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Partnership of PGC-1α and HNF4α in the Regulation of Lipoprotein Metabolism*[S]

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Peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) is a transcriptional coactivator involved in several aspects of energy metabolism. It is induced or activated under different stimuli in a highly tissue-specific manner and subsequently partners with certain transcription factors in those tissues to execute various biological programs. In the fasted liver, PGC-1α is induced and interacts with hepatocyte nuclear factor 4α (HNF4α) and other transcription factors to activate gluconeogenesis and increase hepatic glucose output. Given the broad spectrum of liver genes responsive to HNF4α, we sought to determine those that were specifically targeted by the combination of PGC-1α and HNF4α. Co-expression of these two molecules in murine stem cells reveals a high induction of mRNA for apolipoproteins A-IV and C-II. Forced expression of PGC-1α in mouse and human hepatoma cells increases the mRNA of a subset of apolipoproteins implicated in very low density lipoprotein and triglyceride metabolism, including apolipoproteins A-IV, C-II, and C-III. Coactivation of the apoC-III/A-IV promoter region by PGC-1α occurs through a highly conserved HNF4α response element, the loss of which completely abolishes activation by PGC-1α and HNF4α. Adenoviral infusion of PGC-1α into live mice increases hepatic expression of apolipoproteins A-IV, C-II, and C-III and increases serum and very low density lipoprotein triglyceride levels. Conversely, knock down of PGC-1α *in vivo* causes a decrease in both apolipoprotein expression and serum triglyceride levels. These data point to a crucial role for the PGC-1α/HNF4α partnership in hepatic lipoprotein metabolism.

The liver is central to systemic nutrient metabolism. It regulates both catabolic and anabolic processes that maintain proper blood levels of protein, carbohydrate, and lipid. The liver responds to blood-borne hormones and neurotransmitters, executing biological programs to ensure that the energy demands of peripheral tissues are satisfied. Many of these programs exert metabolic changes through the activation or induction of specific transcription factors, which in turn increase the expression of key enzymes or other downstream regulators.

The peroxisome proliferator-activated receptor γ (PPARγ)[4] coactivator-1α (PGC-1α) (1) has emerged as an important regulator of several liver functions. It positively regulates hepatic heme biosynthesis and links fasting to acute attacks of porphyria (2). PGC-1α induces CYP7A1, the rate-limiting enzyme of bile acid synthesis (3–5). It also partners with liver X receptor α and farnesoid X receptor in modulating cholesterol and triglyceride metabolism (6, 7). In the hepatic response to fasting, PGC-1α coordinates the induction of genes involved in gluconeogenesis, fatty acid oxidation, and ketogenesis (8, 9). Its coactivation of the liver-enriched transcription factors HNF4α and FOXO1, as well as the glucocorticoid receptor, results in the induction of gluconeogenic enzymes and increased hepatic glucose output (10, 11). The functional loss or genetic knock out of either HNF4α or FOXO1 completely abrogates the ability of PGC-1α to activate hepatic gluconeogenesis. PGC-1α interacts tightly with HNF4α *in vitro* and strongly coactivates this transcription factor on the phosphoenolpyruvate carboxykinase and glucose-6-phosphatase promoters (8, 10).

HNF4α has been recognized as a broad regulator of liver function (12, 13). Ectopic expression of HNF4α in de-differentiated hepatoma cells or fibroblasts induces both a morphology and genetic markers characteristic of hepatic epithelium (14, 15). Although the total knock out of HNF4α is embryonic lethal due to defects in gastrulation (16), embryos lacking HNF4α can be rescued in midgestation through tetraploid complementation (17). Analysis of E12.0 fetuses null for HNF4α shows that a lack of HNF4α impairs differentiation into a fully functional hepatic parenchyma that expresses its normal complement of serum and metabolic factors, such as apolipoproteins and fatty acid-binding proteins (18). Conditional knock out of the gene in the liver through a tissue-specific cre-lox system produces viable offspring that suffer from severe defects in lipid homeostasis; they accumulate lipid in the liver and exhibit reduced serum triglyceride levels (19). It is clear that the transcriptional targets of HNF4α are numerous and varied. In fact, using chromatin immunoprecipitation in human hepatocytes combined with promoter microarrays, HNF4α localizes to 12% of 13,000 promoters examined (20).

Given the important functional interaction of PGC-1α with HNF4α and the broad roles of these two molecules in hepatic function, it is quite likely that their partnership influences multiple aspects of liver metabolism beyond that which has been described. Through microarray analysis, we now identify a subset of apolipoproteins as transcriptional targets that are particularly dependent on the *cooperative* action of PGC-1α and HNF4α. Adenoviral infusion of PGC-1α into live mice results in the hepatic induction of these apolipoproteins and a concomitant increase in serum and VLDL triglyceride. Knock

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1 and S2 and supplemental Tables S1–S3.

