Cholesterol 7-α-hydroxylase (CYP7A1) is the key enzyme that commits cholesterol to the neutral bile acid biosynthesis pathway and is highly regulated. In the current studies, we have uncovered a role for the transcriptional co-activator PGC-1α in CYP7A1 gene transcription. PGC-1α plays a vital role in adaptive thermogenesis in brown adipose tissue and stimulates genes important to mitochondrial function and oxidative metabolism. It is also involved in the activation of hepatic gluconeogenic gene expression during fasting. Because the mRNA for CYP7A1 was also induced in mouse liver by fasting, we reasoned that PGC-1α might be an important co-activator for CYP7A1. Here we show that PGC-1α and CYP7A1 are also co-induced in livers of mice in response to streptozotocin induced diabetes. Additionally, infection of cultured HepG2 cells with a recombinant adenovirus expressing PGC-1α directly activates CYP7A1 gene expression and increases bile acid biosynthesis as well. Furthermore, we show that PGC-1α activates the CYP7A1 promoter specifically in oxidative metabolism assays in cultured cells. Thus, PGC-1α is a key activator of CYP7A1 and bile acid biosynthesis and is likely responsible for the fasting and diabetes-dependent induction of CYP7A1. PGC-1α has already been shown to be a critical activator of several other oxidative processes including adaptive thermogenesis and fatty acid oxidation. Our studies provide further evidence of the fundamental role played by PGC-1α in oxidative metabolism and define PGC-1α as a link between diabetes and bile acid metabolism.

The CYP7A1 enzyme converts cholesterol into 7-α-hydroxycholesterol, which is the first specific intermediate in the neutral bile acid biosynthesis pathway in the liver (1). This is a crucial enzyme in mammalian cholesterol metabolism as diversion into the bile acid pathway is the main route for eliminating excess cholesterol from the body. Because of its key role in cholesterol metabolism, the CYP7A1 enzyme and its gene have been studied as an important model for dietary regulation for several years. These studies have revealed that there is a significant amount of regulation at the level of transcription initiation (2); however, post-transcriptional mechanisms for control also occur (1, 3).

The CYP7A1 promoter has been extensively evaluated by several groups and the proximal regions of both the mouse and rat promoters contain two direct repeat type elements that bind several nuclear receptors, some of which are indicated in Fig. 1. There is also a binding site for the monomeric orphan receptor LRH-1 that overlaps the DR-1 element. The DR-4 is a target site for the nuclear receptor LXR, which confers positive regulation by cholesterol to the CYP7A1 promoter in mice and rats (2). However, the DR-4 is not conserved in the human gene which is not subject to feed forward regulation by cholesterol (4).

CYP7A1 is also activated in livers of fasted mice (5). Similarly, the transcriptional co-activator PGC-1α is induced by fasting in liver where it activates transcription of the PEPCK and glucose-6-phosphotase genes during hepatic gluconeogenesis (6). Because both PGC-1α and CYP7A1 were co-induced during fasting, we reasoned that the induction of CYP7A1 might be dependent on PGC-1α. We also reasoned that CYP7A1 might be induced under other physiologically important situations where PGC-1α is also induced such as in the liver of diabetic animals (6). In the current report we show that expression of the CYP7A1 gene is not only co-induced with PGC-1α during fasting but also when animals are made diabetic through streptozotocin injection. We also show that infection of HepG2 cells with an adenovirus expressing PGC-1α results in a robust activation of endogenous CYP7A1 gene expression, which in turn results in an overall increase in bile acid biosynthesis. Finally, we show that the CYP7A1 promoter is directly activated by PGC-1α co-expression in transient transfection assays.

MATERIALS AND METHODS

Plasmids—The pcDNA3.1.rHNF-4a1 has been described previously (7). The pcDNA3.1.rHNF-4a1 was a kind gift from F. Sladek (University of California, Riverside, CA). The pcDNA3.1(−)PGC-1α, was from D. Kelly (Washington University in St. Louis) and has been described previously (8). The adenovirus expressing PGC-1α was from Bruce Spiegelman (Dana Farber Cancer Center, Boston) and was described before (9).

