this study, we present data demonstrating that pyruvate dehydrogenase kinase (PDK)4 is a STAT5A target gene.

PDK is a family of kinases that negatively regulate the activity of the pyruvate dehydrogenase complex (PDC) (rev. in 13). There are four tissue-specific isoforms of PDK, PDK1–4, that have been identified in mammals, and each has different patterns of gene expression (14–16). The specificity in distribution, expression, and activity of each PDK isoform contributes to the long-term regulation of PDC in a given tissue and thus, in part, regulates glucose metabolism. There are several conditions that result in the short-term regulation of PDC activity (17–19). The long-term regulation of PDK that occurs in starvation (18,20) and diabetes involves an increase in the amount of PDK protein, which results in stable increases in PDK activity (21). An induction of PDK4 leads to decreased glucose oxidation, which results in hyperglycemia. PDK4 expression has also been shown to be induced by high-fat feeding in skeletal muscle (22). A study in nondiabetic Pima Indians, a group prevalently stricken with obesity and type 2 diabetes, has demonstrated that increased levels of muscle PDK4 expression positively correlate with increased fasting plasma levels of insulin and negatively correlate with insulin-mediated glucose uptake (23). It has also been documented that insulin has the ability to suppress PDK4 expression in skeletal muscle and hepatoma cells (23–25). Increased insulin sensitivity in post-obese patients has also been shown to be associated with decreased PDK4 expression (26).

In our present study, we examined the modulation of PDK4 in adipose tissue. Our studies clearly demonstrate that PRL and GH can induce the expression of PDK4 in 3T3-L1 adipocytes. Our studies also show that insulin pretreatment has the ability to attenuate the ability of these hormones to induce PDK4 expression. In addition, we identified a hormone-sensitive region in the murine PDK4 promoter and characterized a STAT5 binding site in this region, which mediates the hormonal effects on PDK4 expression. Also, when PDK4 protein was induced, we observed an inhibition in insulin-stimulated glucose up-

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AOX, acyl Co-A oxidase; DMEM, Dulbecco's modified Eagle's medium; EMSA, electrophoretic mobility shift assay; ERK, extracellular signal–related kinase; FAS, fatty acid synthase; FBS, fetal bovine serum; GH, growth hormone; MAPK, mitogen-activated protein kinase; PDC, pyruvate dehydrogenase complex; PDK, pyruvate dehydrogenase kinase; PGC, peroxisome proliferator–activated γ coactivator; PRL, prolactin; SOCS, suppressors of cytokine signaling.

take. In summary, our studies indicate that PDK4 can be induced in adipocytes and may play a role in insulin resistance.

RESEARCH DESIGN AND METHODS

Dulbecco's modified Eagle's medium (DMEM) was purchased from Invitrogen. Fetal bovine serum (FBS) was purchased from Atlanta Biologicals, and calf serum was purchased from Biosource. PDK4 antibodies were purchased from Abgent. Porcine and human GH and sheep PRL were purchased from Sigma. STAT5A antibodies were purchased from Santa Cruz. An antibody directed against active mitogen-activated protein kinase (MAPK) was purchased from Promega. [α-^{32}P]dCTP was purchased from Perkin-Elmer and Amersham Biosciences. Deoxynucleotide thymine triphosphate, dATP, and dGTP were purchased from Amersham Biosciences. Oligonucleotides were purchased from Integrated DNA Technologies. DNAse polymerase I large (Klenow) fragment was purchased from Promega.

Cell culture. Murine 3T3-L1 preadipocytes were plated and grown to 2 days after confluence in DMEM containing 10% bovine serum. Medium was changed every 48 h. Cells were induced to differentiate by changing the medium to DMEM containing 10% FBS, 0.5 mmol/l 3-isobutyl-methylxanthine, 1 μmol/l dexamethasone, and 1.7 μmol/l insulin (MDI). After 48 h, this medium was replaced with DMEM supplemented with 10% FBS, and the cells were maintained in this medium until used for experimentation. For serum deprivation, cells that were between 7 and 10 days after MDI were placed in DMEM supplemented with 0.15% fatty acid free and growth factor–depleted BSA for 16–20 h.

Preparation of whole-cell extracts. Cell monolayers were rinsed with PBS and then harvested in a nondenaturing buffer containing 10 mmol/l Tris (pH 7.4), 150 mmol/l NaCl, 1 mmol/l EGTA, 1 mmol/l EDTA, 1% Triton X-100, 0.5% Nonidet P-40, 1 μmol/l phenylmethylsulfonyl fluoride, 1 μmol/l pepstatin, 50 trypsin inhibitory mU aprotinin, 10 μmol/l leupeptin, and 2 mmol/l sodium vanadate. Samples were extracted for 30 min on ice and centrifuged at 15,000 rpm at 4°C for 15 min. Supernatants containing whole-cell extracts were analyzed for protein content by BCA analysis (Pierce) according to the manufacturer's instructions.

