DIGITAL ACCESS TO SCHOLARSHIP AT HARVARD
DASH.HARVARD.EDU

HARVARD LIBRARY
Office for Scholarly Communication

PGC-1β in the Regulation of Hepatic Glucose and Energy Metabolism

Citation
Lin, Jiandie, Paul T. Tarr, Ruojing Yang, James Rhee, Pere Puigserver, Christopher B. Newgard, and Bruce M. Spiegelman. 2003. “PGC-1β in the Regulation of Hepatic Glucose and Energy Metabolism.” Journal of Biological Chemistry 278 (33): 30843–48. doi:10.1074/jbc.M303643200.

Permanent link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:41543112

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA

Share Your Story

The Harvard community has made this article openly available.
Please share how this access benefits you. Submit a story .

Accessibility

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 278, No. 33, Issue of August 15, pp. 30843–30848, 2003
Printed in U.S.A.
© 2003 by The American Society for Biochemistry and Molecular Biology, Inc.

PGC-1β in the Regulation of Hepatic Glucose and Energy Metabolism*

Received for publication, April 8, 2003
Published, JBC Papers in Press, June 13, 2003, DOI 10.1074/jbc.M303643200

Jiandie Lin‡§, Paul T. Tarr‡, Ruojing Yang¶, James Rhee‡, Pere Puigserver‡, Christopher B. Newgard¶, and Bruce M. Spiegelman‡‖

From the ‡Dana-Farber Cancer Institute and the Department of Cell Biology, Harvard Medical School, Boston, Massachusetts 02115 and the ¶Sarah W. Stedman Nutrition and Metabolism Center and the Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina 27710

Peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) is a transcriptional coactivator that regulates multiple aspects of cellular energy metabolism, including mitochondrial biogenesis, hepatic gluconeogenesis, and β-oxidation of fatty acids. PGC-1α mRNA levels are increased in both type-1 and type-2 diabetes and may contribute to elevated hepatic glucose production in diabetic states. We have recently described PGC-1β, a novel transcriptional coactivator that is a homolog of PGC-1α. Although PGC-1β shares significant sequence similarity and tissue distribution with PGC-1α, the biological activities of PGC-1β in the regulation of cellular metabolism is unknown. In this study, we used an adenoviral-mediated expression system to study the function of PGC-1β both in cultured hepatocytes and in the liver of rats. PGC-1β, like PGC-1α, potently induces the expression of an array of mitochondrial genes involved in oxidative metabolism. However, in contrast to PGC-1α, PGC-1β poorly activates the expression of gluconeogenic genes in hepatocytes or liver *in vivo*, illustrating that these two coactivators play distinct roles in hepatic glucose metabolism. The reduced ability of PGC-1β to induce gluconeogenic genes is due, at least in part, to its inability to physically associate with and coactivate hepatic nuclear receptor 4α (HNF4α) and forkhead transcription factor O1 (FOXO1), two critical transcription factors that mediate the activation of gluconeogenic gene expression by PGC-1α. These data illustrate that PGC-1β and PGC-1α have distinct arrays of activities in hepatic energy metabolism.

Liver is a key organ in the maintenance of systemic glucose homeostasis in mammals. The liver keeps blood glucose levels nearly constant under various nutritional conditions and provides a crucial source of fuel for the function of many organs and tissues under conditions of food deprivation. Deficient hepatic glucose output may lead to hypoglycemia and cause malfunction of key tissues and organs such as the central nervous system, resulting in coma or death. On the other hand, elevated hepatic glucose secretion contributes very significantly to hyperglycemia in both type 1 and type 2 diabetes (1). Glycogenolysis and gluconeogenesis are both key components of hepatic glucose output; suppression of hepatic gluconeogenesis has been shown to improve overall glycemic control in both human patients and type 2 diabetes animal models (2, 3).

PGC-1α[1] was originally identified as a coactivator of nuclear hormone receptors and was shown to regulate the programs of mitochondrial biogenesis and adaptive thermogenesis in the brown fat and skeletal muscle (4–7). Ectopic expression of PGC-1α in cultured C2C12 myotubes or in cardiomyocytes *in vivo* results in the robust activation of mitochondrial gene expression and increases cellular respiration (6, 8). Transgenic expression of PGC-1α in skeletal muscle at near physiological levels of protein induces mitochondrial biogenesis and leads to a functional switch of skeletal myofibers from glycolytic type IIb to the more oxidative types IIa and I (9).

Although hepatic expression of PGC-1α mRNA and protein is minimal under fed conditions, the abundance of PGC-1α is rapidly increased in response to fasting. PGC-1α mRNA is also elevated in the livers of type 1 and type 2 diabetes mellitus (10, 11). Adenoviral-mediated expression of PGC-1α to the levels observed in livers of fasted animals activates the entire program of gluconeogenesis in both cultured primary hepatocytes and live rats. Importantly, increased glucose secretion was observed *in vitro*, and elevated glucose levels occurred *in vivo* in response to PGC-1α (11). Thus, it is very likely that elevated PGC-1α activity in the liver contributes to hyperglycemia observed in diabetes. Recent studies indicate that HNF4α and FOXO1, transcription factors that regulate gluconeogenic gene expression (11–13), are both critical targets of PGC-1α coactivation in the promoter of gluconeogenic genes (14, 15).

