PGC-1α coactivates estrogen-related receptor-α to induce the expression of glucokinase

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1National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and School of Basic Medicine, Peking Union Medical College, Beijing; 2Hubei Province Key Laboratory of Biotechnology of Chinese Traditional Medicine, Hubei University, Wuhan, China; and 3Department of Molecular Parasitology, Humboldt University, Berlin, Germany

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Zhu L, Liu Y, Cui A, Shao D, Liang J, Liu X, Chen Y, Gupta N, Fang F, Chang Y. PGC-1α coactivates estrogen-related receptor-α to induce the expression of glucokinase. Am J Physiol Endocrinol Metab 298: E1210–E1218, 2010. First published March 9, 2010; doi:10.1152/ajpendo.00633.2009.—Peroxisome proliferator-activated receptor-γ (PGC-1α) is a master regulator of cellular energy metabolism and regulates processes such as adaptive thermogenesis, hepatic gluconeogenesis, fatty acid oxidation, and mitochondrial biogenesis by coactivating numerous nuclear receptors and transcription factors. Here, we demonstrate the presence of the ERRα binding site in the regulatory sequence of the glucokinase gene and that PGC-1α coactivates ERRα to stimulate the transcription of glucokinase. Simultaneous overexpression of PGC-1α and ERRα potently induced the glucokinase gene expression and its enzymatic activity in primary hepatocytes; however, expression of either PGC-1α or ERRα alone had no significant effect. Electrophoretic mobility shift and chromatin immunoprecipitation assays revealed the interaction of ERRα with the glucokinase promoter. Finally, the knockdown of endogenous ERRα with specific siRNA (siERRα) or pharmacological inhibition of ERRα with XCT790 attenuated insulin-induced glucokinase expression. Taken together, this research identifies glucokinase as a novel target of PGC-1α/ERRα and underscores the regulatory function of ERRα in insulin-dependent enzyme regulation.

peroxisome proliferator-activated receptor-γ; coactivator-1α; transcriptional regulation; glucose metabolism; insulin

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-γ (PPARγ) coactivator-1α (PGC-1α) is a master regulator of cellular energy homeostasis (5, 26). Although PGC-1α is expressed in a variety of tissues, including the liver, brain, and kidney, it is enriched in tissues dependent on oxidative metabolism for ATP (heart, skeletal muscle) or heat (brown adipose tissue) generation (31). PGC-1α is highly induced in liver and heart during the fasting period, in brown adipose tissue following the exposure to cold temperature, and in skeletal muscle during exercise (1, 24, 33). Proteins interacting with PGC-1α are not confined to the nuclear receptor superfamily, since it also associates with numerous transcription factors of the insulin- and glucagon-signaling pathways (5, 26). For example, it induces the expression of glucose transporter (GLUT4) elevating the sugar import (30). PGC-1α is also known to activate the mitochondrial biogenesis and respiration rates in myogenic cell lines (39, 45). Its overexpression in primary cardiac myocytes or in the hearts of transgenic mice increases the oxidation of fatty acids and mitochondrial volume density (24). The PGC-1α-knockout mice exhibit multiple metabolic defects, including its involvement in numerous disorders such as obesity, diabetes, neurodegeneration, and cardiomyopathy (25, 27).

Estrogen-related receptor-α (ERRα) is the founding member of a small family of orphan nuclear receptors that also includes ERRβ and ERRγ (7, 11, 13). ERRα is enriched in metabolically active tissues such as the skeletal muscle, heart, kidney, and liver (48). ERRα is known to recognize the estrogen response elements with its preference for the sites composed of a single half-site preceded by three nucleotides with the consensus sequence TGACCTTNA, referred to as an ERR response element (ERRE) (18). The transcriptional activity of ERRα is independent of ligand and regulated by PGC-1α and PGC-1β (19, 36). PGC-1α is also capable of coactivating ERRα in addition to inducing its expression (36).

ERRα-knockout mice display a reduced body weight and peripheral fat deposition and are resistant to high fat-diet-induced obesity (28). The precise physiological function of ERRα remains unclear; however, its contribution in the cellular energy metabolism through regulation of fatty acid oxidation (medium-chain acyl-CoA dehydrogenase), oxidative phosphorylation (cytochrome c), and mitochondrial biogenesis (nuclear factor-E2-related factor 2) is well understood (15, 35, 37, 42).