RNA analysis. Total RNA was isolated from cell monolayers with Trizol (Invitrogen) according to manufacturer's instructions with minor modifications. For Northern blot analysis, 15 μg total RNA was denatured in formamide and electrophoresed through a formaldehyde/agarose gel. The RNA was transferred to Zeta Probe-GT (Bio-Rad) in a buffer containing 75 mmol/l sodium citrate tribasic, 10 mmol/l NaOH, and 750 mmol/l NaCl. Probes were labeled by random priming using Klenow fragment and [α-^{32}P]dCTP.

Plasmid constructs. A 973-nucleotide segment of the PDK4 promoter (−949 to 24) was cloned from murine Sv/129 liver genomic DNA using the following PCR primers: forward, ATGACCCGCTAGCATGTTTC; reverse, GGTGAA GGGTTGACACTTGG. Nucleotides were added to the 5′ ends of each primer so that the forward primer could be digested by *Kpn*I and the reverse primer by *Sac*I for directional ligation into the multiple cloning site of pGL3-basic *Kpn*I/*Sac*I to create pGL3-PDK4pro. The STAT element in pGL3-PDK4pro (TTCTTGGAA) was mutated to TTTATGGAA using the QuickChange II Site Directed Mutagenesis kit (Stratagene) with the following primer and its antisense, CACGCTCCGCGGTGAGATTTATGGAAACAGTTTCTGGCTAG, to create pGL3-PDK4mut. Plasmids were sequenced for verification of wild-type and mutagenic nucleotide sequences using Big Dye Terminator Extension Reactions (ABI).

Transfections and luciferase reporter assays. 3T3-L1 adipocytes in six-well plates were transfected on day 3 of differentiation after addition of DMEM containing 10% FBS and 425 nmol/l insulin. Either 2.5 μg pGL3-PDK4pro or 2.5 μg pGL3-PDK4mut was cotransfected with 250 ng pRL-CMV/renilla vector to control for transfection efficiency in each well using Fugene 6 (Roche). After 32 h, cells were serum deprived overnight in DMEM with 0.15% BSA, untreated or treated with PRL for 2 or 4 h, and harvested. Cell lysates were assayed for firefly and renilla luciferase using the Dual Luciferase Reporter System (Promega). Relative light units were calculated by dividing firefly luciferase activity values by renilla luciferase.

Preparation of nuclear and cytosolic extracts. Cell monolayers were rinsed with PBS and then harvested in a nuclear homogenization buffer and centrifuged to isolate cytosolic and nuclear extracts as previously described (27).

Gel electrophoresis and immunoblotting. Proteins were separated in 7.5% polyacrylamide (National Diagnostics) gels containing SDS according to Laemmli (28) and transferred to nitrocellulose (Bio-Rad) in 25 mmol/l Tris, 192 mmol/l glycine, and 20% methanol. After transfer, the membrane was blocked in 4% milk overnight at 4°C. Results were visualized with horseradish peroxidase–conjugated secondary antibodies (Jackson ImmunoResearch Laboratories) and enhanced chemiluminescence (Pierce).

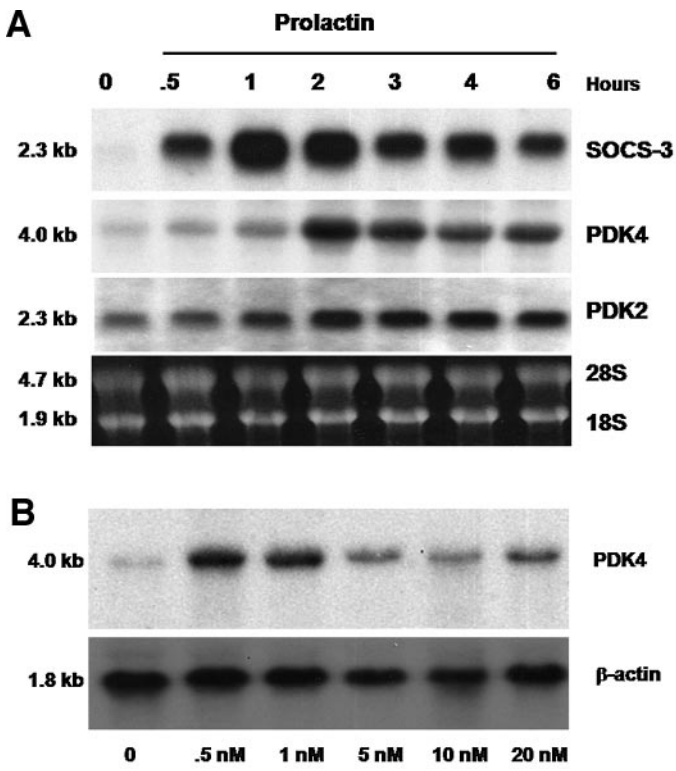


FIG. 1. PRL induces PDK4 expression in a time- and dose-dependent manner in 3T3-L1 adipocytes. *A*: Total RNA was isolated from serum-deprived 3T3-L1 adipocytes after 20 nmol/l (480 ng/ml) PRL treatment for the times indicated. Untreated cells (0) were also harvested at the start of the time course. Fifteen micrograms total RNA was electrophoresed, transferred to nylon, and subjected to Northern blot analysis with radiolabeled probes for PDK4, PDK2, and SOCS-3. Ethidium bromide staining of 28S and 18S RNA is also included. This is a representative experiment independently performed four times. *B*: Total RNA was isolated from serum-deprived 3T3-L1 adipocytes after a 2-h PRL treatment with the doses indicated. Untreated cells (0) were also harvested at the start of the experiment. Fifteen micrograms total RNA was subjected to Northern blot analysis. This is a representative experiment independently performed three times.