PGC-1β is a recently described coactivator in the PGC-1 gene family and shares extensive sequence identity with PGC-1α (16, 17). PGC-1β mRNA is expressed at high levels in the brown fat and heart, both of which have very high levels of oxidative metabolism (16). Hepatic mRNA for PGC-1β, like PGC-1α, is increased in response to fasting; however, the biological function of this coactivator in the regulation of cellular energy metabolism or gluconeogenesis has not been determined. In this study, we use adenoviral-mediated expression of PGC-1β to assess its role in the regulation of hepatic energy metabolism, in particular the genetic programs of gluconeogenesis and mitochondrial metabolism. We report here that

* This work was supported by National Institutes of Health Grants R01DK61562 (to B. M. S.) and P01DK58398 (to C. B. N.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Supported by a postdoctoral fellowship from the American Heart Association.

‖ To whom correspondence should be addressed: Smith Bldg., Rm. 958, Dana-Farber Cancer Institute, One Jimmy Fund Way, Boston, MA 02115. Tel.: 617-632-3567; Fax: 617-632-4655; E-mail: bruce_spiegelman@dfci.harvard.edu.

[1] The abbreviations used are: PGC-1, PPARγ coactivator-1; PPAR, peroxisome proliferator-activated receptor; HNF4α, hepatic nuclear receptor 4α; PEPCK, phosphoenolpyruvate carboxylkinase; G6Pase, glucose-6-phosphatase; GFP, green fluorescence protein; CPT I, carnitine palmatoyl transferase-1; luc, luciferase; FOXO1, forkhead transcription factor O1.

Downloaded from http://www.jbc.org/ by guest on October 14, 2019

PGC-1β is a powerful activator of mitochondrial gene expression, including those regulating the β-oxidation of fatty acids, but has relatively little ability to activate the program of gluconeogenesis. This difference can be at least be partly ascribed to distinct abilities of PGC-1α and PGC-1β to physically associate with and augment the activities of certain hepatic transcription factors involved in gluconeogenesis.

MATERIALS AND METHODS

Cell Culture and Transient Transfections—FAO hepatoma cells were maintained in RPMI 1640 medium containing 10% fetal bovine serum and infected with various titers of adenoviral vectors at 75% confluence as described previously (11). Primary hepatocytes (In Vitro Technologies, Baltimore, MD) were cultured in serum-free hepatocyte media. The cells were treated with various agents (10 μM forskolin; 1 μM dexamethasone; 100 nM insulin) for 12 h before RNA isolation.

SV40 T-antigen immortalized mouse hepatoma cells (18) were maintained in Dulbecco's modified Eagle's medium containing 4% fetal bovine serum supplemented with 0.2 μM dexamethasone (CRL- ATCC, Manassas, VA). These cultures were transiently transfected with various combinations of plasmids using Superfect (Qiagen, Valencia, CA) as described previously (19). Luciferase activity was measured 24–36 h following transfection. In the case of PPARα, Wy-14643 was added to some cultures to a final concentration of 10 μM for 24 h before cells are harvested for luciferase assays, 1995

Adenoviral-mediated Gene Transfer—Recombinant adenoviral vector containing PGC-1β was constructed by placing the full-length PGC-1β cDNA under the control of a CMV promoter. The construction of recombinant adenoviruses containing the cDNAs encoding PGC-1α (11), green fluorescent protein (11), or β-galactosidase (20) has been described previously. Recombinant adenoviruses were amplified in 293 cells, and total cell lysates containing viral particles were used to transduce FAO hepatoma cells or primary hepatocytes. The efficiency of transduction was monitored by GFP expression. For expression of PGC-1β in liver of live rats, the AdCMV-PGC-1β and AdCMV-β-galactosidase (control) adenoviruses were purified by CsCl centrifugation, as described previously (21).

RNA Expression Analysis—Total RNA was isolated from FAO hepatoma cells, primary rat hepatocytes, or rat liver following treatments and/or adenoviral infection using TRIzol (Invitrogen). For northern hybridization, 20 μg of total RNA were separated by gel electrophoresis, transferred to a nylon membrane, and subsequently hybridized with specific probes for various genes. For real-time PCR analysis, total RNA was treated with RNase-free DNase to remove genomic DNA contamination, reverse-transcribed, and subjected to PCR analysis using SyberGreen (Roche Applied Science). The primers for rat PGC-1α are: 5′-ATGCACTGACAGATGGAGACGTGAC-3′ and 5′-GTTCCTATACC-ATAGTCATGCATTG-3′. The primers for rat PGC-1β are: 5′-ACTATG-ATCCCACGTCTGAAGAGTC-3′ and 5′-CCTTGTCTGAGGTATTGAG-GTATTC-3′. The primers for 18 S RNA, used as an internal control, are: 5′-AGTCCCTGCCCTTTGTACACA-3′ AND 5′-CGATCCGAGGG-CCTCACTA-3′.

Coimmunoprecipitation Assays—FLAG-tagged PGC-1s, HNF4α, and PPARα were generated using coupled transcription-translation kit (Promega, Madison, WI). HNF4α and PPARα were labeled with [^{35}S]methionine. Protein interaction and subsequent immunoprecipitation were performed in a buffer containing 100 mM HEPES (pH = 7.6), 150 mM KCl, 2 mM $MgCl_2$, 10% glycerol, and 0.5% Triton X-100. Briefly, 10 μl of lysates containing FLAG-PGC-1α or FLAG-PGC-1β were incubated with 3, 8, or 15 μl of lysates containing HNF4α or PPARα in the presence of anti-FLAG M2 beads at room temperature for 2 h (Sigma). 10 μM Wy-14643 was included in the reactions containing PPARα. The beads were extensively washed with binding buffer and analyzed by SDS-PAGE. Bound HNF4α and PPARα were detected by exposing the gels to x-ray films. PGC-1α and PGC-1β input was detected by immunoblotting using specific antibodies.