Glucokinase (Gck) participates in glucose utilization by stimulating glycolysis and glycogen synthesis in liver. Two alternate promoters regulate the tissue-specific expression of Gck in liver and in pancreatic β-cells; of these, the upstream promoter is specific to β-cells, and the downstream sequence controls the hepatic Gck (17, 29). The pancreatic Gck acts as a glucose sensor that controls the secretion of insulin in β-cells. Therefore, a minor modulation of Gck activity can lead to a measurable impact on the blood glucose level (4, 22). In addition, sterol regulatory element-binding protein-1 (SREBP-1) has been reported to regulate the insulin-dependent transcription of Gck in liver (6, 21), although it remains disputed by others (9). The overexpression of FOXO1 can inhibit the expression of Gck in liver by a yet-unidentified mechanism (47). Furthermore, Roth et al. (34) have postulated hepatocyte nuclear factor-4α (HNF-4α) and hypoxia-inducible factor-1 as the activators of insulin-dependent Gck transcription. PPARγ, specificity protein (SP)1, and SP3 have also been proposed to control the expression of the hepatic Gck (3, 22).
This study demonstrates that PGC-1α can coactivate ERRα and induces the transcription as well as the enzyme activity of Gck. Inhibition of ERRα compromises insulin-mediated induction of the Gck gene. Taken together, our research underscores ERRα to be a novel and important regulator of glucose metabolism.

MATERIALS AND METHODS

Animals, cells, and reagents. All animal experiments were performed with prior approval from the Animal Care and Welfare Committee of the Peking Union Medical College. Male Wistar rats (200–260 g) were maintained on a 12:12-h day-night rhythm with unrestricted access to water and food. HepG2 cell lines were purchased from ATCC. Cell culture reagents were procured from Life Technologies, and other chemicals were from Sigma Chemical unless otherwise specified. Western blot or chromatin immunoprecipitation (ChIP) assays were performed using antibodies against Gck (ab37796; Abcam), Flag (F3165; Sigma), β-actin (A5316; Sigma), and PGC-1α (516557; Calbiochem).

Plasmid constructs. The rat Gck promoter (−498 to +109 bp, referred to as −498 Luc) was amplified as described previously (20) using rat genomic DNA and cloned at KpnI and XhoI sites of pGL3-basic reporter gene vector (Promega). The deletion and mutation constructs (−152, −101, −36, Gck101mut1, and Gck101mut2 Luc) were generated by PCR amplification of −498 Luc followed by cloning into pGL3-basic vector (Fig. 1A). To this end, the different forward primers 5′-ACATGGGTACCTCCAACCAGTGTTCTGTCATCC-3′ (−152 Luc), 5′-ACATGCGTACCAGCCAAGGATTTCCAGGACG-3′ (−101 Luc), and 5′-ACATGGGTACCGGCAGGGTATTTCAGGAGC-3′ (−36 Luc) were used with the identical reverse primer 5′-ACACTCGAGGGGTCTTTGGGAGTAGA-3′. Two site-directed mutants of the rat Gck promoter were used using the Gck-101 Luc as a template. The forward primers for the Gck-101mut1 and the Gck-101mut2 Luc constructs were 5′-CTGGGCCCTGTCGGAATATTGT-GACACTAGGCAG-3′ and 5′-CTGGGCCCTGTCGGAATATTGT-GACACTAGGCAG-3′, respectively; the reverse primers were 5′-CTGGCTAGTGTGCAATTCCAGGCCAGGGGCAG-3′ and 5′-CCCTGCTAGTGTGCAATTCCAGGCCAGGGGCAG-3′, respectively.

Cell culture, transient transfection, and luciferase assay. HepG2 cells were maintained at 37°C under 5% CO2 in MEM-NEAA respectively. GACACTAGGCAGGG-3
GACACTAGGCAG-3 and 5101mut2 Luc constructs were 5 template. The forward primers for the Gck-101mut1 and the Gck-101mut2 Luc constructs (0.3 152 bp, SRE, sterol regulatory site-directed mutations of the rat Gck promoter and 20 ng of an internal control Renilla luciferase reporter plasmid pRL-TK (Promega) were cotransfected with 0.6 μg of the expression plasmids.