Electrophoretic mobility shift analysis. Double-stranded oligonucleotides were end-labeled with [α-^{32}P]dCTP using Klenow. Binding reactions were performed with nuclear extracts, according to Ritzenthaler et al. (29). The sequence of the oligonucleotide used was 5′-GTGAGATTCTTGGAAACAGTT-3′. Protein-DNA complexes were resolved and visualized as previously described (12). For supershift analysis, nuclear extracts were preincubated with 4 μg antibody for 1 h at room temperature.

Determination of [^{3}H] 2-deoxyglucose uptake. The assay of [^{3}H] 2-deoxyglucose uptake was performed as previously described (30). Before the assay, fully differentiated 3T3-L1 adipocytes were serum deprived for 4 h. Uptake measurements were performed in triplicate under conditions where hexose uptake was linear. The results were corrected for nonspecific uptake, and absorption was determined by [^{3}H] 2-deoxyglucose uptake in the presence of 5 μmol/l cytochalasin B. Nonspecific uptake and absorption was always <10% of the total uptake.

RESULTS

Several previous studies have demonstrated an induction of PDK4 in skeletal muscle (22,24); but, the induction of PDK4 expression in adipocytes has not been previously examined. In the course of studying activators of STAT5A, we observed an induction of PDK4 mRNA by PRL. Total RNA was isolated from fully differentiated 3T3-L1 adipocytes after treatment with PRL for the times indicated. PRL is known to induce the expression of suppressors of cytokine signaling (SOCS)-3. As shown in Fig. 1*A*, the

efficacy of PRL was demonstrated via the induction of SOCS-3, which occurred after a 30-min treatment. In addition, PRL induced the expression of PDK4 mRNA, as evidenced by the robust expression at 2 h. Between 2 and 6 h, the induced PDK4 mRNA was apparent. In addition, PRL did not have any substantial effect on the expression of PDK2. Ethidium bromide staining of 28S and 18S RNA was included as a loading control. To further examine the modulation of PDK4 by PRL, fully differentiated 3T3-L1 adipocytes were treated with various doses of PRL for 2 h. As shown in Fig. 1*B*, PRL treatment resulted in a dose-dependent increase in PDK4 mRNA. Physiological levels of PRL, 0.5 nmol/l (12 ng/ml), were capable of inducing PDK4 mRNA. An analysis of β-actin mRNA levels is included as a loading control.

Because PRL is a potent STAT5 activator, we also examined the effect of GH, another hormone known to activate STAT5 in adipocytes. As shown in Fig. 2*A*, a 2-h treatment with sheep PRL or porcine GH resulted in an induction of PDK4 mRNA. Yet, human GH did not have any effect on PDK4. Further analysis revealed that PRL and pig GH resulted in an equivalent level of STAT5 phosphorylation (Fig. 2*B*), but human GH was also capable of activating STAT5 to a lesser extent. Interestingly, all of the hormones resulted in equivalent levels of extracellular signal–related kinase (ERK) 1 and 2 (active MAPK) activation.

To further examine the specificity of the induction of PDK4, we examined the effects of several STAT1 and STAT3 activators. We have previously shown that these cytokines are potent activators of STAT1 or STAT3 or both of these STATs in 3T3-L1 adipocytes but do not activate STAT5 proteins (27,31). Total RNA was isolated from serum-deprived 3T3-L1 adipocytes after a 2-h treatment with ciliary neurotrophic factor, cardiotrophin-1, leukemia inhibitory factor, interferon γ, or PRL. As shown in Fig. 3, we observed that PRL, but not any of the other cytokines, induced PDK4 expression. β-Actin is included as a loading control. The efficacy of the cytokines was confirmed by examining STAT activation (data not shown).

Because previous studies suggest that insulin has the ability to suppress PDK4 expression in other tissues (23–25), we examined the PRL- and GH-mediated induction of PDK4 mRNA after an insulin pretreatment in adipose tissue. Fully differentiated 3T3-L1 adipocytes were treated with porcine GH or PRL for 2 h in the presence or absence of a 20-min insulin pretreatment. The robust activation of PDK4 mRNA after the treatment with either PRL or GH was evident with no insulin preincubation, but the induction of PDK4 mRNA was substantially attenuated by the insulin pretreatment (Fig. 4).