RESULTS

Expression of PGC-1α and PGC-1β in Hepatic Development and Diabetes—The ability to activate gluconeogenesis, β-oxidation of fatty acids, and ketogenesis are all characteristic of mature hepatocytes, which are functionally different from the embryonic liver cells; the embryonic liver is primarily responsible for hematopoiesis during fetal development and undergoes many metabolic changes just before and after birth (22).

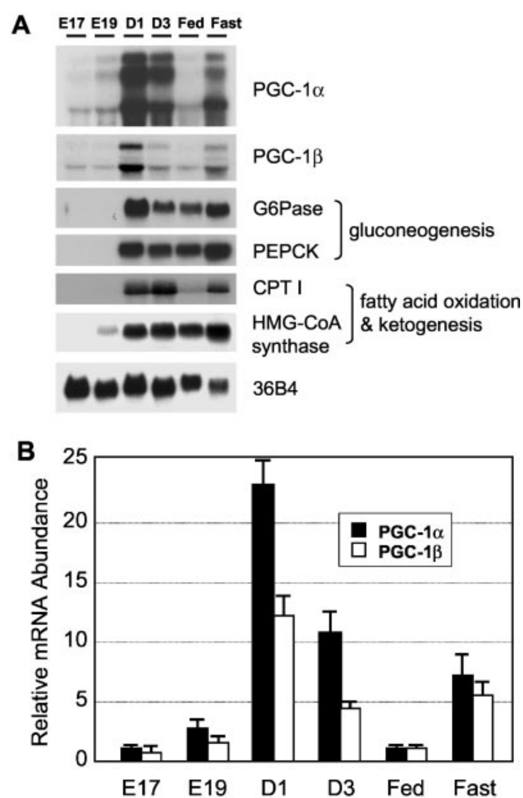

FIG. 1. **Regulation of PGC-1 expression during liver development.** As shown in *A*, total RNA was isolated from embryonic days 17 (*E17*) and 19 (*E19*) fetal liver, day 1 and 3 neonatal liver (*D1* and *D3*), and *ad libitum* (*Fed*) or fasted (*Fast*) adult liver. MRNA expression of PGC-1s and several genes involved in hepatic gluconeogenesis (PEPCK and G6Pase) and fatty acid metabolism (CPT I and hydroxymethylglutaryl (*HMG*)-CoA synthase) was analyzed by hybridization using specific probes. Hybridization to 36B4 is shown as a control for RNA loading. *B*, quantitative PCR analysis of hepatic PGC-1α and PGC-1β mRNA levels during liver development and in response to fasting.

To determine whether the PGC-1 coactivators may play a role in the metabolic maturation of hepatocytes, the expression of PGC-1α and PGC-1β mRNA was examined at different stages of mouse liver development. As shown in Fig. 1*A*, the mRNA levels of both PGC-1s are relatively low at days 17 and 19 (postconception) of the embryonic liver; however, there is a burst of PGC-1α expression at day 1 after birth and decreases to lower levels in the fed adult liver. PGC-1α expression is then rapidly induced in response to fasting in the adult, as was shown earlier. PGC-1β mRNA is also increased in the neonatal liver at day 1 after birth, but this induction is less dramatic than PGC-1α, and is reduced at day 3 after birth (Fig. 1). As shown previously, the expression of PGC-1β mRNA is induced in adult liver in the fasted state. Importantly, the induction of the PGC-1 coactivators at day 1 after birth closely coincides with the transcriptional activation of genes involved in gluconeogenesis (PEPCK and G6Pase), fatty acid β-oxidation (CPT I), and ketogenesis (mitochondrial hydroxymethylglutaryl-CoA synthase, Fig. 1), consistent with the demonstrated role of PGC-1α in regulating these metabolic functions of hepatocytes.

PGC-1α expression is rapidly induced in the liver during fasting, a process that is mediated by cAMP and glucocorticoid signaling pathways (11). To explore the hormonal regulation of PGC-1β expression, cultured primary rat hepatocytes were treated with modulators of these and related pathways, and the expression of PGC-1 mRNAs was determined by quantitative PCR analysis. As observed previously, the levels of PGC-1α mRNA are strongly increased by treatment with forskolin (an