Fig. 1. Regulation of the rat glucokinase (Gck) promoter by estrogen-related receptor-α (ERRα) and peroxisome proliferator-activated receptor-γ (PPARγ) coactivator-1α (PGC-1α). A: schematic representation of the 5′ deletion/mutation series of the rat Gck promoter from nucleotides −498, −152, −101, and −36 to +109 bp. The +1 bp represents transcription start site of hepatic Gck. The relative positions of known transcription factors in the hepatic Gck promoter region are shown. The constructs denoted as Gck-101mut1 and Gck-101mut2 Luc harbor mutations in putative ERR response element (ERRE; −52 to −44 bp). Mutated nucleotide letters are lowercase. B: the promoter-reporter constructs (0.3 μg) and plasmids expressing ERRα (0.6 μg) and PGC-1α (0.6 μg) were cotransfected into HepG2 cells. Transfections were normalized to the Renilla luciferase activity expressed from a cotransfected plasmid pRL-TK (20 ng). The results are presented as the fold increase of luciferase activity over the value obtained with the control plasmid pcDNA3.1 alone. C: the promoter-reporter constructs (0.3 μg of the wild-type Gck-101, Gck-101mut1, or Gck-101mut2 Luc) were cotransfected with plasmids expressing ERRα (0.6 μg) and PGC-1α (0.6 μg) or pcDNA3.1 (1.2 μg) as basal level (control). The results are presented as the fold induction of luciferase activity over the value obtained with the wild-type Gck-101 Luc at basal level. **p < 0.01 and ***p < 0.005, statistical difference. SRE, sterol regulatory element-binding protein-1 response element; PPRE, PPARγ response element; P2, upstream stimulatory factor (USF) binding element; HRE, hypoxia-inducible factor-1 response element; RLA, relative luciferase activity.
Cells were harvested and analyzed by luciferase reporter assay 48 h posttransfection.

Adenovirus constructs and RNA interference. The recombinant adenovirus expressing ERRE-Flag (Ad-ERRx-Flag) was generated using the forward primer 5'-TGCAGACATGGCAGTTCCGAGGTGGT-3' and the reverse primer 5'-TCGAGTAATACGCTTGTATCGTCGCTATCGGCCTGAGC-3'. The PCR fragments were cloned into the shuttle vector pAdTrack-CMV. Ad-PGC-1α was constructed by cloning full-length PGC-1α cDNA into pAdTrack-CMV vector, as described previously (23). Recombination with pAdEasy-1 and propagation of adenovirus expressing PGC-1α and ERREx was performed in 293A cell line was performed as described previously (23). The adenoviral vector expressing siRNA against ERREx (siERRx) was a kind gift from Dr. Anastasia Krali (Vanderbilt University School of Medicine and Veterans Affairs Medical Center). The siERRx is designed to target the sequence 5'-GAGCATGCCAGTGCTCTCC-3' of mouse and rat ERREx (11). The nucleotide sequences for siRNA against luciferase (si-control) are 5'-GATCCCTTAGCTGATTGTGATCAAGGAGTCGAGTACTCCAGCATACTGGAAGTACCTAGGTAAGC-3' (forward) and 5'-AGCTTAAACCATGGCAGTTACCTGAGTGTGAATCGAGTCAGTACCTGGAAGTACCTAGGTAAGC-3' (reverse). Inserts were cloned into the pAdTrack-U6 shuttle vector, and adenovirus constructs were created by recombination of the shuttle and pAdEasy vectors in BJ5183 bacteria. For adenoviral siRNA infection, cells were infected with the adenovirus expressing siERRx (siERRx) or control adenovirus (si-control) at a multiplicity of infection sufficient to infect 100% of the cells 2 h postinfection. The medium was supplemented with DMEM-F-12 containing 10% FBS, and cells were cultured for an additional 48 h. Cells were then incubated with 100 nM insulin for 6 h. For green fluorescent protein (GFP) expression adenovirus, cells were infected at a multiplicity of infection sufficient to infect >95% of cells.

Isolation and treatment of primary hepatocytes. Hepatocytes were isolated from the male Wistar rats by a two-step in situ liver perfusion. Rats were anesthetized by 60 mg/kg pentobarbital. The hepatic portal vein was cannulated, and the liver was flushed with Hanks’ solution at 30 ml/min for 8 min. Then the liver was perfused by collagenase-dissolved in Hanks’ solution and 5 mM CaCl2 (15 ml/min, 30 min). The liver was dissected in 10 ml of DMEM supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. Finally, the liver capsule was disrupted, and the cells were dispersed. The culture was adjusted to 4 × 10⁵ cells/ml, and cells were allowed to attach onto gelatin-coated culture plates at a density of 1 × 10⁶ cells/cm². Liver cells were maintained under standard conditions in DMEM-F-12 containing 10% FBS, and cells were cultured for an additional 48 h. Cells were harvested for the RNase isolation and protein extraction at 48 h postinfection.