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[4] The abbreviations used are: PPAR, peroxisome proliferator-activated receptor; PGC-1α, PPARγ coactivator-1 α; HNF4α, hepatocyte nuclear factor 4 α; VLDL, very low density lipoprotein; RNAi, RNA interference; GFP, green fluorescent protein; DR-1, direct repeat separated by one nucleotide.

TABLE 1
Highest scoring targets of AdPGC1α + AdHNF4α co-expression

		Fold expression over GFP		
	GFP alone	PGC-1α alone	HNF4α alone	PGC-1α + HNF4α
Apolipoprotein C-II	1	1	18.51	810.56
Aminoacylase 1	1	31.21	82.36	674.17
RIKEN cDNA 4932432N11 gene	1	36.6	1	270.45
Purinergic receptor P2X, ligand-gated ion channel, 3	1	1	201.21	229.43
Apolipoprotein A-IV	1	1	1	156.09
Retinol dehydrogenase 7	1	1	2.35	118.08
C-type lectin-related f	1	4.02	2.11	105.13
PGC-1α	1	43.08	NA	94.08
G_0/G_1 switch gene 2	1	NA	5.85	71.01
HNF4α	1	4.75	67.1	70.52

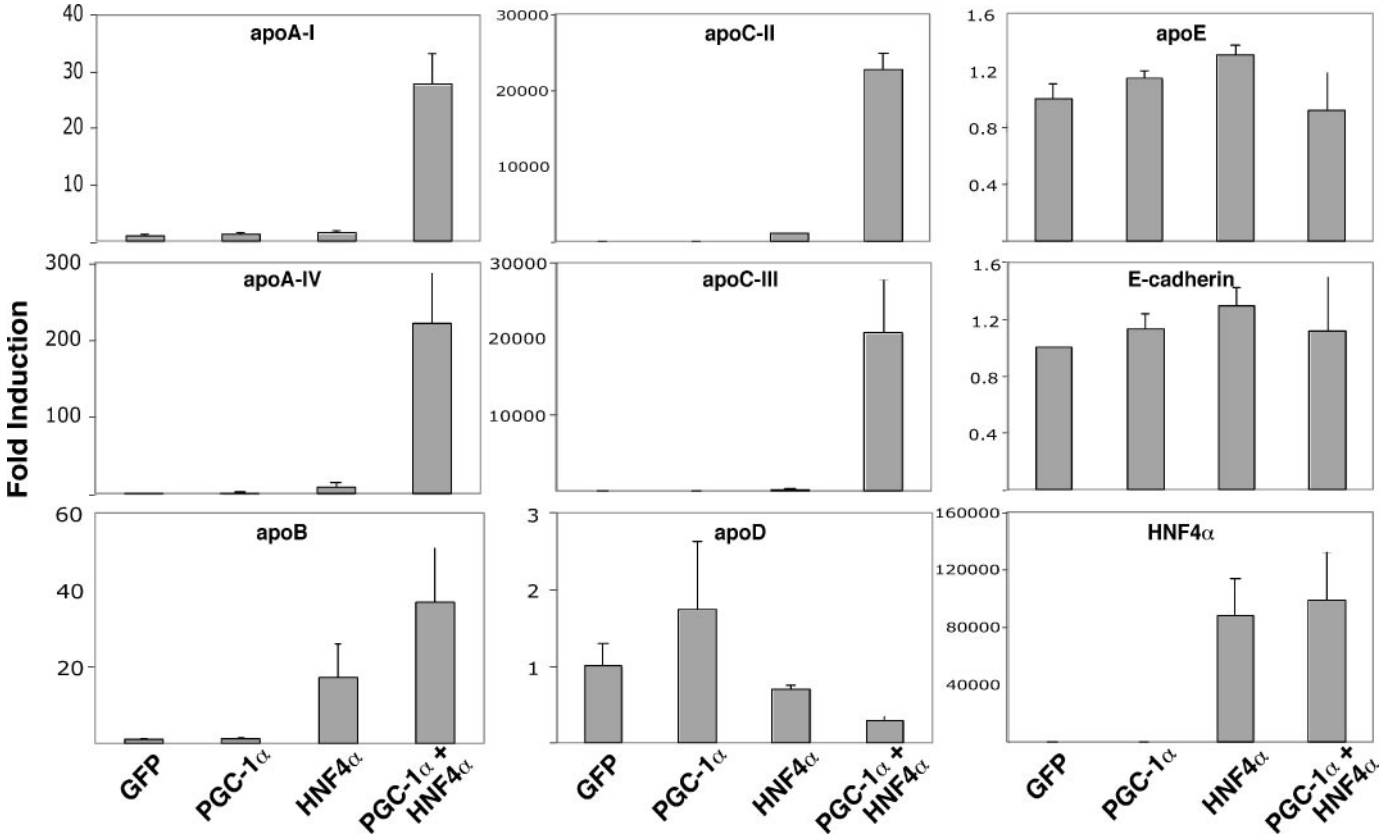

FIGURE 1. **The ectopic combination of PGC-1α and HNF4α induces apolipoproteins in F9 cells.** RNA taken from F9 teratocarcinoma cells infected with adenoviruses encoding GFP, PGC-1α, HNF4α, or PGC-1α plus HNF4α was subjected to quantitative PCR analysis. Primer sets were used that amplify a select group of apolipoproteins and other liver genes. Graphs represent -fold change over gene expression in cells receiving only GFP.