Downloaded from http://www.jbc.org/ by guest on October 14, 2019

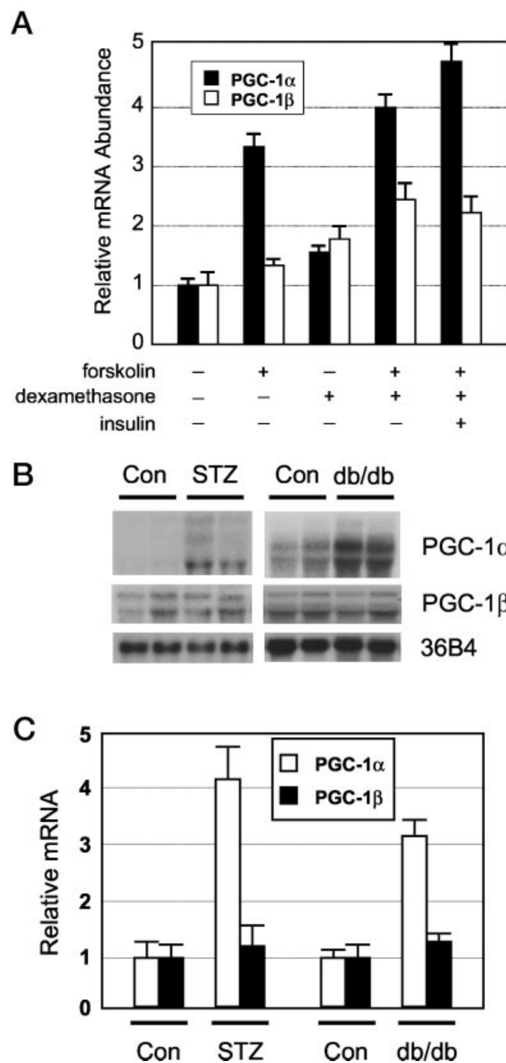

FIG. 2. **Hormonal and pathophysiological modulation of PGC-1 expression. As shown in** *A*, cultured primary rat hepatocytes were treated with various hormonal combinations (10 μM forskolin, 1 μM dexamethasone, and 100 nM insulin) for 12 h. Gene expression was analyzed by quantitative PCR using primer sets specific for PGC-1α, PGC-1β, and 18 S RNA as an internal control. *B*, analysis of PGC-1 mRNA levels in the liver from control (*Con*) or streptozotocin-treated (*STZ*) diabetic mice and control or db/db mice. *C*, quantitative PCR analysis of PGC-1α and PGC-1β mRNA levels in the control and diabetic mouse liver.

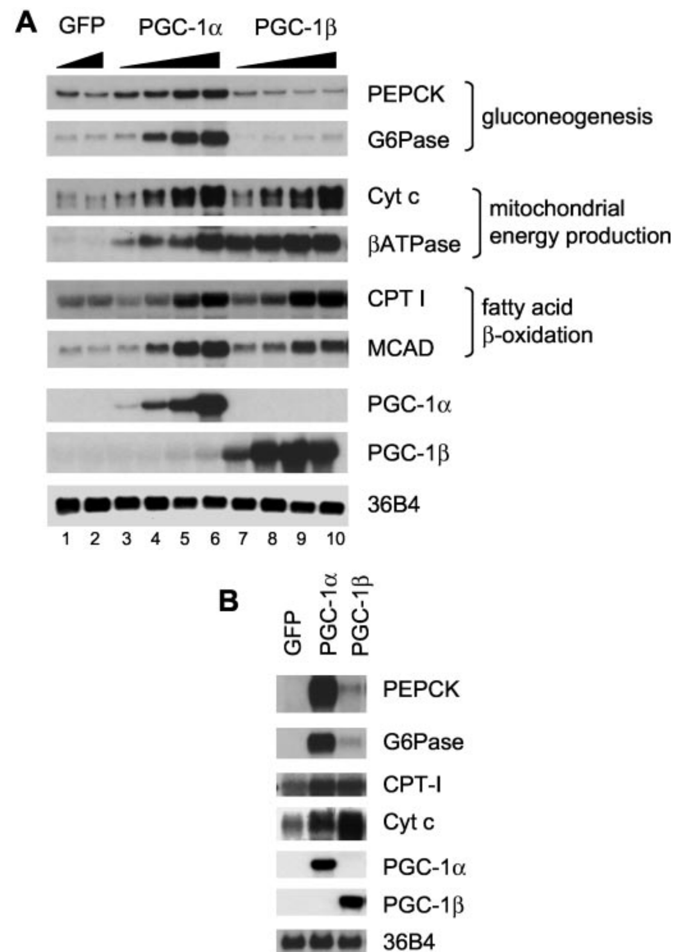

FIG. 3. **Activation of mitochondrial but not gluconeogenic gene expression by PGC-1β.** As shown in *A*, FAO hepatoma cells were infected with different doses of recombinant adenoviruses expressing GFP (*lanes 1–2*), PGC-1α (*lanes 3–6*), or PGC-1β (*lanes 7–10*). Total RNA was isolated and analyzed by hybridization using specific probes. *Cyt c*, cytochrome *c*. As shown in *B*, primary rat hepatocytes were infected with adenoviruses expressing GFP, PGC-1α, or PGC-1β and analyzed for gene expression. Hybridization to 36B4 is shown as a control for RNA loading.

activator of adenylate cyclase) and modestly increased by dexamethasone, a synthetic glucocorticoid (Fig. 2*A*). In contrast, PGC-1β mRNA levels are not elevated by forskolin and are increased modestly by dexamethasone, but the combination of forskolin and dexamethasone increases PGC-1β expression by ~2–3-fold. Insulin appears to have no effect on PGC-1α or PGC-1β mRNA levels under these conditions.

It has been shown previously that PGC-1α expression is elevated in both type 1 and type 2 diabetic mouse models. To determine hepatic PGC-1β levels in pathophysiological states, liver from streptozotocin-induced type 1 diabetic mice and leptin receptor-deficient db/db diabetic (type 2) mice was analyzed for PGC-1β expression. In contrast to PGC-1α, which is induced in both of these mouse models, PGC-1β mRNA levels are not altered (Fig. 2*B*). These results were also confirmed by quantitative real-time PCR analysis (Fig. 2*C*).

Regulation of Hepatic Gene Expression by PGC-1b—These data illustrating that PGC-1β is regulated distinctly from PGC-1α suggested that they might have distinct biological functions. To examine this, we treated cultured cells or live rats with recombinant adenoviruses expressing PGC-1β or a control green fluorescent protein (GFP). FAO hepatoma cells have been shown to respond to various hormonal stimuli and provide a useful culture model to study hepatic gene expression. As shown in Fig. 3*A*, ectopic expression of PGC-1α in FAO cells resulted in a dose-dependent increase of PEPCK and G6Pase mRNA levels. In striking contrast, PGC-1β has no effect on the expression of these two gluconeogenic genes. However, PGC-1β, like PGC-1α, potently induces the expression of genes of mitochondrial energy metabolism such as cytochrome *c* and βATP synthase. In addition, genes involved in fatty acid oxidation, *i.e.* medium chain acyl-CoA dehydrogenase (MCAD) and CPT I, are also induced by both PGC-1β and PGC-1α (Fig. 3*A*). This divergence of PGC-1 action on gluconeogenic and mitochondrial gene expression is also observed in adenovirally infected primary rat hepatocytes (Fig. 3*B*). Although both PGC-1 coactivators enhance the expression of certain mitochondrial genes, including CPT I and cytochrome *c*, only PGC-1α robustly activates the expression of PEPCK and G6Pase. Weak but detectable increases in the PEPCK and G6Pase mRNA levels were observed in PGC-1β-infected hepatocytes.

