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‡

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

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). Glycolysis 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‡ 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 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...
PGC-1β is a powerful activator of mitochondrial gene expression, including those regulating the β-oxidation of fatty acids, and 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 vectors expressing PGC-1α was constructed by placing the full-length PGC-1α 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-1α in liver of live rats, the AdCMV-PGC-1α 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 blotting 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).
activator of adenylate cyclase) and modestly increased by dexamethasone, a synthetic glucocorticoid (Fig. 2A). In contrast, PGC-1α/H9252 mRNA levels are not elevated by forskolin and are increased modestly by dexamethasone, but the combination of forskolin and dexamethasone increases PGC-1α/H9252 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. 2B). These results were also confirmed by quantitative real-time PCR analysis (Fig. 2C).

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. 3A, 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 ATP 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. 3A). This divergence of PGC-1 action on gluconeogenic and mitochondrial gene expression is also observed in adenovirally infected primary rat hepatocytes (Fig. 3B). Although both PGC-1 coactivators enhance the expression of certain mitochondrial genes, including CPT 1 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

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 18S 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.
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. 4A). 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. 4B). 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. 5A, 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-1α 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. 5C, 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. 5C). 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 35S-labeled PPARα or HNF4α and subsequently immunoprecipitated with anti-FLAG antibodies. As shown in Fig. 6, both PGC-1α 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 communoprecipitate 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. 2A). 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. 2B), 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...
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 vivo 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 co-activate 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
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-1αs 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.

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