down of PGC-1α via adenoviral RNAi decreases apolipoprotein mRNA and serum triglyceride levels. These data indicate that the PGC-1α/HNF4α partnership plays a critical role in hepatic lipoprotein synthesis and export.

EXPERIMENTAL PROCEDURES

Microarray Analysis—Affymetrix array hybridization and scanning were performed by the Core Facility at Dana-Farber Cancer Institute using Murine 430 2.0 chips. Array data were analyzed with d-CHIP software.

RNA Isolation and Analysis—Total RNA was isolated from liver or cultured cells using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. For quantitative real-time PCR analysis, 2 μg of total RNA was reverse transcribed with Moloney murine leukemia virus reverse transcriptase (Invitrogen) and random hexamer primers (Roche Applied Science). Relative mRNA abundance normalized to 18 S rRNA levels was determined with the ΔΔCt method after amplification using an iCycler iQ real-time PCR detection system (Bio-Rad) and SYBR Green (Bio-Rad). For Northern hybridizations, 20 μg of each sample of RNA was resolved on a formaldehyde gel, transferred to nylon membrane using a TurboBlotter (Schleicher & Schuell), and hybridized with ^{32}P-labeled gene-specific probes. Hybridization to ribosomal protein 36B4 was included as a loading control.

Animal Experiments—All animal experiments were performed according to procedures approved by the Institutional Animal Care and Use Committee. Animals were fed standard rodent chow and housed in a controlled environment with 12-h light and dark cycles. Fasts were started at the beginning of a dark cycle, and mice were deprived of food for the indicated amount of time before being sacrificed.

Adenoviral Infection—Adenoviral GFP and PGC-1α have been described previously (54). An adenoviral vector encoding HNF4α was generated by cloning an HNF4α cDNA into AdTrack-CMV (Invitro-

gen). Cultured cells were infected with the indicated adenovirus at a multiplicity of infection of 50 at 90% confluence. 36 h after infection, cells were harvested and their RNA isolated. Female, 8-week-old C57 BL/6 mice were transduced with CsCl-purified adenovirus via tail vein injection. Mice were each tail vein injected with 0.2 OD of adenovirus ($\sim 2 \times 10^{11}$ viral particles/mouse). Four days later, they were subjected to a 24-h fast. The mice were sacrificed on the fifth day and their livers and plasma harvested.

Cell Culture, Transfections, and Reporter Gene Assays—F9 teratocarcinoma cells were cultured on gelatinized plates in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. H2.35 mouse hepatoma cells were kept in Dulbecco's modified Eagle's medium supplemented with 4% fetal bovine serum and 1 μM dexamethasone. HepG2 cells were cultured in 10% fetal bovine serum in Dulbecco's modified Eagle's medium. Cells were transfected in 6-well dishes at 70% confluence using Superfect (Qiagen) according to the manufacturer's protocol. Each transfection involved a 1:1:10 ratio of reporter gene:transcription factor(s):coactivator for a total of 3.6 μg of DNA. Equal amounts of DNA were used for all transfection combinations by using appropriate vector DNA. For reporter gene assays, cells were harvested 24 h after transfection. Luciferase levels were determined and normalized to β-galactosidase expression. All transfection experiments were repeated at least three times in triplicate.

Site-directed Mutagenesis—Site-directed mutagenesis of the apoC-III/A-IV luciferase reporter (28) was performed using overlapping primers and the Expand Long Template PCR system (Roche Applied Science). HNF4α-binding Site 1 was mutated from 5′-TGGGCAAAG-GTCA-3′ to 5′-TGGGCAAtGcgCA-3′, which introduced an FspI site. Site 2 was mutated from 5′-TGGACCTTGTTCT-3′ to 5′-TGGACCT-TcTaga-3′, which introduced an XbaI site.

Chromatin Immunoprecipitation—Experiments were performed using a chromatin immunoprecipitation kit (EZ ChIP) following the manufacturer's protocol (Upstate Biotechnology). H2.35 cells were transfected in 15-cm plates at 70% confluence with plasmids expressing GFP, FLAG-tagged PGC-1α, or FLAG-tagged PGC-1α plus HNF4α. Two days later cells were harvested, DNA-protein complexes cross-linked in formaldehyde, and immunoprecipitation reactions performed using M2 anti-FLAG resin (Sigma). After reverse cross-linking, DNA was purified by spin columns and subsequently analyzed by PCR.