Cell Culture and Transient Transfection Assay—HepG2 cells were maintained in minimum essential medium supplemented with 10% fetal bovine serum at 37 °C and 5% CO2. One day before transfection, cells were seeded in 6-well plates at a density of 350,000 cells/well. After incubation for 16 h, cells were transfected by the calcium phosphate co-precipitation. Co-precipitates contained 2 μg/well of the luciferase reporter, 2 μg/well of the β-galactosidase plasmid as an internal control for transfection efficiency, and the indicated amount of expression vector as described in the legend to Fig. 5. Salmon sperm DNA was added to equalize total amounts of plasmid DNA transfected into cells. After 4–6 h, cells were treated with 10% glycerol, washed three times with...
phosphate-buffered saline, refed with fresh normal medium, and incubated for an additional 40–48 h before harvest. 293T cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum at 37 °C and 5% CO₂. Cells were seeded in 6-well plates at a density of 300,000 cells/well the day before transfection. After an overnight incubation, cells were transfected for 16 h as described above. The next day, cells were washed three times with phosphate-buffered saline, refed with fresh normal medium, and cultured for an additional 24 h before harvest.

**Enzyme Assays**—Luciferase assays were performed using a luminometer with a luciferin reagent (Promega). β-Galactosidase assays were carried out by a standard colorimetric procedure with 2-nitrophenyl-β-D-galactopyranoside as a substrate. Luciferase activities were corrected and presented as the ratio of luciferase activity over β-galactosidase activity. Values represent the mean of duplicate S.D. Each experiment was repeated at least three times.

**Animal Feeding Studies**—For the fasting experiments, B6129 male mice were obtained from Taconic and maintained on a 12-h light/dark cycle (dark cycle was from 7 p.m. to 7 a.m.) with free access of food and water. Mice were allowed to adapt to the new environment at least 1 week before experiments. Mice (7–8 weeks old) were randomly divided into three groups. One group was fed ad libitum with regular diet, a second group was fasted for 24 h, and a third group was fasted for 24 h followed by refeeding regular diet for 12 h before sacrifice. All mice were sacrificed at the same time between 8 and 10 AM. Livers were removed and immediately frozen in liquid nitrogen and stored at −80 °C until RNA was extracted as described below.

For the streptozotocin (STZ) experiments, 7–8-week-old mice were prepared as above and separated into two groups. After they were adapted, one group received daily intraperitoneal injections of STZ (100 μg/g of body weight) for 3 days. Ten days later, blood glucose levels were measured with a glucometer (One Touch Ultra from Lifescan), animals were sacrificed at 9:30 a.m., and livers were removed and processed as above.

**Adenovirus Infection of HepG2 Cells**—Replication defective recombinant adenoviruses were propagated and titered in 293 cells. They were used to infect HepG2 cells that were plated in 100-mm dishes at the multiplicities of infection indicated in the legend to Fig. 4A. Medium was replaced and cells were processed at various times post-infection for total RNA harvest followed by blot hybridization studies as described below.

**Bile Acid Synthesis**—Bile acid synthesis rates were quantified essentially as described previously (10). Briefly, HepG2 cells were seeded on 60-mm plates at 2.5 × 10⁶ cells/plate. Twenty-four hours later cells were infected at the indicated multiplicity of infection in 0.5 ml of serum-free medium. After 2 h, the virus was diluted with 1.5 ml of medium, and 0.13 μCi of [14C]cholesterol (50 mCi/mmol) was added and incubation was continued for 40 h. Conversion of [14C]cholesterol to [14C]methanol/
water-soluble products was determined by scintillation counting after Folch extraction (11) of the culture medium with chloroform/methanol (2:1, v/v).