Because PRL and GH are potent activators of STAT5, we searched the murine PDK4 promoter for STAT binding motifs in the promoter region 2.5 kb upstream of the transcription start sites. Typically, STAT proteins bind a core palindromic sequence, TTC NNN GAA, and selective variation can occur in particular nucleotide positions (32). One consensus STAT binding motif was identified at −389 in the PDK4 promoter. An oligonucleotide probe containing 9 bp of the core STAT motif with 6 bp of flanking sequence on either end was incubated with cytoplasmic or nuclear extracts from PRL-treated 3T3-L1 adipocytes. To evaluate this potential STAT5 binding site, we performed electrophoretic mobility shift assays (EMSAs). As shown in Fig. 5*A*, we observed PRL-induced binding when the labeled probe was incubated with nuclear extracts from

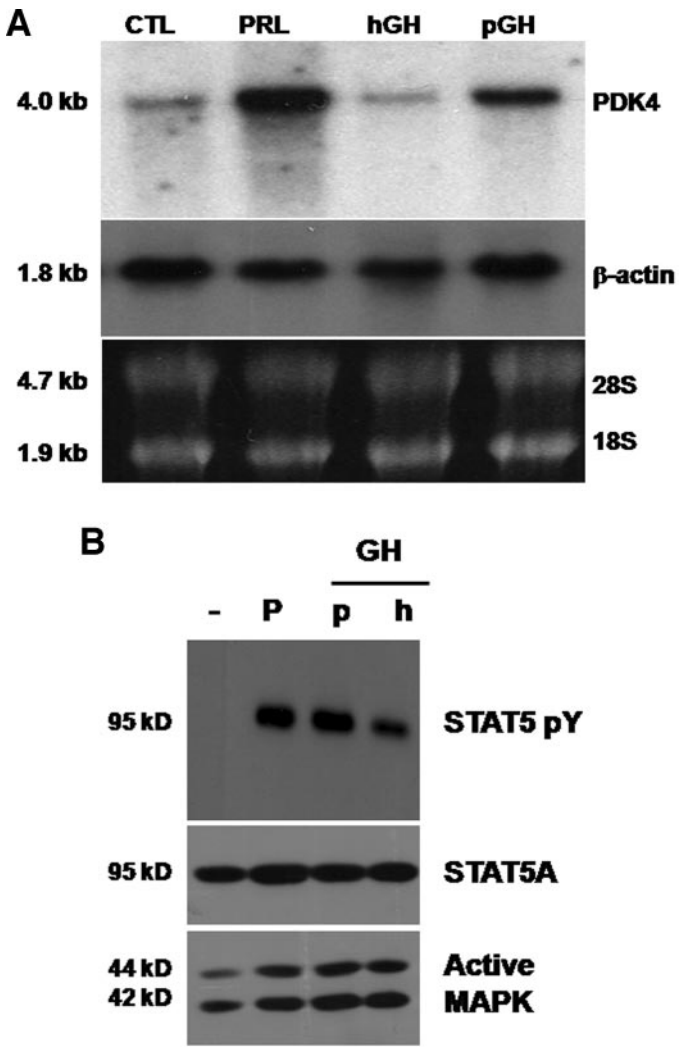


FIG. 2. Both PRL and porcine GH induce PDK4 expression in 3T3-L1 adipocytes. *A*: Total RNA was isolated from serum-deprived 3T3-L1 adipocytes after a 2-h treatment with 0.5 nmol/l PRL (P), human GH (h), or porcine GH (p). Untreated cells (CTL) were also harvested at the start of the experiment. Fifteen micrograms total RNA was electrophoresed, transferred to nylon, and subjected to Northern blot analysis with radiolabeled probes for PDK4 and β-actin. Ethidium bromide staining of 28S and 18S RNA (gel photo) is also shown. This is a representative experiment independently performed four times. *B*: From the same group of cells, whole-cell extracts were isolated after a 15-min treatment with the same doses of the hormone listed above. Twenty-five micrograms protein from each sample was subjected to SDS-PAGE and then was transferred to nitrocellulose for immunoblot analysis. This is a representative experiment independently performed four times.

PRL-treated adipocytes. Specific binding to the probe was also confirmed by results demonstrating that cytosolic proteins did not bind to this site, and preincubation with unlabeled probe decreased binding intensity (Fig. 5*A*, *last lane*). Preincubation of nuclear extracts with specific antibodies to individual STAT proteins in a supershift EMSA indicated that STAT5A was present in the binding complex induced by PRL (Fig. 4*B*). In addition, STAT1 and STAT3 did not participate in binding to the STAT element in the PDK4 promoter. Our data clearly demonstrate that the −389 to −378 region of the murine PDK4 promoter binds nuclear PRL-activated STAT5A proteins in vitro. To determine whether this region of the PDK4 promoter contributed to the regulation of PDK4 by STAT5 activators in living cells, we performed site-specific mutagenesis to