Triglyceride Measurements and Lipoprotein Fractionation—Total serum triglyceride was measured using a colorimetric assay kit (337; Sigma). For fractionation experiments, serum was diluted 1:1 (v/v) with phosphate-buffered saline and centrifuged to remove any debris. 100 μl of diluted sample was injected into a Superose 6 HR 10/30 gel filtration column for analysis of plasma lipoproteins, performed using a fast protein liquid chromatography system (Pharmacia ÄKTA) at 4 °C (55). Samples were eluted at a flow rate of 0.4 ml/min in buffer containing 0.05 M phosphate and 0.15 M NaCl, pH 7.0. Fractions of 0.3 ml were collected for analysis. Triglyceride levels in each fraction were measured enzymatically using an Infinity Triglyceride (GPO-Trinder) kit (Sigma).

RESULTS

Combined Expression of PGC-1α and HNF4α Induces Apolipoproteins in Cultured Cells—To determine the genes that are dependent upon cooperation between PGC-1α and HNF4α, we exogenously expressed these two molecules in F9 teratocarcinoma stem cells using adenoviral vectors. These cells have the ability to produce all three germ layers and have been used heavily as a model system for differentiation (21). Table 1 is a partial list of the genes most highly induced by the

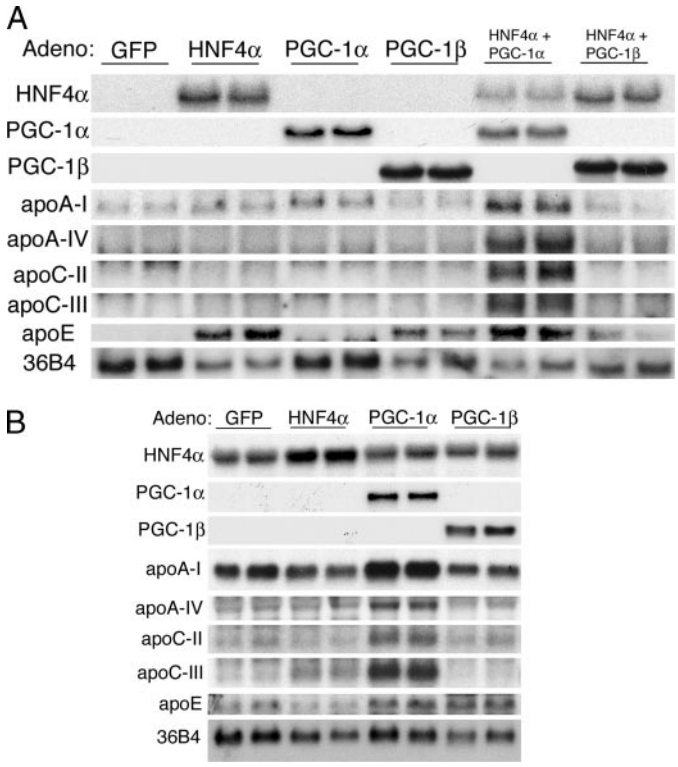

FIGURE 2. **The ectopic combination of PGC-1α and HNF4α induces apolipoproteins in hepatoma cells.** *A*, H2.35 mouse hepatoma cells were infected with the indicated adenoviruses, and their RNA was analyzed by Northern blotting with radiolabeled mouse cDNA probes. 36B4 was blotted as a loading control. *B*, HepG2 human hepatoma cells were infected with the indicated adenoviruses, and their RNA was analyzed by Northern blotting with radiolabeled human cDNA probes. 36B4 was blotted as a loading control.

combined ectopic expression of PGC-1α and HNF4α as determined by microarray analysis. Apolipoproteins C-II and A-IV are highly induced by the *combination* of HNF4α and PGC-1α, whereas either molecule alone does not induce them to nearly the same extent. Other apolipoproteins detected by the microarray are not significantly increased by these two molecules (supplemental Table S1).

To confirm the results of the microarray analysis, we analyzed the RNA from the F9 cells with quantitative PCR. As shown in Fig. 1, apoC-II and apoA-IV are highly induced by PGC-1α and HNF4α, much more so than by either protein alone. Interestingly, apoA-I and apoC-III are also significantly induced by PGC-1α and HNF4α in this context, despite their relatively low expression in the arrays. HNF4α activates the apoB promoter (22), and although there is a 20-fold induction of apoB by HNF4α alone, PGC-1α only further augments this by a modest 2-fold. The expression of apoE, another target of HNF4α (23), is not increased in the presence of ectopic PGC-1α and HNF4α. Likewise, although the epithelial marker E-cadherin is expressed in response to forced HNF4α expression in fibroblasts (14), it fails to be activated in these cells by the co-expression of PGC-1α and HNF4α. In summary, PGC-1α robustly coactivates a subset, but not all, of the downstream targets of HNF4α.