The effects of PGC-1β on hepatic gene expression *in vivo*

Downloaded from http://www.jbc.org/ by guest on October 14, 2019

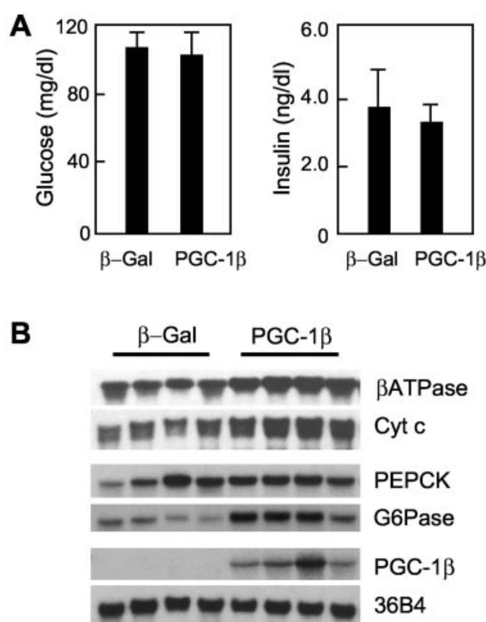

FIG. 4. **Regulation of hepatic metabolism by PGC-1β *in vivo*.** As shown in *A*, normal chow-fed rats were infected with purified recombinant adenoviruses expressing β-galactosidase (*β-gal*) (n = 15) or PGC-1β (n = 6). Plasma glucose and insulin levels were measured 6 days following infection. As shown in *B*, expression of mitochondrial and gluconeogenic genes in the liver was analyzed by hybridization using specific probes with 36B4 as a loading control.

were examined by systemic infusion of purified adenoviruses expressing a β-galactosidase control or PGC-1β into rats via tail veins. Adenovirus predominantly transduces hepatocytes when delivered systemically, resulting in restricted and robust expression of inserted genes in the liver of host animals (20, 23). Previous work has demonstrated that elevating PGC-1α expression via adenoviral infusion into rats leads to mild hyperglycemia and higher circulating insulin levels, accompanied by activation of gluconeogenic gene expression. Ectopic expression of PGC-1β in rat liver does not alter plasma glucose and insulin levels (Fig. 4*A*). Although the expression of cytochrome *c* and βATP synthase is increased in response to PGC-1β, hepatic PEPCK mRNA levels are similar in rats receiving control or PGC-1β viruses. Surprisingly, the expression of G6Pase is consistently elevated by PGC-1β, a divergence from the data obtained in hepatoma and isolated hepatocytes (Fig. 4*B*). The distinct modes of the G6Pase induction by PGC-1β in cultured hepatocytes and *in vivo* have been observed in at least three groups of animals and multiple experiments in cultured hepatocytes. These studies together indicate that PGC-1β is a powerful regulator of the genes of mitochondrial energy metabolism; however, this coactivator does not activate a broad program of gluconeogenic genes, nor does it change systemic glucose or insulin levels.

Comparative Analysis of PGC-1α and PGC-1β on Hepatic Transcriptional Regulators—Recent studies indicate that gluconeogenic activity of PGC-1α is mediated by several transcription factors including the glucocorticoid receptor, HNF4α and FOXO1; the latter two factors are required genetically for PGC-1α action in controlling gluconeogenesis. To gain mechanistic insight as to why PGC-1β does not activate gluconeogenic gene expression in isolated hepatocytes, we examined coactivation of these transcription factors by PGC-1β on reporter plasmids containing their respective binding sites. As shown in Fig. 5*A*, PGC-1α augments the transcriptional activity of HNF4α by over 70-fold, whereas PGC-1β is a much weaker coactivator for HNF4α (~5-fold) under these conditions. Similarly, although PGC-1α enhances the activity of a FOXO1 reporter plasmid by ~3-fold (3× IRE-luc), PGC-1β has little or no effect on the transcriptional activity of FOXO1.

We also examined the effect of PGC-1s on PPARα, a key transcription factor in the activation of β-oxidation, a process activated by both of these coactivators (24, 25). As shown in Fig. 5*C*, both PGC-1α and PGC-1β robustly increase the transcriptional activity of PPARα when assayed on a reporter containing multimerized PPARα binding sites (4× DR1-luc, Fig. 5*C*). The coactivation of PPARα by PGC-1β is more ligand-dependent (12-fold increase in the presence of Wy-14643, a synthetic PPARα agonist) than PGC-1α (2-fold).

It is interesting that PGC-1α can coactivate both nuclear receptors studied here (HNF4α and PPARα), whereas PGC-1β can coactivate only PPARα. To gain additional insight into the differential activities of PGC-1 coactivators, the physical interaction between the PGC-1s and these receptors was examined. Although it has been shown previously that the N termini of both PGC-1α and PGC-1β are able to associate with several nuclear hormone receptors, including HNF4α and PPARα (16), it is unclear from those studies whether these two coactivators display distinct affinity for HNF4α in the context of full-length proteins. FLAG-tagged PGC-1α and PGC-1β translated *in vitro* were incubated with increasing amounts of ^{35}S-labeled PPARα or HNF4α and subsequently immunoprecipitated with anti-FLAG antibodies. As shown in Fig. 6, both PGC-1s are able to precipitate PPARα with similar affinity (*lanes 9–11* for PGC-1α as compared with *lanes 12–14* for PGC-1β). In contrast, only PGC-1α (*lanes 2–4*) but not PGC-1β (*lanes 5–7*) can effectively coimmunoprecipitate with HNF4α. Very weak interaction between PGC-1β and HNF4α can be detected at the highest HNF4α concentrations (*lane 7*). These results clearly indicate that PGC-1α and PGC-1β have distinct affinity for these nuclear hormone receptors.