Electrophoretic mobility shift assay. Rat primary hepatocytes were isolated and infected with Ad-ERREx-Flag. Two hours postinfection, the medium was substituted with DMEM-F-12 containing 10% FBS, and cells were cultured for additional 48 h. Nuclear proteins were extracted. Liver capsule was disrupted, and the cells were dispersed. The culture was adjusted to 4 × 10⁵ cells/ml, and cells were allowed to attach onto gelatin-coated culture plates at a density of 1 × 10⁶ cells/cm². Liver cells were maintained under standard conditions in DMEM-F-12 containing 10% FBS, insulin and 100 μM dexamethasone for 3 h. The primary hepatocytes were incubated with the indicated adenovirus cultures in DMEM at 37°C for 2 h, followed by their culture with 10% FBS. The virus-infected cells were harvested within 48 h postinfection.

Western blot analysis. The whole cell proteins (30–60 μg) were fractionated on 10% SDS-PAGE and then electroblotted to polyvinylidene difluoride membranes for 2 h at 300 mA. The blots were incubated with blocking buffer (50 mM Tris-Cl, pH 7.4, 150 mM NaCl, 0.05% Tween-20, and 0.5% skimmed milk powder) for 2 h at room temperature, followed by overnight treatment with the primary antibody at 4°C and then with the horseradish peroxidase-conjugated secondary antibody. Following a 30-min wash step, the protein bands were detected by enhanced chemiluminescence. Quantitative densitometry analysis of protein bands was performed using Genetool software (Syngene, Cambridge, UK).

Gck activity assay. Gck activity was measured essentially as described previously (41). Briefly, hepatocytes were washed with 0.9% NaCl, scraped in a buffer containing 20 mM K-HPO₄, 1 mM EDTA, 110 mM KCl, and 5 mM dithiothreitol (pH 7.4), and homogenized by sonication. The homogenate was centrifuged at 12,000 g for 20 min at 4°C. Ten microliters of the supernatant was added to 200 μl of the assay buffer containing 50 mM HEPES (pH 7.4), 100 mM KCl, 7.4 mM MgCl₂, 15 mM β-mercaptoethanol, 0.5 mM Na⁺D₅, 0.05% BSA, 3 μg/ml glucose-6-phosphate dehydrogenase, 5 mM ATP, and either 0.5 or 100 mM of glucose for the low or the high K₆ measurements, respectively. The reaction was incubated for 90 min at 30°C and terminated by 1 ml of 500 mM NaHCO₃ (pH 9.4). The enzyme activity was monitored using a YSI Model 2700 (Yellow Springs Instruments, Yellow Springs, OH).

Statistical analysis. Data are presented as means ± SD of three independent experiments. For Western blot assay, X-ray films were quantified in the linear range by densitometry using UVIsoft-UVIP software.
and Application V97.04. Statistical differences were determined by Student’s t-test. Significance was accepted at a P value of <0.05.