Apolipoprotein biosynthesis is most abundant in the liver *in vivo*. To examine the effects of PGC-1α and HNF4α in cells derived from liver, we exogenously expressed the two molecules in H2.35 mouse hepatoma cells through adenoviral transduction and subsequently harvested RNA. Northern analysis in Fig. 2*A* shows that the mRNAs for apolipoproteins A-IV, C-II, and C-III are induced to detectable levels *only* in the presence of both PGC-1α and HNF4α. ApoA-IV and apoC-II are expressed 11-fold over their levels with HNF4α alone, whereas apoC-III is increased 14-fold as

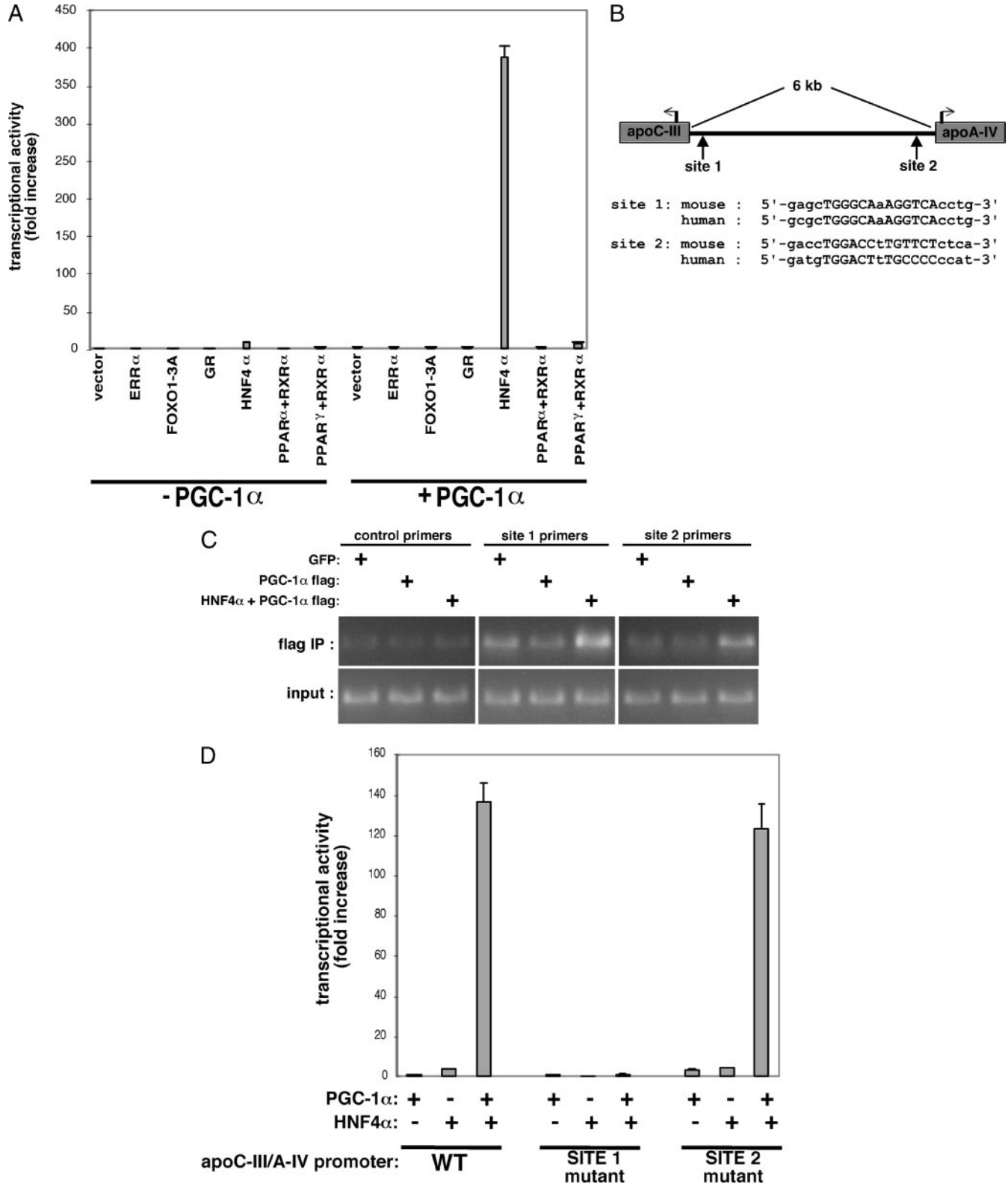

FIGURE 3. **PGC-1α coactivates and is recruited to the apoC-III/A-IV promoter region.** *A*, H2.35 cells were transfected with an apoC-III/A-IV luciferase reporter and plasmids encoding the transcription factors estrogen-related receptor α (*ERR*α), FOXO1, GR, HNF4α, PPARα, or PPARγ. Reporter activity was measured for each of these transcription factors in the absence and presence of PGC-1α and depicted as –fold change over the first lane (*vector alone*). Transfections were repeated at least three times in triplicate. *B*, a diagram of the apoC-III/A-IV intergenic region. The full-length mouse promoter is 6 kb long, and two previously identified HNF4α response elements are depicted. Conservation of these sites between human and mouse genomes is also shown. *C*, H2.35 cells were transfected with plasmids expressing GFP, FLAG epitope-tagged PGC-1α, or a combination of HNF4α and tagged PGC-1α. Following formaldehyde cross-linking, chromatin immunoprecipitation assays were performed using anti-FLAG resin. Co-immunoprecipitated DNA and 5% of input DNA were used as template for PCR primers flanking site 1, site 2, and a control region on chromosome 9 downstream of the apoA-IV gene. All PCR reactions were carried out to 23 cycles. *D*, H2.35 cells were transfected with PGC-1α, HNF4α, and either a wild-type or mutant apoC-III/A-IV luciferase reporter. HNF4α-binding sites 1 and 2 were mutated as described under "Experimental Procedures." PGC-1α coactivation of HNF4α was measured on each of the different reporters and depicted as –fold change over the first lane (*PGC-1α alone*). Transfections were repeated at least three times in triplicate.

measured by quantitative PCR (supplemental Table S2). Unlike its effect in F9 cells, ectopic HNF4α in H2.35 cells is, alone, sufficient to increase apoE levels. However, the addition of PGC-1α does not significantly enhance this induction. Likewise, HNF4α alone is able to induce apoB by 18-fold, and PGC-1α does not further increase its levels (supplemental Table S2). PGC-1β, a closely related homolog of PGC-1α that has been shown to regulate hepatic lipid synthesis and export (24), fails to induce these same genes. This is most likely due, at least in part, to the fact that PGC-1β coactivates HNF4α poorly (25).