**RNA Isolation and Northern Blot Analysis**—Total RNA was isolated from mouse livers using TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. 20 μg of total RNA was fractionated on 1% agarose-formaldehyde gels and transferred to Nytran (Schleicher & Schuell). The membranes were hybridized with 32P-labeled cDNA probes overnight at 42 °C. A 80 base HindIII/EcoRI fragment of rat ribosomal protein L32 cDNA was used as a probe to normalize total amounts of RNA per lane. After hybridization, the blots were exposed to a Kodak BioMAX film, and mRNA levels were quantified by Quan-tity one (Bio-Rad). The following cDNA probes were used: CYP7A1, a 0.7-kb AccI/EcoRI fragment from the pBSK7a; PGC-1α, a 1.5-kb BglII/NcoI fragment from the pSV-SPORT-PGC-1 (gift of Peter Ton-tonoz, UCLA); fatty acid synthase (FAS), a 0.2-kb EcoRI fragment from mouse FAS cDNA; SHP, a 0.8-kb XhoI fragment from CDM8-SHP; PEPCK, a 1.3-kb SphI/SalI fragment from PC116 (gift of D. Granner, Vanderbilt University of School of Medicine); LDLR, a 0.3-kb BamHI/PstI fragment from mouse LDLR cDNA; h-CYP7A1, a 0.3-kb fragment from human CYP7A1 cDNA isolated by PCR from HepG2 cDNA.

**RESULTS**

For over 2 decades, a fasting/refeeding protocol has been used to evaluate lipogenic and gluconeogenic gene responses. We noted that CYP7A1 gene expression was stimulated by fasting and returned to normal during the refeeding phase of this dietary manipulation (Fig. 2A). This pattern is exactly opposite to the expression of FAS, which was repressed by fasting and activated by the refeeding protocol as is typical for lipogenic genes (Fig. 2C). As mentioned in the introduction, expression of the mRNA for the transcriptional co-activator PGC-1α was induced in mouse liver by a similar fasting protocol (9), and we noted this response in our experiment as well (Fig. 2B). SHP is a known negative regulator of CYP7A1 (2), and although SHP levels were mildly reduced by fasting, they did not rebound by the refeeding protocol (Fig. 2D). Taken together, these observations are consistent with induction of CYP7A1 during fasting through a PGC-1α-dependent mechanism.

We reasoned that if PGC-1α is a physiological regulator of CYP7A1, then they should also be co-induced under other conditions where PGC-1α expression increases in the liver such as during STZ-induced diabetes (9). Therefore, we evaluated CYP7A1 expression in this diabetic model. In Fig. 3, we show that both PGC-1α and CYP7A1 are robustly induced in livers of mice following STZ injection. As expected, PEPCK was also co-induced, however, levels of LDL receptor and SHP RNAs were either unaffected or slightly lower in the STZ treated animals, respectively.

The experiments so far demonstrate that PGC-1α and CYP7A1 are co-induced during two different physiologically stressful situations: food restriction and diabetes. This strongly suggests that PGC-1α is a physiologically important co-activator for CYP7A1. To directly evaluate the response of CYP7A1 to an acute increase in PGC-1α we measured CYP7A1 mRNA levels in HepG2 cells following infection with a recombinant adenovirus engineered to express PGC-1α. The experiment in Fig. 4A demonstrates that CYP7A1 was robustly activated by the PGC-1α virus in a dose-dependent manner. Importantly, mRNA levels from other genes were not altered by the PGC-1α virus, and infection with a control virus had no effect on CYP7A1.

Because CYP7A1 is the rate-controlling step of the neutral bile acid biosynthetic pathway, it was important to evaluate whether the induction of CYP7A1 by PGC-1α also resulted in
increased production of bile acids. To evaluate this, bile acid synthesis was monitored in HepG2 cells following infection with recombinant adenoviruses expressing PGC-1α, CYP7A1, or a control virus (Fig. 4B). Infection with either the PGC-1α- or CYP7A1-expressing viruses resulted in a dose-dependent increase in bile acid production and there was no response to the control virus. These results indicate that not only is PGC-1α a critical co-activator for CYP7A1, but changes in its expression level can influence flux through the entire bile acid pathway as well.