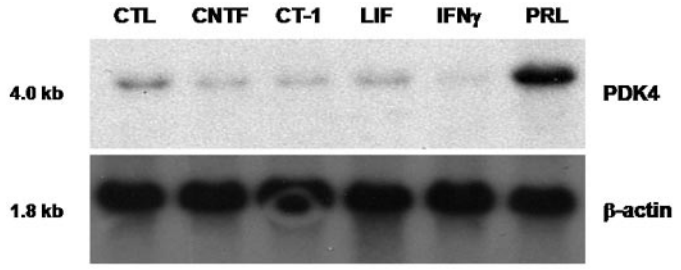


FIG. 3. STAT1 and STAT3 activators do not induce PDK4 expression in 3T3-L1 adipocytes. Total RNA was isolated from serum-deprived 3T3-L1 adipocytes after 2 nmol/l ciliary neurotrophic factor (CNTF), 0.2 nmol/l cardiotrophin-1 (CT-1), 0.5 nmol/l leukemia inhibitory factor (LIF), 100 units/ml interferon γ (IFNγ), or 0.5 nmol/l PRL treatment. Untreated cells (CTL) were also harvested at the start of the experiment. Fifteen micrograms total RNA was subjected to Northern blot analysis. This is a representative experiment independently performed two times.

alter 2 bp within the murine PDK4 promoter/luciferase construct. We have shown that this mutation abolished binding of PRL-induced proteins to this site (data not shown). Transfection of the wild-type and mutant constructs into 3T3-L1 cells revealed that the basal level of luciferase activity was not significantly affected by mutation of the STAT5A site that we identified in the PDK4 promoter (Fig. 6). In addition, a two- to fourfold increase in luciferase activity was observed after a 2-h PRL or GH (porcine) treatment and measurement of the wild-type construct. In addition, the construct containing the mutated STAT5 site was unresponsive to induction by GH or PRL. Thus, these data clearly indicate that the −389 to −378 site of the PDK4 promoter is sensitive to GH and PRL, and these data also suggest that this site confers the positive regulation of PDK4 by GH- and PRL-activated STAT5A protein complexes.

The expression and modulation of PDK4 in skeletal muscle is well documented, yet, the induction of PDK4 in adipocytes has not been previously observed. Because our studies indicate that PRL increased PDK4 mRNA levels, we hypothesized that PDK4 protein levels might be increased after PRL treatment. Hence, we exposed fully differentiated serum-deprived 3T3-L1 adipocytes to PRL and isolated whole-cell extracts to examine PDK4 expression. Chronic treatments (22 h or more) did not result in

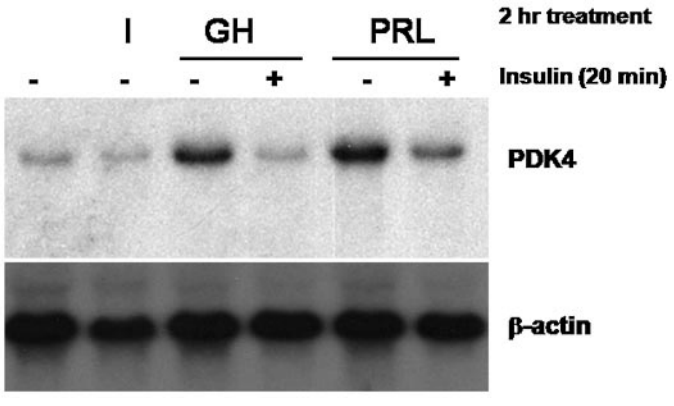
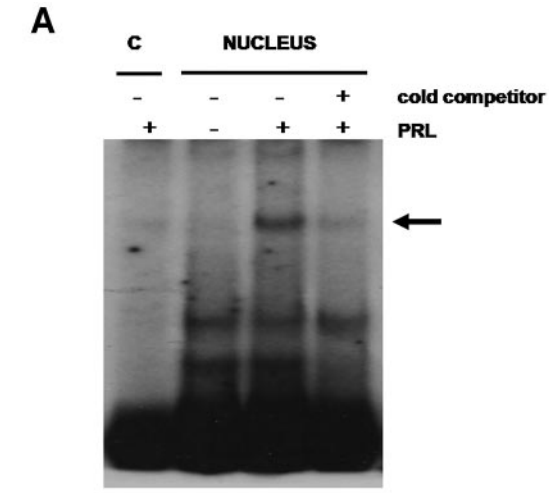


FIG. 4. The induction of PDK4 by PRL or GH is attenuated by insulin pretreatment in 3T3-L1 adipocytes. Total RNA was isolated from serum-deprived 3T3-L1 adipocytes after 2-h treatments with 0.5 nmol/l porcine GH or 0.5 nmol/l PRL in the presence or absence of a 20-min 50 nmol/l insulin pretreatment. Fifteen micrograms total RNA was subjected to Northern blot analysis. This is a representative experiment independently performed three times.



