Fine-tuned regulation of the PGC-1α gene transcription by different intracellular signaling pathways

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Hong T, Ning J, Yang X, Liu H, Han J, Liu Z, Cao W. Fine-tuned regulation of the PGC-1α gene transcription by different intracellular signaling pathways. Am J Physiol Endocrinol Metab 300: E500–E507, 2011. First published December 14, 2010; doi:10.1152/ajpendo.00225.2010.—It has previously been known that transcription of the PGC-1α gene can be either inhibited or stimulated by p38 MAP kinase (p38 MAPK). To determine whether p38 MAPK plays an inhibitory or stimulatory role in PGC-1α gene transcription, we further investigated the role of p38 MAPK in this study. Our results showed that the basal level of p38 MAPK phosphorylation was increased in gastrocnemius of mice under HFD and that p38 MAPK stimulated PGC-1α gene transcription in C2C12 myotubes. Our results also provided new mechanisms in myotubes that the p38 MAPK-induced PGC-1α gene transcription was prevented by insulin. Insulin suppression of PGC-1α gene transcription was neutralized by overexpression of the constitutively nuclear form of FoxO1. Finally, we located three insulin response elements (IREs) in the PGC-1α promoter, and mutations of these IREs abolish or blunt activity of the PGC-1α promoter. Together, our results show that transcription of the PGC-1α gene is balanced by different intracellular signaling pathways.

p38; Akt; insulin; insulin signaling; peroxisome proliferator-activated receptor-γ coactivator-1α

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-γ COACTIVATOR-1α (PGC-1α) is a multifaceted transcriptional coactivator. It is expressed in all major tissues/organs that are involved in metabolism, such as brown adipose tissue, liver, and skeletal muscles. In the brown adipose tissue, PGC-1α promotes energy dissipation through upregulation of the uncoupling protein-1 gene (35). In the liver, PGC-1α stimulates de novo synthesis of glucose via gluconeogenesis to provide a fuel source for cells (neurons and red blood cells) that can only use glucose as their fuel source (32). In the skeletal muscle, PGC-1α converts the low energy-consuming white muscles into the high energy-consuming red muscles (18, 42). In all these tissues, PGC-1α promotes the production of new mitochondria, the “power house” of a cell (34). Thus, the main function of PGC-1α in metabolism is to burn off the stored energy. Excess energy storage due to overeating and/or lack of physical activity is an equivalent to obesity, which is almost always associated with insulin resistance/hyperinsulinemia. Insulin resistance/hyperinsulinemia in turn is either a precursor or key component of numerous modern health problems such as metabolic syndrome, cardiovascular disorders, nonalcoholic fatty liver, Alzheimer’s disease, depression, asthma, some cancers, and aging (1, 4, 6, 9, 10, 25, 26, 31, 38). Therefore, a full understanding of PGC-1α gene transcription will certainly help find new ways to prevent and eliminate the excess energy storage and its numerous associated diseases.

It is known that p38 MAPK can stimulate the activity of PGC-1α (8, 33, 41). Additionally, p38 MAPK plays a role in PGC-1α expression. It has been shown that p38 MAPK may mediate the reduced expression of PGC-1α-induced free fatty acids (FFA) in skeletal muscles (11). In contrast, activation of p38 MAPK has been shown to be involved in the exercise-induced expression of the PGC-1α gene in skeletal muscles (2, 40). Furthermore, we have shown previously that both p38 MAPK activity and PGC-1α expression are increased in the brown adipose tissue by cold exposure (8). Considering these opposing results, whether p38 MAPK plays a positive or negative role in PGC-1α gene transcription is still being debated. Besides, the exact mechanism by which p38 MAPK regulates PGC-1α expression has not been fully established.