To directly evaluate whether the PGC-1α protein was acting directly as a co-activator of the CYP7A1 promoter, we performed a series of co-transfection studies in cultured cells. In the experiment in Fig. 5A, the rat CYP7A1 promoter was stimulated 20-fold when an expression vector for PGC-1α was co-transfected with a CYP7A1 promoter-luciferase promoter construct in HepG2 cells. Because PGC-1α does not bind DNA directly, it must be recruited to a promoter through a separate DNA bound protein. To determine which critical DNA binding protein(s) might be responsible for PGC-1α activation of CYP7A1, we utilized exogenous expression vectors for DNA-binding proteins that are known to activate CYP7A1 (2). When an expression vector for HNF-4 was simply added along with the wild type promoter, there was minimal stimulation on its own (lane 2), and there was no additional stimulation when the HNF-4 construct was added on top of PGC-1α (lane 6). Similarly, addition of a COUP TFII expression vector alone was ineffective (lane 3). However, when the COUP TFII expression plasmid was added together with PGC-1α a significant stimulation over the level achieved by PGC-1α alone was observed (compare lane 3 with lanes 5 and 7). Adding expression constructs for both HNF-4 and COUP TFII stimulated the promoter about 20-fold in agreement with a previous report (12),

**Fig. 4.** PGC-1α expression from a recombinant adenovirus induces CYP7A1 and bile acid synthesis. A. HepG2 cells were infected with a control adenovirus or a recombinant expressing PGC-1α at the indicated multiplicity of infection. Cells were harvested at the indicated times and processed for RNA extraction and blot hybridization for PGC-1α, CYP7A1, and L32 mRNAs as described under “Materials and Methods.” B. HepG2 cells were infected at the indicated multiplicity of infection, and bile acid synthesis was measured as described under “Materials and Methods.”

**Fig. 5.** PGC-1α activation of CYP7A1 promoter. A. HepG2 cells were co-transfected with the wild type rat CYP7A1 luciferase reporter (−342 to +59) along with expression vectors for PGC-1α (3 μg), HNF-4 (0.5 μg), or COUP TFII (0.5 μg) as indicated. pCMV-β-galactosidase was added to each transfection as an internal control for transfection efficiency. Luciferase activities were measured as described under “Materials and Methods” and were corrected for β-galactosidase activity. Results are expressed as fold change relative to those of cells transfected with luciferase reporter alone. In B and C, 293T cells were transfected with the CYP7A1 promoter. The LRH-1 expression construct was added at 3 μg. All other constructs were added at the same levels as in A. Luciferase activities are plotted as relative light units divided by the β-galactosidase activity as indicated under “Materials and Methods.”
and when both expression constructs were added on top of PGC-1α a further significant increase in activation was observed (lane 8).

HepG2 cells have endogenous HNF-4, COUP TFII, and LRH-1, which is known to be a critical co-activator for CYP7A1 (7, 13, 14). To evaluate the protein requirements for CYP7A1 promoter stimulation in a naïve setting where endogenous liver expressed proteins are absent, we used 293T cells. When the CYP7A1 promoter was transfected into 293T cells, a low level of activity was observed (Fig. 5, B and C). To achieve high levels of stimulation, expression vectors for HNF-4, LRH-1, COUP TFII, and PGC-1α were all required (Fig. 5, B and C). Elimination of any one of the four protein expression constructs resulted in a significant drop in promoter activity.

**DISCUSSION**

There are several important observations in the current investigation. First, we have established a role for the transcriptional co-activator PGC-1α in activation of the CYP7A1 promoter. Based on the fasting-dependent induction of CYP7A1 mRNA (Ref. 5 and Fig. 2), we proposed that PGC-1α is a critical co-activator of oxidative metabolic genes involved because it is a co-activator protein that is induced by fasting in the liver (Ref. 9 and Fig. 2). While we were in the finishing stages of our work, a paper was published on-line in the JBC by De Fabiani et al. (15) demonstrating that PGC-1α stimulated CYP7A1. Importantly, our studies have gone further to demonstrate that CYP7A1 is co-induced with PGC-1α during STZ-induced diabetes (Fig. 3) and that infection of HepG2 cells with a recombinant adenovirus expressing PGC-1α also induces CYP7A1 mRNA expression and the entire pathway of bile acid biosynthesis (Fig. 4).