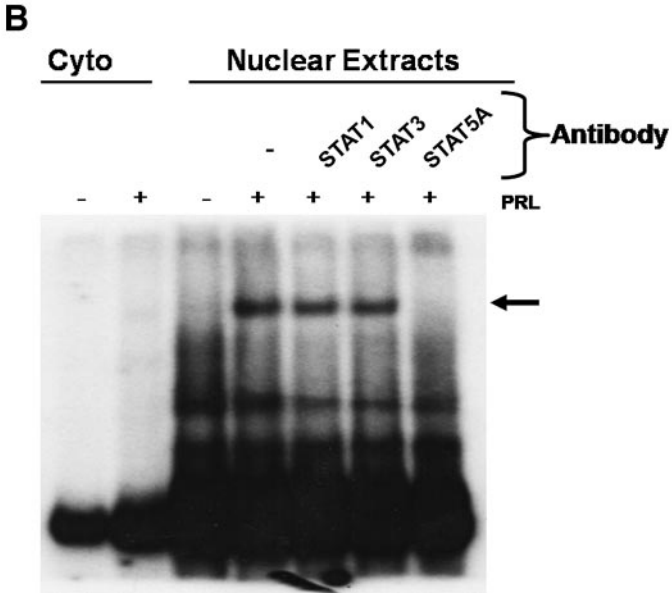


FIG. 5. PRL induces binding to a potential STAT site at −389 to −378 of the mouse PDK4 promoter in vitro. *A*: Cytosolic and nuclear extracts were prepared from differentiated serum-deprived 3T3-L1 adipocytes that were untreated or treated with PRL for 15 min. For each sample, 10 μg protein was incubated with 50,000 cpm/ml −389 to 378 ^{32}P-labeled probe of the murine PDK4 promoter. The protein-DNA complexes were resolved by EMSA. For cold competition, nuclear extracts were preincubated with an excess of the indicated unlabeled oligonucleotides (10 μmol/l). The PRL-sensitive shift is indicated with an arrow. This is a representative experiment independently performed three times. *B*: Cytosolic and nuclear extracts were prepared from 3T3-L1 adipocytes treated with PRL for 15 min. For supershift analysis, 10 μg protein was preincubated with 4 μg indicated antibody and then incubated with 50,000 cpm/ml indicated ^{32}P-labeled probe of the −389 to −378 site in the murine PDK4 promoter. The protein-DNA complexes were resolved by EMSA. This is a representative experiment independently performed two times.

any substantial changes in PDK4 protein levels (data not shown). Because the effects of STATs on gene expression are usually transient, we examined the ability of shorter PRL treatments to modulate PDK4 expression. As shown in Fig. 7, we observed a significant induction of PDK4 protein levels after a 6- and 8-h treatment with PRL. After 24 h, this induction was not substantial. We did observe some fluctuation in the time in which PRL induced levels of PDK4 protein. In some experiments, we observed a substantial induction at 4 h, whereas in most experiments,

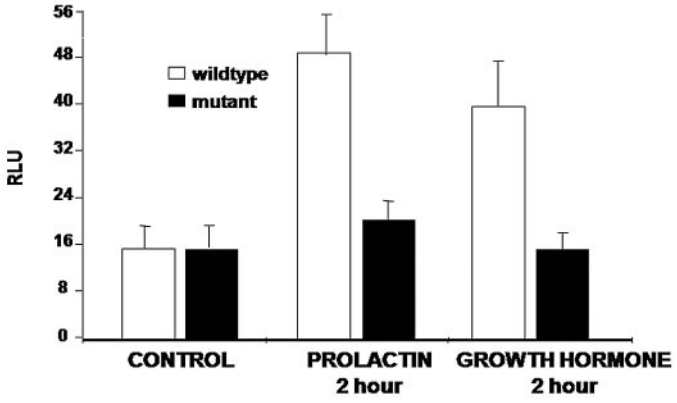


FIG. 6. PRL and GH induce the activity of the murine PDK4 promoter in 3T3-L1 cells via a STAT5 binding site. Proliferating 3T3-L1 cells were transiently transfected with murine PDK4 promoter (−949 to 24)/ luciferase constructs (wild type or mutant) and the TK/renilla vector to control for transfection efficiency. After 48 h of transfection, cells were stimulated with 0.5 nmol/l PRL or 0.5 nmol/l porcine GH for 2 h. Relative light units (RLU) were calculated by dividing firefly luciferase activity by renilla luciferase activity. Results are shown as ±SD. For each experiment, three plates of cells were used for each particular condition. In addition, each experiment was performed in triplicate on three independent batches of cells with similar results.

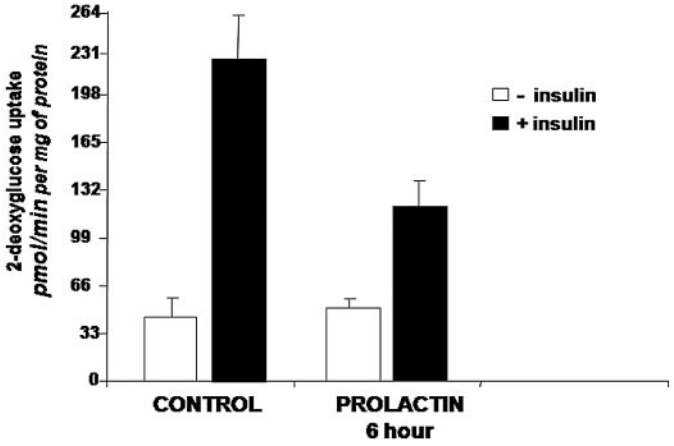


FIG. 8. The effects of PRL on insulin-stimulated glucose uptake in 3T3-L1 adipocytes. Serum-deprived, fully differentiated 3T3-L1 adipocytes were untreated or incubated with 0.5 nmol/l PRL for 6 h. Cells were then exposed to 50 nmol/l insulin or saline control for 10 min. Glucose uptake was measured as described in RESEARCH DESIGN AND METHODS. Values shown represent the means ± SE (light bars, saline control; dark bars, insulin treatment) of triplicate determinations from four independent experiments.

the levels of PDK4 protein were the greatest after an 8- to 10-h treatment. However, in all of our experiments, there was no significant effect observed after a 22-h PRL treatment. The efficacy of the PRL treatment was demonstrated by showing the activation of STAT5 and ERK 1 and 2 (active MAPK).