It is known that the Akt-dependent signaling may inhibit the activity of PGC-1α protein (17). There are indications that the Akt-dependent classical insulin signaling may play an inhibitory role in PGC-1α expression. For example, PGC-1α expression is decreased in skeletal muscles of subjects with insulin resistance/hyperinsulinemia (11, 29). The cold-induced expression of PGC-1α is accompanied by reduced insulin signaling (27). We have shown recently that PGC-1α expression is increased significantly when insulin production is prevented by streptozotocin (24). Our sequence analysis of the PGC-1α promoter identified several putative insulin response elements (IREs) or FoxO binding sites. In this study, we examined roles of the p38 MAPK- and Akt/FoxO1-
dependent intracellular signaling pathways in transcription of the PGC-1α gene.

MATERIALS AND METHODS
Reagents. Antibodies against total/phospho-Akt (Ser473), phospho-FoxO1 (Thr24), phospho-ATF2 (Thr73), phospho-CAMP response element-binding protein (CREB; Ser133), total/phospho-p38 (Thr180/Tyr182), and total/phospho Erk1/2 (Thr202/Tyr204) were from Cell Signaling Technology (Danvers, MA). The antibodies against PGC-1α (H-300) and α-tubulin (AA12) were from Santa Cruz Biotechnology (Santa Cruz, CA). The antibody against laminin receptor (COOH terminus, L1293) was from Sigma-Aldrich (Milwaukee, WI). LY-294002 was from Calbiochem (San Diego, CA). Protein assay kits were from Bio-Rad (Hercules, CA). Other materials were all obtained commercially and are of analytical quality. The constitutively nuclear form of FoxO1 encoded by adenovirus and the adenoviral vector encoding green fluorescent protein (GFP) were kind gifts from Drs. Domenico Accili and Christopher Newgard, respectively (5, 13). The constitutively activated form of MKK6 (MKK6E) encoded by adenovirus was a gift from Dr. Jiahua Han.

Animal experiments. C57BL/6 (B6) were housed under the usual day (12 h of daylight) and night (12 h of darkness) circadian and fed ad libitum. Mice were fed with either the normal rodent chow diet (ND) or high-fat diet (HFD) (Research Diets D12330, 58.0 kcal/100 kcal fat, 16.0 kcal/100 kcal proteins, and 26 kcal/100 kcal carbohydrates) for 4 wk. All animal studies were approved by the Institutional Animal Care and Use Committee of The Hamner Institutes for Health Sciences and fully complied with the guidelines from the National Institutes of Health. Insulin sensitivity was evaluated by measuring fasting levels of blood glucose and plasma insulin and then calculated by homeostasis model assessment (HOMA), as described previously (20, 21).

Construction of PGC-1α promoter expression vectors. The plasmids with a 2.6-kb fragment of the mouse PGC-1α promoter in the pGL3-basic vector containing the luciferase reporter gene and cAMP response element (CRE) or myocyte enhancer factor 2 (MEF2) response element (MEF2RE) deletion were purchased from Addgene (Cambridge, MA). The constructs with mutated IREs were constructed by site-directed mutagenesis with QuikChange Site-Directed II Mutagenesis Kit from Stratagene (La Jolla, CA). Primers used for mutagenesis were listed in Supplemental Table S1 (Supplemental Material for this article can be found online at the AJP-Endocrinology and Metabolism web site). Sequences of all constructs were verified by DNA sequencing.

Transient DNA transfections, luciferase assays, and adenoviral infections. Plasmid DNAs were introduced into cells by lipofectamine 2000 transfection agents. Promoter activities were measured by a luciferase assay system (Promega) with a Wallac 1420 Multilabel Counter (PerkinElmer Life Sciences) and normalized to the activity of pGL3-basic empty vector under the same treatments. Recombinant adenoviruses encoding the nuclear form of FoxO1 (Ad-FoxO1) or the constitutively active form of MKK6 (MKK6E) or GFP (Ad-GFP) were introduced into cells in six-well plates, as described previously (7, 22, 23). The typical infection rate of C2C12 cells under our conditions is 70–80%.