To determine whether PGC-1α-mediated induction of apolipoproteins occurs within human cells as well as those from mouse, we expressed PGC-1α in HepG2 hepatoma cells. As in the H2.35 cells, PGC-1α is able to induce the same subset of apolipoproteins (Fig. 2*B*). However, in contrast to its expression in H2.35 cells, ectopic PGC-1α in HepG2 cells is alone sufficient to activate expression of these genes. In fact, adenoviral PGC-1α is much more effective in inducing these genes than is adenoviral HNF4α. For example, the level of apoA-IV is more than 30 times higher in the setting of adenoviral PGC-1α than in the presence of adenoviral HNF4α (supplemental Table S3). This is likely the result of a high endogenous level of HNF4α (Fig. 2*B*) through which the exogenously expressed PGC-1α may act.

PGC-1α Coactivates HNF4α on the apoC-III/A-IV Promoter—The *apoA-IV* and *apoC-III* genes are separated in the mouse and human genomes by an ~6-kb intergenic region (26). Because apoA-IV and apoC-III are transcribed in opposite directions, this intergenic region serves as a common 5′-flanking sequence for both genes (27). To test whether this region is responsive to PGC-1α coactivation of HNF4α, we transfected plasmids encoding these two factors along with an apoC-III/A-IV promoter luciferase construct into H2.35 hepatoma cells. Recent studies in intestinal epithelial cells report that PGC-1α coactivates estrogen-related receptor α on this promoter construct (28). Fig. 3*A* shows that PGC-1α robustly coactivates HNF4α on this reporter to nearly 400-fold over base line. No significant activation occurs when PGC-1α is co-expressed with other liver transcription factors, including a constitutively active allele of FOXO1, glucocorticoid receptor (GR), PPARα, and PPARγ (Fig. 3*A*). Previous reports have shown that PGC-1α coactivates these factors in other contexts (1, 11, 29).

We next identified the HNF4α response elements through which PGC-1α exerts its effects. Fig. 3*B* shows two previously characterized HNF4α-binding sites, labeled sites 1 and 2, located in the proximal promoters of apoC-III and apoA-IV, respectively (30, 31). These two direct repeats (DR-1) were also identified by NUBIScan, a computer algorithm that predicts DNA recognition sites for nuclear receptors (32). Site 1 is a DR-1 that is 100% conserved between mouse and human. Chromatin immunoprecipitations were performed with antibodies against FLAG epitope-tagged PGC-1α, expressed alone or with HNF4α in H2.35 hepatoma cells. Only the immunoprecipitates containing cross-linked complexes of PGC-1α and HNF4α with genomic DNA are enriched with fragments encompassing site 1 and site 2, indicating that PGC-1α is recruited to both of these sites through HNF4α (Fig. 3*C*). Primers that amplify a control region on chromosome 9 downstream from the *apoC-III*/*apoA-IV* gene cluster show no PGC-1α-specific enrichment.