RESULTS

PGC-1α and ERRα can coactivate the Gck promoter. Gck is a key enzyme for glucose utilization. Previous studies have shown that several transcription factors, including SREBP-1, PPARγ, HNF-4, and upstream stimulatory factor (USF), can activate hepatic Gck transcription (22, 34), and PGC-1α is known to interact directly with and coactivate HNF-4 as well as PPARγ (31, 46). Initially, we hypothesized potential stimulation of Gck by PGC-1α via its interaction with these two factors and used the rat hepatic Gck promoter-luciferase reporter construct (Gck-498 Luc) to test this assumption. Unexpectedly, transfection of PGC-1α alone in HepG2 cells did not activate the Gck promoter (Fig. 1), motivating us to search for the potential unidentified transcription factors. Hence, the nucleotide sequence of the hepatic Gck promoter was inspected. In addition to the previously identified factors (the relative positions of their binding elements are shown in Fig. 1A), a putative ERRE (TGACCTTGT) from −52 to −44 bp of the transcription start site was revealed. Since PGC-1α is also known to coactivate ERRα (36), we were prompted to examine whether ERRα alone or together with PGC-1α can induce the Gck promoter. The luciferase reporter assays depicted in Fig. 1B reveal that transfection of ERRα alone did not influence the Gck-498 Luc reporter gene containing −498/+109 bp region of hepatic Gck promoter. In contrast, cotransfection of PGC-1α and ERRα led to a strong activation of the promoter activity. To confirm the involvement of putative ERRE in the PGC-1α/ERRα-dependent activation of the Gck promoter, we generated reporter constructs that contained serial 5’ deletions in the promoter. The PGC-1α/ERRα-mediated coactivation was compromised significantly upon deletion of a region from −101 to −36 bp harboring the ERR binding element (Fig. 1, A and B). Mutational studies were also performed to investigate the involvement of ERRs in the mentioned phenomenon. The potential ERR binding element was mutated in the Gck-101mut1 and Gck-101mut2 Luc constructs (Fig. 1A), which have previously been demonstrated to inactivate this element (43). We observed that mutation in the ERRE alleviated the effect of PGC-1α and ERRα on the Gck promoter (Gck-101mut1 and Gck-101mut2 Luc; Fig. 1C). These results demonstrated that the ERRE is crucial for the activation of Gck by PGC-1α and ERRα (Fig. 1C). Interestingly, we also observed a reduction in the basal promoter activity of the Gck-101mut1 Luc, indicating the contribution of ERRα in the basal expression of Gck in HepG2 cells (Fig. 1C).

ERRα interacts with the ERRE in the Gck promoter. To determine whether ERRα protein binds to ERRE identified within the Gck promoter, EMSA was performed using the nuclear extracts of primary hepatocytes and the synthetic double-stranded oligonucleotides outlined in Fig. 2A. Nuclear proteins interacted with the labeled DNA probe, and increasing amounts of unlabeled wild-type probes reduced the formation of the protein-DNA complex (Fig. 2B). A 200-fold excess of competing DNA completely reversed the phenomenon. However, a similar excess of unlabeled mutant probes did not influence the designated complex, confirming the ERR specificity of this interaction.

To determine whether ERRα and PGC-1α occupy the endogenous promoter site, ChIP assays were performed with chromatin isolated from the primary hepatocytes infected with Ad-ERRα-Flag and Ad-PGC-1α. Cross-linked chromatin fragments were
precipitated with antibodies against Flag or PGC-1α or with control IgG for subsequent PCR analysis (Fig. 2C). The Gck promoter sequence containing ERRE (proximal region) could be amplified from the precipitates obtained using the anti-Flag antibody but not from the control IgG sample. Immunoprecipitation with anti-PGC-1α antibody also led to a weaker but reproducible amplification of the proximal region of Gck promoter (Fig. 2C). As expected, the distal promoter region without ERRE used as a negative control could not be amplified from any of the immunoprecipitates. Collectively, these data confirm the in vivo and in vitro interaction of ERRα with ERRE in the Gck promoter and reveal the recruitment of PGC-1α to the proximal region and its colocalization with ERRα.

Induced expression of Gck is mediated by ERRα and PGC-1α. To examine whether PGC-1α coactivation of ERRα also induces Gck transcript, we isolated the rat primary hepatocytes and infected them with adenoviruses expressing ERRα-Flag (Ad-ERRα-Flag) and PGC-1α (Ad-PGC-1α). Western blot results showed that ERRα and PGC-1α were expressed effectively (Fig. 3A). The total mRNA was isolated from hepatocytes and analyzed for Gck transcript. As depicted in Fig. 3B, Gck expression is potently induced upon overexpression of ERRα and PGC-1α compared with GFP control. We also observed that Gck expression was further stimulated by insulin, which appears to have an additive effect. Moreover, as anticipated, the overexpression of ERRα or PGC-1α alone did not affect the transcript. Next, we examined the kinetics of mRNA levels following treatment with insulin. Cells overexpressing ERRα/PGC-1α exhibited an elevated Gck expression compared with GFP control in the absence of insulin, and its mRNA level was increased rapidly in response to insulin (Fig. 3C). Insulin-induced Gck expression was apparent within 2 h and reached a peak within 6 h of treatment. Western blot analysis also demonstrated an induced Gck protein in HepG2 cells cotransfected with plasmids expressing PGC-1α and ERRα (Fig. 3, D and E). A similar result was obtained with the rat primary hepatocytes infected with Ad-PGC-1α and Ad-ERRα or control Ad-GFP (Fig. 3, F and G).