Fig. 1. Decreased peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) expression is associated with increased basal levels of both Akt- and p38 MAPK-dependent signaling pathways in skeletal muscle of mice under high-fat diet (HFD). C57BL/6 (B6) mice were fed either the normal chow diet (ND; n = 8) or HFD (n = 7) for 4 wk. After an overnight fast, the following measurements were performed. A: food consumption. B: body weight. C: blood glucose. D: plasma insulin. E: evaluation of insulin sensitivity by HOMA. F: PGC-1α mRNAs in gastrocnemius were quantified by real-time PCR and normalized to 36B4. G: PGC-1α proteins in gastrocnemius were quantified by immunoblotting and normalized to β-actin. H: levels of phosphorylated and total Akt in gastrocnemius were determined by immunoblotting. I: levels of phosphorylated and total p38 MAPK (p38) in gastrocnemius were measured by immunoblotting.
**Cell culture and treatments.** C2C12 cells were cultured and differentiated as described previously (15). Briefly, cells were maintained in DMEM containing 10% FBS and 1× antibiotic-antimycotic. When cells reached 90% confluence, recombinant adenoviruses or plasmids were introduced into cells via standard infections or transient transfections. Six hours later, they were switched to DMEM containing 2% horse serum and 1× antibiotic-antimycotic and treated for 24–36 h with either vehicle or target reagents (insulin, SB-203580, or LY294002 as noted).

**Chromatin immunoprecipitation assays.** Chromatin immunoprecipitation (ChIP) assays were conducted using the EZ ChIP Chromatin Immunoprecipitation Kit from Upstate (Lake Placid, NY) according to the manufacturer’s instructions. Briefly, cells in three 150-mm plates per condition were cross-linked with 1% formaldehyde for 10 min at room temperature. The reaction was stopped with the addition of glycine solution and then washed with ice-cold 1× PBS. Cells were pooled, precipitated, and then resuspended in cold 1× SDS lysis buffer supplemented with the protease inhibitor cocktail mixture. Cells were then sonicated (6 pulses, 10 s/pulse at 40% power) to break DNA into fragments in the range of 200–1,000 bp. Sheared chromatin samples (100 μl) were incubated with protein G agarose on ice for 1 h and then incubated with an antibody against histone H3 antibody (positive control), IgG (negative control), or anti-CREB antisera (P16220; Millipore) and anti-MEF2 antisera (sc-13266x; Santa Cruz Biotechnology) overnight at 4°C. Aliquots of chromatin that was not incubated with an antibody were used as the input sample control. Antibody-bound protein/DNA complexes were washed, eluted, reverse-cross-linked, and treated with proteinase K to digest proteins. The chromatin samples were then used in PCR analyses. Primers amplifying the mouse PGC-1α promoter fragment containing CRE were 5′-AAG CGT TAC TTC ACT GAG GCA GAG G-3′ (forward) and 5′-ACG GCA CACACT CAT GCA GGC AAC C-3′ (reverse), generating a 206-bp product. Primers amplifying the mouse PGC-1α promoter fragment containing MEF2RE were 5′-CGC TGC ATT TCT TTC TTC CTT TGA C-3′ (forward) and 5′-AAC CAG CTC ATT TTC TTT ACT GTA C-3′ (reverse), generating a 229-bp product. PCR reactions were visualized in 4% agarose gels.

**Immunoblotting.** Cells were lysed in Nonidet P-40 (NP-40) lysis buffer [1% NP-40, 150 mM NaCl, 10% glyceral, 2 mM EDTA, 20 mM Tris (pH 8.0), 1 mM dithiothreitol, 1 mM sodium orthovanadate, 1 mM phenylmethysulfonyl fluoride, 2 μg/ml leupeptin, and 10 μg/ml aprotinin]. Cell lysates (15 μg/total protein or 30 μg/lane for nuclear extracts) were resolved in 4–20% Tris-glycine gels (Invitrogen) and transferred to nitrocellulose membranes (Bio-Rad). After blocking with 5% skim milk (catalog no. 25010602; GE Healthcare, Piscataway, NJ), the membranes were incubated overnight with primary antibodies. Target proteins were detected by immunoblotting with specific antiserum as indicated and alkaline phosphatase-conjugated secondary antiserum. Fluorescent bands were visualized with a Typhoon 9410 variable mode Imager from GE Healthcare and then quantified by densitometry analysis using Image-Quant 5.2 software from Molecular Dynamics (Piscataway, NJ).