Expression of SHP mRNA was lower during fasting and STZ treatment. Because SHP is a negative regulator of CYP7A1 (14, 15), part of the increase in CYP7A1 expression in our study could be due to the lower levels of SHP. However, SHP levels did not rebound during the refeeding phase in Fig. 2 when CYP7A1 levels returned to normal. Therefore, alterations in SHP cannot adequately account for the effects observed in the current report.

Our cell culture-based transfection studies with a PGC-1α expression vector support a direct role for this co-activator in activation of CYP7A1 (Fig. 5). In these experiments, PGC-1α stimulated expression from the CYP7A1 promoter in HepG2 cells, and the effect was significantly enhanced by co-expression of COUP TFII and HNF-4. In 293T cells, high level expression of the promoter required co-transfection of expression vectors for HNF-4, COUP TFII, LRH-1, and PGC-1α. These results indicate that PGC-1α stimulation of CYP7A1 is likely mediated by more than one DNA binding protein acting on the promoter. This is similar to PEPCK where several different DNA binding factors are required for maximal stimulation by PGC-1α (9).

CYP7A1 commits cholesterol to the bile acid pathway and represents a key control point in metabolism. Even though there are many irreversible enzymatic steps in metabolism, relatively few of these are defined as “committing” their substrate to a singular metabolic pathway or fate (16). CYP7A1 represents one of these key metabolic points, and similar to other committed steps, there is a complex mechanism in place to ensure that metabolic flux can be integrated smoothly with variations in nutrient availability to provide balanced metabolism. The nutrient deprivation associated with fasting disrupted this balance, and CYP7A1 mRNA was induced. The physiological reason why CYP7A1 should be increased by fasting is not immediately apparent. In fact, there is evidence that bile acid synthesis and CYP7A1 enzyme activity are down-regulated by prolonged fasting in the rat (17). The differences with our studies may be related to different experimental parameters or timing and the species differences. Nonetheless, CYP7A1 was activated by fasting in both our study and a previous report (5).

CYP7A1 and PGC-1α were also co-induced during streptozotocin-induced diabetes (Fig. 3). Similar to nutrient deprivation, this is another physiologically stressful situation that throws lipid metabolism out of balance. The co-induction by fasting and STZ treatment provide strong correlative evidence that PGC-1α is a co-activator for CYP7A1. Evidence for a direct role was provided by the recombinant adenovirus and promoter studies of Figs. 4 and 5. Importantly, infection with the PGC-1α adenovirus not only resulted in an induction of CYP7A1 mRNA but also a significant stimulation in flux through the entire bile acid pathway. In fact, the increase was similar in magnitude to that obtained when HepG2 cells were infected with an adenovirus expressing CYP7A1 directly (Fig. 4B).

These observations are particularly relevant to the mechanism connecting diabetes with bile acids. It has long been known that bile acid metabolism is altered in diabetes (18), and the increased levels of circulating bile acids may be partially responsible for increased cholesterol absorption and atherosclerosis in diabetic patients. Insulin is also a potent negative regulator of CYP7A1 in primary hepatocytes (19). Additionally, there is a strong clinical association between type II diabetes plus obesity on the one hand and gallstone disease on the other (20). However, whether the increased gallstone formation was due to the diabetes or other complications associated with obesity has been somewhat controversial (21). Our studies demonstrating that CYP7A1 is increased in livers of diabetic mice support a direct association between diabetes and bile acid metabolism that could contribute directly to gallstone production independent of obesity.

Taken together, our results indicate that PGC-1α is an important co-activator in the regulation of CYP7A1 and bile acid metabolism. Like bile acid synthesis, the other metabolic pathways simulated by PGC-1α are associated with oxidative metabolism (22). Thus, our observations provide further evidence that CYP7A1 is a critical co-activator of oxidative metabolic processes.

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