Because PRL induced PDK4 proteins levels, we hypothesized that glucose uptake might be regulated under these conditions. The results in Fig. 8 demonstrate that a 10-min treatment with insulin results in a fivefold increase in glucose uptake in fully differentiated 3T3-L1 adipocytes. Also, a 6-h treatment with PRL resulted in a substantial decrease (2.5-fold) in insulin-stimulated glucose uptake. The induction of PDK4 protein under these conditions was confirmed by Western blot analysis (data not shown).

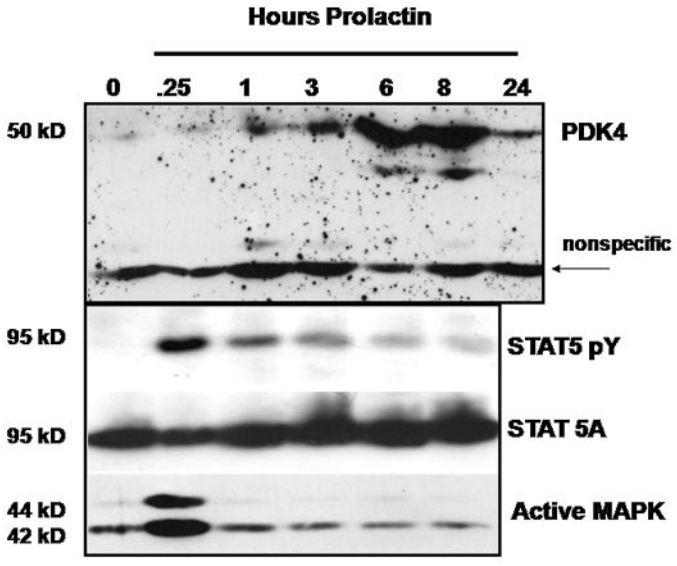


FIG. 7. PRL induces the expression of PDK4 protein in 3T3-L1 adipocytes in a time-dependent manner. Fully differentiated 3T3-L1 adipocytes were serum deprived overnight and then exposed to 20 nmol/l PRL for the times indicated. One hundred micrograms protein from whole-cell extracts was loaded into the gel for each sample. The samples were subjected to SDS-PAGE and then were transferred to nitrocellulose for immunoblot analysis. PDK4 polyclonal antibodies were purchased from Abgent and used at a dilution of 1:200. STAT5A polyclonal antibodies were purchased from Santa Cruz and used at a 1:500 dilution. A STAT5 $pY^{694/699}$ polyclonal antibody was purchased from Upstate Cell Signaling and used at 1:500 dilution. Active MAPK polyclonal antibodies was purchased from Promega and used at a 1:5,000 dilution. This is a representative experiment independently performed four times.

DISCUSSION

The novel findings in this study include data demonstrating that PDK4 levels are induced in adipocytes after stimulation with two STAT5-activating hormones, the identification of a hormone-responsive region in the murine PDK4 promoter, and the characterization of a STAT5 binding site in this region. These results strongly suggest that STAT5A directly activates the expression of PDK4 in adipocytes. Moreover, our data indicate that PRL and STAT5A could induce conditions of insulin resistance by inducing PDK4 expression and attenuating insulin-stimulated glucose uptake.

Although GH is a well-known modulator of metabolism and is a known effector of insulin action (rev. in 2 and 33), the role of PRL in these processes is less understood. PRL is primarily a pituitary secreted hormone, yet in humans, PRL is also produced and secreted from additional tissues, such as human glandular and adipose breast tissues (34) and human decidua (35), and is a circulating hormone (rev. in 36). The presence of PRL receptors in almost all organs, including adipocytes (3), contributes to the diverse roles of PRL. Recently, a novel human adipocyte cell line, LS14, has been developed that produces and responds to PRL (37). In morbidly obese patients, the amount of PRL secreted from subcutaneous adipose tissue is much less than in lean patients, but, surprisingly, there are no apparent differences in PRL secretion between men and women (rev. in 36).

Studies have also implicated PRL as a contributor to insulin resistance. One study conducted in lean pregnant women demonstrated that PRL could cause insulin resistance in adipocytes by suppressing adiponectin production and secretion (38). This mechanism is plau-

sible, because low levels of adiponectin can be associated with obesity and insulin resistance. Although the cross-talk between PRL and leptin action is not well understood, PRL has been shown to suppress insulin-induced leptin secretion in cultured adipocytes (39). PRL also decreases insulin-stimulated glucose uptake in pregnant rat adipose tissue (40). Likewise, severe hyperprolactinemia has been shown to induce an insulin-resistant state by decreasing insulin binding in human subjects (41). Our data present another plausible mechanism by which PRL may cause insulin resistance in adipocytes by modulating PDK4 levels.