**RNA extraction and real-time PCR.** Total RNAs were extracted from cells or tissues with an RNeasy Mini Kit (Qiagen) and reverse-transcribed into cDNAs, which were then quantified by real-time PCR with specific probes and primers and normalized to levels of 36B4. The oligo sequence for PGC-1α quantification is forward: 5′-AAT CAGACCG TGACCAAGCG-3′; reverse: 5′-GATTTTCTT-CAATTTCACCAA-3′. The oligo sequence for 36B4 quantification is forward: 5′-GGACGCAACGTGGCCTCCAAAGC GAT-3′; reverse: 5′-GGTCCTCCTTGGTGAACACGAACC-3′.

**Statistical analysis.** Data are presented as means ± SEM. Data were compared by Student’s t-test with GraphPad Prism version 4.0 for Windows (GraphPad, San Diego, CA). Differences at values of P ≤ 0.05 were considered statistically significant.
RESULTS

Decreased expression of PGC-1α in the skeletal muscle is associated with increased basal levels of Akt- and p38 MAPK-dependent signalings in mice on HFD. It has previously been shown that expression of PGC-1α is decreased in skeletal muscles in subjects with insulin resistance and hyperinsulinemia (29). In contrast, PGC-1α expression is increased in both liver and skeletal muscle when insulin secretion is deleted by streptozotocin (24). We have shown recently that the basal level of Akt phosphorylation is increased in both liver and skeletal muscle in mice with HFD-induced insulin resistance and hyperinsulinemia (20). Furthermore, the basal p38 MAPK activity may be also increased in skeletal muscles in subjects with insulin resistance/hyperinsulinemia (16). To further investigate the mechanism of the decreased PGC-1α expression in subjects with insulin resistance and hyperinsulinemia, C57BL/6 (B6) mice were fed either ND or HFD for 4 wk, followed by evaluations of PGC-1α level and basal levels of p38 MAPK and Akt phosphorylation. Food consumption and body weight were increased by HFD feeding for 4 wk (Fig. 1, A and B). Fasting plasma insulin level was significantly increased by HFD, whereas fasting blood glucose level was not yet altered (Fig. 1, C and D). As a result, insulin sensitivity evaluated by HOMA was significantly decreased by HFD (Fig. 1E). As shown in Fig. 1, F and G, levels of both mRNA and protein of the PGC-1α gene in gastrocnemius were reduced by the 4-wk HFD. Basal levels of both Akt and p38 MAPK phosphorylations were also increased in gastrocnemius by HFD (Fig. 1, H and I). Together, these results imply that PGC-1α expression may be balanced by the p38 MAPK- and Akt-dependent intracellular signaling pathways.