To test the functional significance of these two putative HNF4α-response elements, we disrupted one of the two half-sites within each DR-1 through site-directed mutagenesis. Whereas mutation of site 2 leaves PGC-1α coactivation completely intact, mutation of site 1 utterly abolishes the PGC-1α effect (Fig. 3*D*). The absolute loss of response to PGC-1α when site 1 is mutated is observed in both the full-length reporter as well as in a truncated version containing 700 bp of apoC-III

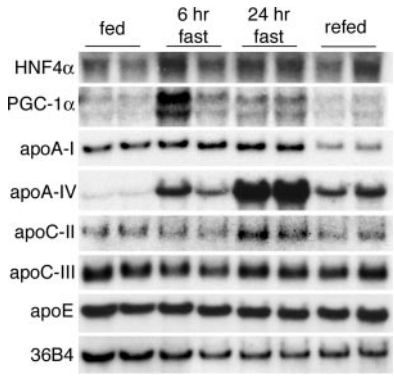

FIGURE 4. **Fasting induces hepatic mRNA for PGC-1α, apoA-IV, and apoC-II.** Mice were fasted for 6 and 24 h and sacrificed or refed for another 24 h and then sacrificed. Liver mRNA was analyzed for PGC-1α and apolipoprotein transcript levels via Northern blotting using radiolabeled mouse cDNA probes. 36B4 was blotted as a loading control.

proximal promoter (data not shown). Taken together, these data show that PGC-1α directly coactivates HNF4α on the apolipoprotein C-III/A-IV intergenic region through an HNF4α-responsive element.

Apolipoproteins A-IV and C-II Are Induced in Fasted Liver—We next investigated the physiological significance of PGC-1α-mediated induction of apolipoproteins. Because PGC-1α is an integral part of the hepatic fasting response, we looked at the expression of apolipoproteins in fasted mice. Previous reports demonstrate mild increases in apoA-I during fasting and more robust increases in apoA-IV (33). Northern blotting in Fig. 4 shows that the genes for PGC-1α and apolipoproteins A-IV and C-II are indeed induced under 6- and 24-h food deprivation. Whereas apoC-II is increased roughly 2-fold at the 24-h time point, apoA-IV is induced over 70-fold as assessed by quantitative PCR (supplemental Fig. S1). ApoA-IV has been closely linked to triglyceride metabolism, and apoC-II is a requisite cofactor for lipoprotein lipase activity and uptake of free fatty acids into extrahepatic tissues. Given the importance of apolipoproteins in lipid metabolism and their regulation by PGC-1α *in vitro*, we next investigated the role of PGC-1α in the regulation of triglyceride levels *in vivo*.

Hepatic PGC-1α Levels Influence Serum and VLDL Triglyceride Levels—We assessed apolipoprotein and lipid levels *in vivo* following either acute gain or loss of PGC-1α function. Adenoviral PGC-1α or adenoviral RNAi targeting PGC-1α was infused into the tail veins of mice and their hepatic mRNAs collected 5 days later immediately following a 24-h fast during which endogenous PGC-1α was induced.

Fig. 5*A* demonstrates that forced expression of PGC-1α causes a roughly 2-fold increase in mRNA levels for apolipoproteins A-IV and C-II compared with control mice and a modest increase in apoC-III expression. These transcriptional events occur in parallel with a 4-fold increase in serum triglyceride level (Fig. 5*B*). We next measured the triglyceride content of VLDL, because this lipoprotein is the major carrier of triglyceride in the endogenous pathway of lipid transport. Fast protein liquid chromatography lipoprotein fractionation and triglyceride measurement display an ~2-fold increase in triglyceride content in the VLDL fraction of mice receiving adenoviral PGC-1α (Fig. 5*C*).

Tail vein delivery of adenoviral RNAi, which knocks down PGC-1α expression *in vivo* (34), allows examination of the requirement for PGC-1α in lipoprotein metabolism. Injection of this RNAi results in a 70% decrease in PGC-1α mRNA *versus* control mice and concomitant decreases in apoA-IV and C-II of 50 and 40%, respectively (Fig. 5*A*, supplemental Fig. S2). Analysis of serum taken from PGC-1α knockdown mice reveals a significant decrease in total serum triglyceride level (Fig. 5*B*) as well as a 2-fold decrease in VLDL triglyceride (Fig. 5*C*).