DISCUSSION

We describe here the regulation of PGC-1β expression in the liver and the biological activities of this coactivator in the control of hepatic energy metabolism. Although PGC-1β shares a similar tissue distribution with PGC-1α, and the mRNA level of PGC-1β also increases in the liver during fasting, the signals that regulate the expression of these two PGC-1 coactivators appear to be quite different. First of all, unlike PGC-1α, which has been shown to be a direct target of the cAMP signaling pathway and cAMP-response element-binding protein activation, PGC-1β expression is not altered by forskolin treatment in cultured primary hepatocytes (Fig. 2*A*). This observation is consistent with previous findings that PGC-1β mRNA is not induced by cold exposure in brown adipose tissue (16), a process known to be accompanied by activation of β-adrenergic receptors and the cAMP pathways. In addition, the expression of PGC-1β remains essentially unchanged in the liver of streptozotocin-treated and db/db diabetic mice (Fig. 2), both of which have alterations in the glucagon/insulin axis. These results suggest that although PGC-1β mRNA levels are increased in the liver in response to fasting, the physiological signals that lead to the induction of PGC-1α and PGC-1β expression are probably different. Free fatty acid levels, which are elevated in all of these physiological states, must be considered as a potential regulator of PGC-1β.

Hepatocytes acquire their specialized metabolic activities such as gluconeogenesis, fatty acid oxidation, and the synthesis of ketone bodies shortly following birth (22). Sustained production of glucose by the liver is essential for the survival of pups, who consume milk relatively low in carbohydrate but rich in fats. The expression of PGC-1α mRNA in the liver is strongly increased at day 1 of postnatal development (Fig. 1), consistent

Downloaded from http://www.jbc.org/ by guest on October 14, 2019

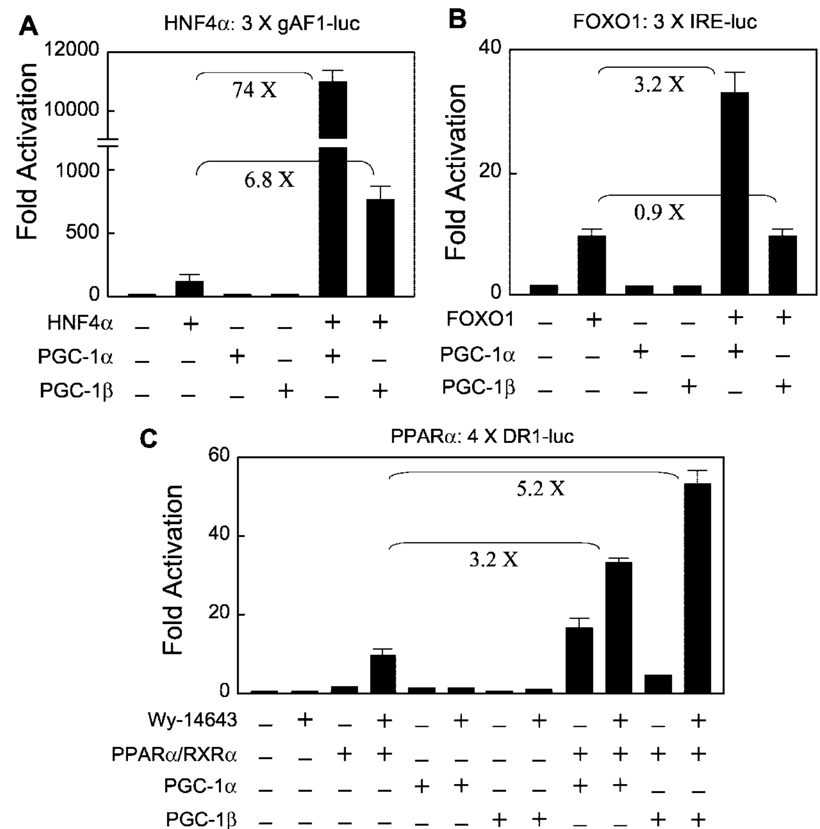

FIG. 5. **Coactivation of hepatic transcription factors by PGC-1β.** SV40 hepatocytes were transiently transfected with a reporter plasmid containing multimerized HNF4α binding sites (*A*, 3× gAF1-luc), FOXO1 binding sites (*B*, 3× IRE-luc), or a PPARα reporter plasmid (*C*, 4× DR-1-luc) along with various combinations of expression constructs as indicated. For PPARα transfection, cells were treated with vehicle or 10 μM Wy-14643 24 h before luciferase assays. Luciferase activity was expressed as -fold activation over vector alone. -Fold activation between different treatments is indicated.