Optimal transcription of the PGC-1α gene requires both p38 MAPK signaling and FoxO transcription factor(s). To further define the mechanism by which p38 MAPK regulates transcription of the PGC-1α gene, p38 MAPK was activated by the constitutively active MKK6 (MKK6E) encoded by recombinant adenoviruses in C2C12 myotubes for 24 h, followed by evaluation of PGC-1α mRNA. Introduction of MKK6E increased the level of MKK6 proteins more than twofold (Fig. 2A). Overexpression of MKK6E activated p38 MAPK as predicted (Fig. 2A). The level of PGC-1α transcripts was increased by the overexpression of MKK6E, and the increase was largely prevented by the p38 inhibitor SB-203580 (Fig. 2B). To examine the effect of p38 activation on the PGC-1α promoter, p38 MAPK was activated by MKK6E or blocked in C2C12 myotube cells by dominant negative p38 MAPK (dn-p38α) encoded by recombinant adenoviruses or SB-203580. Overexpression of MKK6E stimulated the PGC-1α promoter, and the stimulation was blocked by either dn-p38α or SB-203580 (Fig. 2C). To examine activation of the CRE-binding transcription factors activated by p38 MAPK, phosphorylations of CREB and activating transcription factor-2 (ATF-2) were measured by immunoblotting. Overexpression of MKK6E stimulated CREB phosphorylation very obviously with a very mild effect on ATF-2 phosphorylation. These results imply that p38 MAPK stimulation of PGC-1α transcription in C2C12 myotube cells may be mediated by CREB under this condition.

To determine the potential role of CREB in PGC-1α gene transcription in C2C12 myotube cells, interactions between transcription factors CREB and myocyte enhancer factor 2C
(MEF2C) and PGC-1α promoter elements were examined by ChIP assays with specific antibodies against either CREB or MEF2C, which is known to regulate the PGC-1α promoter (3). As shown in Fig. 3, CREB bound the CRE and the PGC-1α promoter upon p38 MAPK activation. The interaction between MEF2C and MEF2RE was slightly influenced by p38 MAPK activation. These results further show that p38 MAPK promotes PGC-1α gene transcription primarily through CREB instead of MEF2C.

To further investigate the mechanism of p38 MAPK stimulation of the PGC-1α gene transcription, CRE, MEF2RE, and three putative IREs (IRE1: \(-204^{\text{atgtttg}}-198\); IRE2: \(-360^{\text{atgtttg}}-360\); IRE3: \(-1860^{\text{atgtttg}}-1863\); see Supplemental Table S1 for details) were mutated one by one, followed by evaluation of p38 MAPK activation of the PGC-1α promoter. As shown in Fig. 4A, mutation of either the CRE or IREs decreased the promoter activity significantly, whereas mutation of MEF2RE reduced the promoter activity less dramatically. Next, the combined effects of p38 MAPK and IRE-binding transcription factors (FoxO) were examined. As shown in Fig. 4B, overexpression of either MKK6E or Ad-FoxO1 stimulated the PGC-1α promoter activity moderately. However, coexpression of MKK6E and Ad-FoxO1 activated PGC-1α promoter dramatically. Together, these results indicate that full activation of the PGC-1α gene transcription requires both the p38 MAPK-mediated activation of transcription factors like CREB and the presence of FoxO transcription factor(s).

Insulin blunts the p38 MAPK-mediated expression of the PGC-1α gene. As described above, PGC-1α expression was decreased in the skeletal muscle when the basal phosphorylation levels of both Akt and p38 MAPK were increased (Fig. 1). Since Akt phosphorylation is a key step of insulin signaling and the mice under HFD always have hyperinsulinemia, we examined the role of insulin in the decreased PGC-1α expression. As shown in Fig. 5A, overexpression of MKK6E stimulated phosphorylation of both p38 MAPK and CREB while elevating PGC-1α protein level. The elevated PGC-1α protein level was reduced by the treatment with insulin, which stimulated Akt phosphorylation. Similarly, overexpression of MKK6E enhanced PGC-1α mRNA level, but the enhancement was blocked by insulin (Fig. 5B). To determine the mechanism of insulin suppression of PGC-1α expression, all three putative IREs of the PGC-1α promoter were mutated, followed by evaluations of the wild-type and mutant PGC-1α promoters.