ERRα and PGC-1α can induce Gck activity. Following these observations, we were motivated to determine whether an elevated Gck transcript correlates with an enhanced enzymatic activity. Rat primary hepatocytes were isolated and infected with Ad-PGC-1α and Ad-ERRα-Flag in the presence or absence of insulin. The expression of PGC-1α as well as ERRα proteins was confirmed prior to enzyme assays (Fig. 4A). In agreement with previous reports, insulin was able to induce the basal enzyme activity (Fig. 4B). Cotransfection of PGC-1α and ERRα not only stimulated the Gck in the absence of insulin but also potentiated the insulin-induced enzymatic activity. As
discussed before in Fig. 3B, these results also suggest the additive effect of PGC-1α/ERRα and insulin on Gck.

**ERRα mediates the inducible effect of insulin on Gck.** The inducible effect of insulin on Gck expression in liver is well established. However, the molecular mechanism by which insulin regulates its expression remains ambiguous and controversial. The cis-acting elements mediating the insulin effect are unknown. SREBP-1c has been reported to govern the process (6, 21), but other studies have failed to confirm these findings (9). Our results indicate ERRα as an important determinant of insulin-mediated regulation of Gck expression. To validate this assumption, we employed a specific siRNA (siERRα) that is known to effectively knock down the ERRα (11). As depicted in Fig. 5A, insulin moderately upregulated the expression of endogenous ERRα; however, it was not statistically significant. The expression of ERRα was potently inhibited by siERRα regardless of the presence or absence of insulin (Fig. 5A). As depicted in Fig. 5A, insulin moderately upregulated the expression of endogenous ERRα; however, it was not statistically significant. The expression of ERRα was potently inhibited by siERRα regardless of the presence or absence of insulin (Fig. 5A). Similarly, the knockdown of ERRα also led to the attenuation of insulin-induced Gck expression in hepatocytes (Fig. 5B). In addition, we also treated primary hepatocytes with XCT790, an inhibitor of ERRα (44), and observed an inhibition of insulin-mediated Gck expression (Fig. 5C).

Fig. 4. Gck activity is enhanced by ERRα and PGC-1α. A: primary hepatocytes were infected with adenoviruses expressing ERRα-Flag and PGC-1α or GFP. Cells were incubated with 100 nM insulin for 6 h before analysis. The protein levels of PGC-1α, ERRα, and β-actin were determined by Western blot. B: primary hepatocytes were treated as in A. Gck activity was determined as described in MATERIALS AND METHODS.* P < 0.05 and ** P < 0.01, statistical difference.

Fig. 5. Insulin-dependent induction of Gck is compromised by inhibition of endogenous ERRα. Primary hepatocytes were isolated and infected with an adenovirus expressing siRNA against ERRα (siERRα) or the control siRNA (si-ctrl). Forty-eight hours later, cells were treated with 100 nM insulin for 6 h. The transcripts of ERRα (A) and Gck (B) were determined by real-time PCR and normalized to β-actin. C: primary hepatocytes were treated with XCT790 (3 μM) for 24 h, and cells were incubated with 100 nM insulin for 6 h. The Gck mRNA was quantified. The results represent the mean ± SD of 3 independent experiments (* P < 0.05 and ** P < 0.01).
These results suggest that ERRα, at least in part, acts as a mediator of the insulin signaling.

In summary, it is reasonable to conclude that ERRα is required for the basal expression of Gck, and it also contributes to the inducible effect of insulin. Since ERRα is a weak transcription factor in the context of Gck promoter, our aforementioned findings advocate its activation by PGC-1α consequently exerting a substantial modulation of Gck expression.