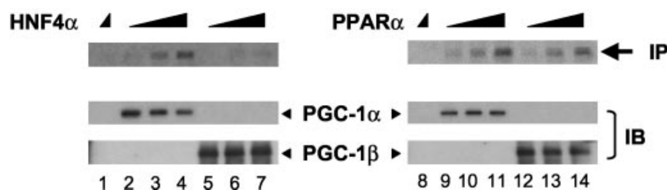

FIG. 6. **Physical interaction between PGC-1 coactivators and nuclear hormone receptors.** *In vitro* transcribed/translated FLAG-PGC-1α (*lanes 2–4* and *9–11*) or PGC-1β (*lanes 5–7* and *12–14*) were incubated with increasing amounts of ^{35}S-labeled HNF4α (*lanes 2–7*) or PPARα (*lanes 9–14*) along with anti-FLAG beads. Beads were also incubated with HNF4α (*lane 1*) or PPARα (*lane 8*) in the absence of PGC-1s as background controls. The immunoprecipitated complexes were resolved by SDS-PAGE followed by exposure to x-ray films (*IP*). Immunoblotting using antibodies against PGC-1α or PGC-1β was shown as a control for immunoprecipitation (*IB*). Note that both PGC-1s interact with PPARα with similar affinity, whereas only PGC-1α associates with HNF4α.

with its known function in the control of gene expression related to β-oxidation of fatty acids, gluconeogenesis, and ketogenesis (6, 11, 14). In contrast, the induction of PGC-1β expression is less striking during this period. Notably, PGC-1α and PGC-1β mRNA levels display different patterns of regulation in both type 1 and 2 diabetes. Taken together, all of these investigations of the expression of the PGC-1s suggest that these two coactivators are likely to play distinct roles in the regulation of hepatic gene expression and metabolism.

Adenoviral-mediated expression of PGC-1β in cultured hepatoma or primary hepatocytes results in robust induction of genes involved in the mitochondrial electron transport system and the β-oxidation of fatty acids, activities that are almost indistinguishable from that of PGC-1α (Fig. 3). These effects on mitochondrial gene expression, in particular, are consistent with previous observations that PGC-1β, like PGC-1α, is a powerful transcriptional coactivator for nuclear respiratory factor-1 (16), a key factor in the expression of many mitochondrial genes and mitochondrial biogenesis *per se* (26). However, in striking contrast to PGC-1α, PGC-1β has no effect on the expression of gluconeogenic enzymes in FAO hepatoma cells and only weakly increases their mRNA levels in primary hepatocytes. Consistent with the studies in cultured cells, hepatic expression of PGC-1β in live rats does not significantly alter glucose homeostasis *in vivo*, as ascertained by plasma glucose and insulin levels. Although the expression of mitochondrial genes such as βATPase and cytochrome *c* was induced by PGC-1β (Fig. 4), PEPCK mRNA levels were essentially unchanged. The expression of G6Pase is increased in response to PGC-1β *in vivo*, suggesting that hormonal milieu in the intact animals might play a critical role in the induction of G6Pase expression by PGC-1β. Alternatively, the induction of G6Pase expression in the liver may be due to indirect mechanisms that result from ectopic PGC-1β expression and probably altered hepatic lipid metabolism. Prior studies have demonstrated that adenovirus-mediated expression of the catalytic subunit of G6Pase in liver of normal rats is sufficient to alter glucose homeostasis and circulating insulin levels (23). The lack of alteration in circulating glucose and insulin levels probably reflects the lack of induction of PEPCK, the initial and rate-limiting step of the gluconeogenic pathway (2, 27).

Several transcription factors have been implicated as mediators of PGC-1α action on gluconeogenic promoters, including the glucocorticoid receptor, HNF4α, and FOXO1 (11, 14, 15). The latter two factors appear to be genetically required for PGC-1α action on the gluconeogenic genes in isolated hepatocytes. Transient transfection analysis indicates that PGC-1β is much weaker in augmenting the activity of HNF4α and FOXO1 when assayed on reporter plasmids containing multimerized respective binding sites (Fig. 5). In contrast, both PGC-1s coactivate PPARα and nuclear respiratory factor-1 to a similar extent under these experimental conditions (Fig. 5 and data not shown), suggesting that PGC-1α and PGC-1β have distinct sets of transcription factor targets. The lack of ability by

Downloaded from http://www.jbc.org/ by guest on October 14, 2019

PGC-1β to robustly enhance the transcriptional activity of HNF4α and FOXO1 probably contributes to its weak gluconeogenic activity. The inability of PGC-1β to strongly coactivate HNF4α is likely to be controlled, at least in part, by weak interaction between these two factors. Although previous experiments have shown that the N terminus of PGC-1β is able to physically associate with a number of nuclear hormone receptors including HNF4α with apparent similar affinity as PGC-1α, our new studies showed that full-length PGC-1β is unable to bind HNF4α, although it is clearly capable of associating with PPARα (Fig. 6). It is likely that the full-length protein may provide certain steric constraints on the availability of the docking sites that eradicate the interaction between HNF4α and PGC-1β. This mechanism may also provide the basis for generating selectivity with regard to other transcription factor coactivation targets for PGC-1α and PGC-1β.

These new data allow some perspective on the role of PGC-1β in the normal physiology of energy homeostasis in the liver. During fasting, as well as in the postnatal period, the liver is called upon to oxidize free fatty acids and utilize the resulting energy to support all other aspects of hepatic metabolism, including gluconeogenesis. Since PGC-1β can activate the genetic program of fatty acid β-oxidation and mitochondrial electron transport, without a strong activation of gluconeogenic genes, it appears to be capable of energetically "charging" the liver. These data are also consistent with bioenergetic analysis recently carried out with PGC-1s in muscle cells, where PGC-1β was found to stimulate a great increase in mitochondrial respiration with no increase in the degree of uncoupling (28). Hence, in both of these tissues, PGC-1β appears to serve, more than PGC-1α, as a molecular machine devoted to providing increased cellular energy via fuel oxidation. Ultimately, the relative role of PGC-1β in the energy metabolism as shown here must be complemented by genetic loss-of-function studies.