**Fig. 5.** Insulin blunts p38 MAPK-mediated expression of the PGC-1α gene. A: p38 was activated by MKK6E encoded by recombinant adenoviruses in C2C12 myotube cells for 24 h. Some cells were treated with either SB-203580 or insulin simultaneously. Protein levels of phosphorylated and total p38, phosphorylated and total Akt, and tubulin in cytoplasm were measured by immunoblotting. PGC-1α, phospho-CREB, and laminin receptor in the nuclear lysates were determined by immunoblotting. Results represent means ± SD of 2 independent experiments. *P < 0.05 and **P < 0.01 vs. vehicle (−); #P < 0.05, ##P < 0.01, and ###P < 0.001 vs. MKK6E alone. B: p38 was activated by overexpression of MKK6E encoded by recombinant adenoviruses in C2C12 myotube cells for 24 h. Some cells were treated with either SB-203580 or insulin simultaneously, followed by measurements of PGC-1α mRNA with real-time RT-PCR. C: the PGC-1α promoter was introduced into C2C12 myotube cells via transient transfection together with MKK6E encoded by recombinant adenoviruses as indicated for 24 h. Some cells were treated with insulin (1 nM) or SB-203580 (SB; 1 μM) simultaneously. The promoter activity was then evaluated by luciferase assays and normalized to β-galactosidase activity. Results represent means ± SD of 3 independent experiments. *P < 0.05 and **P < 0.01 vs. MKK6E alone; #P < 0.05 vs. MKK6E + insulin (4th bar); &P < 0.05 vs. negative control (1st bar).
As shown in Fig. 5C, both insulin and the p38 MAPK inhibitor SB-203580 blunted the p38 MAPK-mediated activation of the PGC-1α promoter, and insulin suppression was largely prevented by LY-294002. SB-203580 did not affect the basal level of the promoter activity, whereas LY-294002 increased it. When the IREs were mutated, the overall activity of the PGC-1α promoter was decreased, and insulin failed to further decrease the activity of the PGC-1α promoter. Together, these results demonstrate that p38 MAPK-mediated activation of PGC-1α gene transcription is suppressed by insulin signaling.

Insulin suppression of the PGC-1α gene transcription is FoxO dependent. Insulin usually inhibits transcription of target genes by phosphorylating and excluding FoxO transcription factors from the nucleus (30). To determine the role of FoxO transcription factors in insulin suppression of the PGC-1α gene, the constitutive nuclear form of FoxO1 encoded by adenoviruses was introduced into C2C12 myoblast cells. Ad-FoxO1 adenovirus infected C2C12 cells effectively (Fig. 6, top). Some cells were treated with insulin as noted. As shown in Fig. 6, insulin did not alter basal levels of PGC-1α and nuclear FoxO1 (compare lanes 1 and 4). Overexpression of Ad-FoxO1 increased levels of both nuclear FoxO1 and PGC-1α proteins. Importantly, insulin failed to suppress the expression of PGC-1α driven by the constitutive nuclear form of FoxO1. These results suggest that insulin suppresses PGC-1α gene expression by excluding FoxO transcription factors from the nucleus.

Insulin suppression of PGC-1α gene transcription is mediated mainly by IRE1 and IRE2. To determine the promoter elements that are involved in insulin inhibition of the PGC-1α gene, three putative insulin response elements (IRE1, -2, and -3) were mutated one at a time or all together. Activities of these mutant promoters were then examined in C2C12 myoblast cells. As shown in Fig. 7, mutation of either IRE1 or IRE2 abolished the promoter activity completely, whereas mutation of IRE3 reduced the promoter activity less dramatically. Mutations of all three IREs together reduced the promoter activity significantly, but apparently less dramatically than mutations of either IRE1 or IRE2 alone. These results suggest that IRE1 and IRE2 are necessary for PGC-1α promoter activation, and IRE3 may be required for the optimal activation of PGC-1α gene transcription.

**DISCUSSION**

Since its discovery by Puigserver et al. (35), PGC-1α has been shown to be involved in many different fundamental processes, such as thermogenesis, gluconeogenesis, maintaining the architecture and functions of skeletal muscles, central control of metabolism, antioxidation, cholesterol metabolism, and aging (14, 18, 19, 28, 35–37, 39, 42). Overall, PGC-1α appears to be a master transcriptional coactivator that promotes the combustion of stored energy primarily by enhancing mitochondrial production and consequently influences other fundamental processes such as oxidative stress and aging. In this...
study, we have found that PGC-1α gene transcription is balanced by different intracellular signaling pathways.