Previous studies support similar or overlapping functions for PRL and GH in mammary gland metabolism (42). Our studies revealed that sheep PRL and porcine GH behaved similarly to modulate PDK4 levels in murine adipocytes. As is commonly known for PRL action, we did observe a bell-shaped curve rather than linear dose-dependence relationship, and higher doses of the hormone were inhibitory. Interestingly, human GH had no substantial effects on PDK4, despite its ability to weakly activate STAT5A and strongly activate ERK 1 and 2. In multiple independent experiments, we demonstrated the induction of PDK4 specifically by sheep PRL and porcine GH but not by human GH in 3T3-L1 adipocytes. These data are intriguing because human GH did result in some activation of STAT5A but did not induce PDK4 levels. A BLAST analysis revealed that human GH and mouse GH have a 69.7% similarity (58.3% identity). However, porcine GH and murine GH have a 94.4% similarity (89.8% identity). Hence, we hypothesize that the human GH does not engage the murine receptor normally because of the substantial difference in homology. Alternatively, the activation of STAT5 by sheep PRL and porcine GH may include an additional factor(s) that participates in the induction of PDK4 expression, and this factor is not similarly modulated by human GH signaling in murine adipocytes.

Although it has been shown that insulin has the ability to suppress PDK4 expression in various other tissues (23–25), our studies demonstrate that insulin has the ability to suppress the PRL- and GH-induced PDK4 expression in adipocytes. Because the basal levels of PDK4 are low in adipocytes, we did not observe a strong repression of PDK4 mRNA after insulin treatment alone (Fig. 2). Our studies provide evidence to support antagonistic cross-talk between PRL or GH and insulin, as well as the possible role of PRL in insulin resistance in adipose tissue. In addition, our results indicate that a 6-h PRL treatment results in an inhibition of insulin-sensitive glucose uptake. This data are complemented by studies showing that reduced PDK4 expression is associated with increased insulin sensitivity (26). In summary, the presence of PDK4 protein in adipocytes correlates with an attenuation of insulin-sensitive glucose uptake and likely plays a role in the ability of PRL to induce insulin resistance.

Studies have shown that other transcription factors, such as FOXO, also bind to the PDK4 promoter and induce PDK4 expression in response to starvation in the skeletal muscle of mice (43). Glucocorticoids, which are implicated in insulin resistance, also increase the expression of PDK4, and insulin antagonizes these effects (25). Additional studies show that insulin suppresses the PDK4 induction mediated by glucocorticoids by inactivation of FOXO transcription factors, which are required for full transcriptional activation of the PDK4 gene by glucocorticoids (44). The transcriptional coactivator peroxisome proliferator–activated γ coactivator (PGC)1α has also been shown to induce the expression of PDK4 in primary rat hepatocytes and ventricular myocytes (45). In C2C12 myoblasts, PGC1α induces PDK4 expression in a manner that is dependent on ERRα (46). Hence, in muscle, the modulation of PDK4 can be influenced by FOXO transcription factors and PGC1α. Our studies demonstrate a novel induction of PDK4 in adipocytes that is mediated by a STAT5A binding site in the mPDK4 promoter. Other studies from our laboratory have shown that adipocyte STAT5A can inhibit the expression of FAS (12) and bind to the promoter of the acyl Co-A oxidase (AOX) gene, which correlates with an increase in AOX expression (47). Hence, the role of STAT5A in mature adipocytes appears to be one that attenuates lipid accumulation and favors lipid oxidation. Interestingly, our studies on PDK4 also suggest that STAT5A may contribute to energy homeostasis by decreasing glucose oxidation and increasing insulin resistance in adipocytes. The differential capabilities of STAT5A are apparent from several studies that demonstrate that in various preadipocytes or nonprecursor cells, STAT5A can promote lipid accumulation and adipogenesis (7–9). Although the mechanisms that account for the ability of STAT5A to have lipogenic or lipolytic effects have not been clearly defined, collectively, these studies indicate that STAT5A is likely an important contributor to maintenance of energy balance, particularly in response to hormones.

Because PDK inhibitors have been shown to be effective potential therapeutics in improving glycemic control and type 2 diabetes (48), these kinases warrant more in-depth analysis. In particular, PDK4 proves to be an object of necessary consideration because it is expressed in substantial amounts in skeletal muscle and adipose tissue. In summary, we have observed that PRL and GH can induce the expression of PDK4 in adipocytes and positively regulate the murine PDK4 promoter. Our identification of a STAT5 binding site in the promoter of PDK4 characterizes a novel mechanism of regulating its expression. In summary, we hypothesize that the regulation of PDK4 by PRL and GH via STAT5A is likely an important contribution to the maintenance of energy homeostasis.

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