**DISCUSSION**

Glucose can regulate the expression of glucose-responsive genes through Gck, since the latter functions as an enzyme as well as a sugar sensor in hepatocytes and pancreatic β-cells, respectively (8, 22). It is well documented that insulin stimulates the expression of the liver Gck, and the induction is a rapid and strictly hormone-dependent process (9, 16). Although the transcription factors participating in this phenomenon have received some attention, the underlying molecular mechanisms remain unclear. This study has identified an ERR in the proximal region of the Gck promoter. The mutations in ERR attenuated the basal transcriptional activity of Gck, suggesting ERRα to be essential for the process, although its overexpression failed to influence the transcript. Interestingly, ERRγ, which also binds to ERR, was capable of moderately inducing the transcript (data not shown). It appears as though the endogenous ERRα is sufficient to activate the promoter. It is also plausible that ERRα itself is too weak to exert its effect alone. We also found a transcriptional repressor in the Gck promoter between −498 and −152 bp. The basal transcriptional activity is increased upon deletion of this region (data not shown). Therefore, the regulatory mechanism that underlies the expression of the Gck gene appears to be quite complex.

It is noteworthy that, despite the deletions or mutations in ERR, cotransfection of cells with PGC-1α and ERRα can still induce Gck. This is indicative of the involvement of other transcription factors that may be induced by PGC-1α and/or ERRα and cause the secondary activation of Gck. Previous studies have demonstrated that PGC-1α interacts and coactivates HNF-4 (46) and thus mediates the effect of insulin (34). SREBP-1c is also reported to mediate the effect of insulin on Gck expression (6, 21). However, the findings of Gregori et al. (9) have revealed that insulin-dependent induction of Gck occurs at least 4 h before the accumulation of mature SREBP-1c in the nucleus. In addition, unlike its influence on fatty acid synthase, the knockdown of SREBP-1c failed to modulate the Gck transcript. These results contradict SREBP-1c as a mediator of the early insulin signaling to the Gck promoter.

The results presented in this report demonstrate the association of ERRα with insulin stimulation of Gck expression. However, the physiological role of PGC-1α in Gck expression in hepatocytes could not be established since endogenous PGC-1α is expressed at low levels and overexpression of either PGC-1α or ERRα did not influence the Gck promoter. Our EMSA and ChIP experiments demonstrate clearly that ectopically expressed ERRα binds to the ERR identified in the hepatic Gck promoter. Consistent with these data, the RNAi-mediated knockdown of endogenous ERRα inhibited the Gck induction by insulin. However, these findings require further validation with the use of a specific and high-affinity antibody against the endogenous ERRα. The activation of Gck by insulin occurs within 2 h (9); hence, it could be argued as a secondary response through elevated expression of downstream transcription factors. However, this appears unlikely in light of the fact that insulin exerted only a weak, ~1.7-fold induction of ERRα transcript, and the overexpression of ERRα did not induce the Gck transcription. Previous studies have shown that ERRα can be phosphorylated (40). We postulate the phosphorylation of ERRα followed by its conformation changes, leading to an increase in its transcriptional activity through optimal interaction with the ERRE. Further research is necessary to validate this assumption.

This report revealing the contribution of ERRα in insulin-mediated Gck regulation suggests ERRα-null mice to be diabetic, and yet ERRα-knockout mice are viable and fertile and exhibit no apparent anatomic alterations. The only visible phenotype is their reduced body weight and peripheral fat deposits. Notably, the ERRα-null mice were resistant to high-fat diet-induced obesity, indicating that the loss of ERRα might indirectly influence the activity of other nuclear receptor-based signaling pathways. Thus, ERRα appears to have an intriguing regulation of in vivo lipid metabolism (28).

Recent studies have shown that PGC-1α is a critical regulator of mitochondrial and nonmitochondrial reactive oxygen species (ROS) detoxification pathway (38). ERRα serves as its partner protein in regulating ROS detoxification genes (32). The generation of ROS is presumed to play a central role in insulin resistance (14). Hence, enhancing the activity of ERRα may have therapeutic effects in prevention or treatment of diabetes. Accordingly, this study demonstrates that PGC-1α/ERRα can stimulate Gck gene expression, and ERRα partly mediates the insulin signaling of Gck expression. In type 2 diabetic patients, hepatic glucose production is increased and glycogen synthesis is decreased due to insulin resistance. Enhancing the transcriptional activity of ERRα by means of a synthetic agonist may have beneficial consequences on glucose metabolism through suppression of the hepatic gluconeogenesis and simultaneous activation of glycolysis and glycogen synthesis together with an elevated fatty acid oxidation and oxidative phosphorylation. Hence, further investigation on the aforementioned function of ERRα should facilitate the treatment of diabetes and obesity.

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**DISCLOSURES**

We declare that there is no conflict of interest on the research reported here.

**REFERENCES**