Expression of PGC-1α is increased under different situations. First, PGC-1α expression is increased in the brown adipose tissue after cold exposure, during which insulin signaling is reduced (27). Second, PGC-1α expression is increased in the liver when plasma insulin level is low during fasting and when insulin secretion is deficient in diabetes (7, 24). Third, PGC-1α expression is increased in skeletal muscles after physical exercise (2, 41). In all of these situations, the increased PGC-1α expression is accompanied by the elevated activity of p38 MAPK. In some cases, it has been shown that blockade of p38 MAPK can prevent the increased expression of PGC-1α. Results from this study also show that p38 MAPK plays a stimulatory role in PGC-1α in myotubes. The stimulatory effect of p38 MAPK has previously been shown to be mediated by ATF-2 (2). Our results in this study show that CREB plays a primary role of p38 activation of PGC-1α promoter. The reason for the difference is currently unknown.

Expression of PGC-1α is at a low level during the fed and unprovoked state (7, 35). A lot of attention has been paid to why PGC-1α expression is increased under various conditions whereas little attention has been paid to the question of why PGC-1α expression is low under the fed and unprovoked states. It can be due to lack of stimulators such as adrenelines, glucagon, p38 MAPK, or others. But it can certainly be due to the presence of an inhibitor(s). One such likely candidate is insulin. Insulin is increased in the blood during the fed state and is the single most dominant inhibitor of energy spending, which is contrary to the master effect of PGC-1α in promoting fuel combustion. Decreased insulin signaling is accompanied by cold-induced increase of PGC-1α expression (27). Deletion of insulin secretion leads to increased PGC-1α expression (24). Here we have found that addition of insulin can prevent p38 MAPK-mediated expression of PGC-1α. Insulin suppression of PGC-1α expression is neutralized by the phosphatidylinositol 3-kinase inhibitor LY-294002 or overexpression of the constitutively nuclear form of FoxO1. Furthermore, our results demonstrate that the optimal activation of the PGC-1α promoter requires both p38 MAPK- and FoxO-mediated activities. Thus, the low FoxO activity in the presence of insulin signaling may contribute to the low level of PGC-1α expression during the fed and unprovoked states. This may help explain why PGC-1α expression in skeletal muscles is decreased in subjects with insulin resistance and hyperinsulinemia (11, 29). Since both PGC-1α and FoxO transcription factors are known to be required for maintaining muscle mass, our results may also help explain why subjects with insulin resistance/hyperinsulinemia generally lose their muscle mass while gaining fat mass.

The functions of FoxO transcription factors are carried out through their interactions with various IREs. Previous studies identified three IREs in the PGC-1α promoter (12). They are located at −976 to −970, −586 to −580, and −354 to −348 (12). In this study, we also identified three IREs, but they are located at −1,869 to −1,863 (IRE1), −366 to −360 (IRE2), and −204 to −198 (IRE3). Mutations of either IRE1 or IRE2 almost abolished the promoter activity, whereas mutations of IRE3 only slightly diminished the promoter activity. Unexpectedly, mutations of all three IREs appear to have less effect than mutations of either IRE1 or IRE2 alone. The reason is unclear now. It is possible that the three-dimensional structure of the PGC-1α promoter is altered by mutations of all three IREs, and the alteration may increase the access of other transcriptional stimulators such as CREB, leading to more promoter activity.

In summary, results from this study provide new understanding about p38 MAPK-mediated transcription of PGC-1α. More importantly, our results provide explanations about the low expression level of PGC-1α gene under various physiological and pathophysiological situations. Therefore, results from this study may provide new strategies to prevent and reverse many modern health problems associated with the positive energy imbalance caused by overeating and/or lack of physical activity.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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