REFERENCES

1. Saltiel, A. R., and Kahn, C. R. (2001) *Nature* **414,** 799–806
2. Nordlie, R. C., Foster, J. D., and Lange, A. J. (1999) *Annu. Rev. Nutr.* **19,** 379–406
3. Moller, D. E. (2001) *Nature* **414,** 821–827
4. Knutti, D., Kaul, A., and Kralli, A. (2000) *Mol. Cell. Biol.* **20,** 2411–2422
5. Puigserver, P., Wu, Z., Park, C. W., Graves, R., Wright, M., and Spiegelman, B. M. (1998) *Cell* **92,** 829–839
6. Wu, Z., Puigserver, P., Andersson, U., Zhang, C., Adelmant, G., Mootha, V., Troy, A., Cinti, S., Lowell, B., Scarpulla, R. C., and Spiegelman, B. M. (1999) *Cell* **98,** 115–124
7. Puigserver, P., and Spiegelman, B. M. (2003) *Endocr. Rev.* **24,** 78–90
8. Lehman, J. J., Barger, P. M., Kovacs, A., Saffitz, J. E., Medeiros, D. M., and Kelly, D. P. (2000) *J. Clin. Invest.* **106,** 847–856
9. Lin, J., Wu, H., Tarr, P. T., Zhang, C. Y., Wu, Z., Boss, O., Michael, L. F., Puigserver, P., Isotani, E., Olson, E. N., Lowell, B. B., Bassel-Duby, R., and Spiegelman, B. M. (2002) *Nature* **418,** 797–801
10. Herzig, S., Long, F., Jhala, U. S., Hedrick, S., Quinn, R., Bauer, A., Rudolph, D., Schutz, G., Yoon, C., Puigserver, P., Spiegelman, B., and Montminy, M. (2001) *Nature* **413,** 179–183
11. Yoon, J. C., Puigserver, P., Chen, G., Donovan, J., Wu, Z., Rhee, J., Adelmant, G., Stafford, J., Kahn, C. R., Granner, D. K., Newgard, C. B., and Spiegelman, B. M. (2001) *Nature* **413,** 131–138
12. Hall, R. K., Sladek, F. M., and Granner, D. K. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 412–416
13. Nakae, J., Kitamura, T., Silver, D. L., and Accili, D. (2001) *J. Clin. Invest.* **108,** 1359–1367
14. Rhee, J., Inoue, Y., Yoon, J. C., Puigserver, P., Fan, M., Gonzalez, F. J., and Spiegelman, B. M. (2003) *Proc. Natl. Acad. Sci. U. S. A.*
15. Puigserver, P., Rhee, J., Donovan, J., Walkey, C. J., Yoon, J. C., Oriente, F., Kitamura, Y., Altomonte, J., Dong, H., Accili, D., and Spiegelman, B. M. (2003) *Nature* **423,** 550–555
16. Lin, J., Puigserver, P., Donovan, J., Tarr, P., and Spiegelman, B. M. (2002) *J. Biol. Chem.* **277,** 1645–1648
17. Kressler, D., Schreiber, S. N., Knutti, D., and Kralli, A. (2002) *J. Biol. Chem.* **277,** 13918–13925
18. Zaret, K. S., and Stevens, K. A. (1990) *Mol. Cell. Biol.* **10,** 4582–4589
19. Puigserver, P., Rhee, J., Lin, J., Wu, Z., Yoon, J. C., Zhang, C. Y., Krauss, S., Mootha, V. K., Lowell, B. B., and Spiegelman, B. M. (2001) *Mol. Cell* **8,** 971–982
20. Herz, J., and Gerard, R. D. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 2812–2816
21. Becker, T. C., Noel, R. J., Coats, W. S., Gomez-Foix, A. M., Alam, T., Gerard, R. D., and Newgard, C. B. (1994) *Methods Cell Biol.* **43,** 161–189
22. Duncan, S. A. (2000) *Dev. Dyn.* **219,** 131–142
23. Trinh, K. Y., O'Doherty, R. M., Anderson, P., Lange, A. J., and Newgard, C. B. (1998) *J. Biol. Chem.* **273,** 31615–31620
24. Hashimoto, T., Cook, W. S., Qi, C., Yeldandi, A. V., Reddy, J. K., and Rao, M. S. (2000) *J. Biol. Chem.* **275,** 28918–28928
25. Kersten, S., Seydoux, J., Peters, J. M., Gonzalez, F. J., Desvergne, B., and Wahli, W. (1999) *J. Clin. Invest.* **103,** 1489–1498
26. Huo, L., and Scarpulla, R. C. (2001) *Mol. Cell. Biol.* **21,** 644–654
27. Pilkis, S. J., and Granner, D. K. (1992) *Annu. Rev. Physiol.* **54,** 885–909
28. St-Pierre, J., Lin, J., Krauss, S., Tarr, P. T., Yang, R., Newgard, C. B., and Spiegelman, B. M. (2003) *J. Bio. Chem.* **278,** 26597–26603

Downloaded from http://www.jbc.org/ by guest on October 14, 2019

PGC-1β in the Regulation of Hepatic Glucose and Energy Metabolism
Jiandie Lin, Paul T. Tarr, Ruojing Yang, James Rhee, Pere Puigserver, Christopher B. Newgard and Bruce M. Spiegelman

J. Biol. Chem. 2003, 278:30843-30848.
doi: 10.1074/jbc.M303643200 originally published online June 13, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M303643200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 26 references, 10 of which can be accessed free at
http://www.jbc.org/content/278/33/30843.full.html#ref-list-1

Downloaded from http://www.jbc.org/ by guest on October 14, 2019