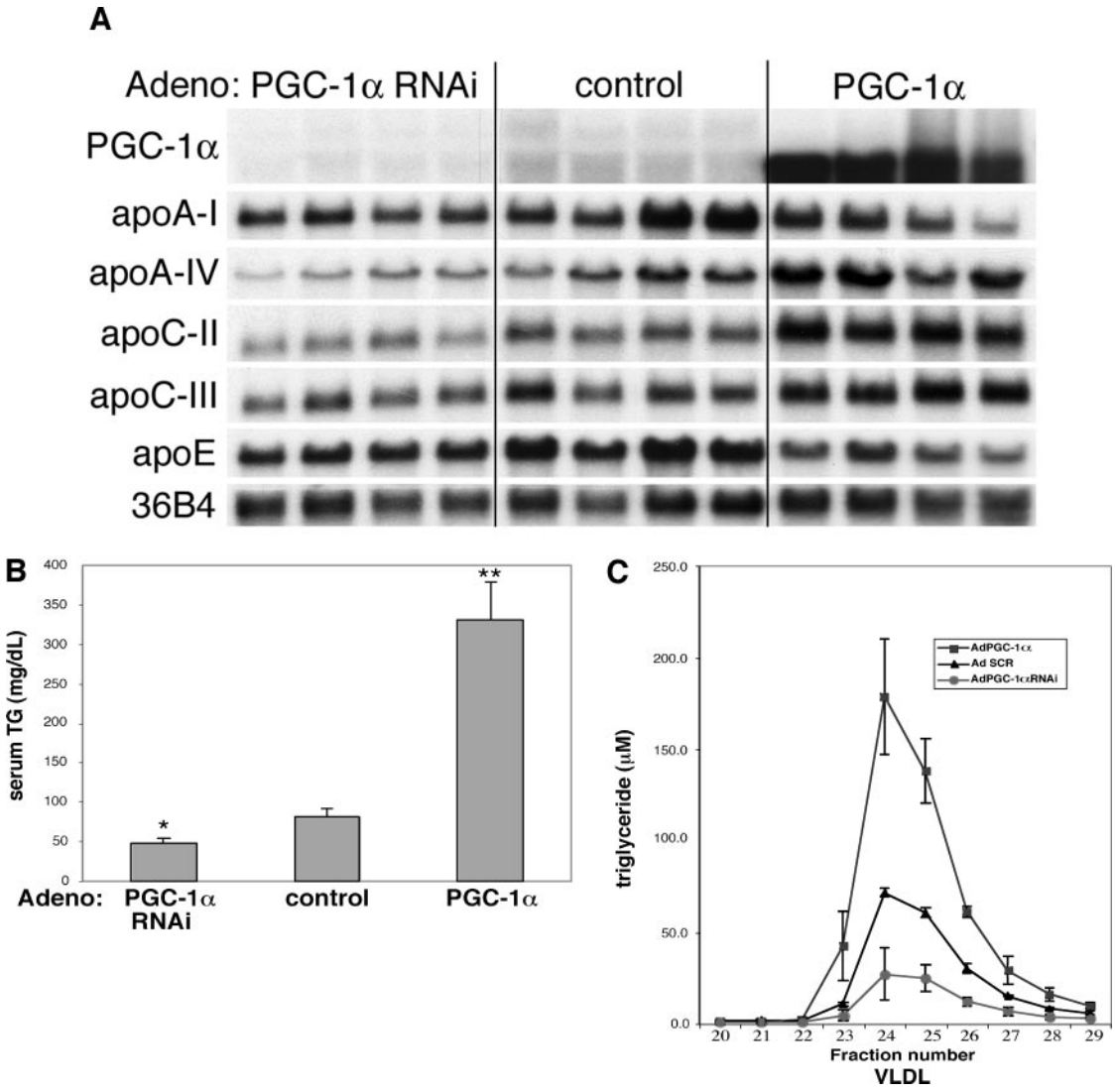

FIGURE 5. **Apolipoprotein mRNA levels, VLDL triglyceride levels, and serum triglyceride levels were determined *in vivo* by PGC-1α.** Mice were tail vein injected with adenovirus encoding PGC-1α, a scrambled RNAi control, or an RNAi that targets and knocks down PGC-1α transcript. Livers and plasma were harvested. *A*, liver mRNA was analyzed for PGC-1α and apolipoprotein transcript levels via Northern blotting using radiolabeled mouse cDNA probes. 36B4 was blotted as a loading control. *B*, serum was separated from other plasma components and analyzed for triglyceride content. Data represent the mean ± S.E. *, $p < 0.01$; **, $p < 0.001$. *C*, serum was subjected to fast protein liquid chromatography to fractionate lipoprotein classes. VLDL fractions were collected and analyzed for triglyceride content. Data represent mean ± S.E.

These data together support an important role for PGC-1α in regulating hepatic lipoprotein export.

DISCUSSION

Given the versatile roles in liver biology attributed to both PGC-1α and HNF4α, we looked for genes with a particular requirement for the *combination* of these two factors. We found that PGC-1α and HNF4α robustly target a subset of apolipoproteins in F9 stem cells and human and mouse hepatoma cells. These apolipoproteins are involved in the metabolism of VLDL, the major carrier of hepatic triglyceride, and are induced during fasting. Forced expression of PGC-1α *in vivo* induces these apolipoproteins and increases both serum and VLDL triglyceride levels. Knock down of PGC-1α *in vivo* leads to a parallel decrease in apolipoprotein expression and reduced serum and VLDL triglyceride. Liver-specific HNF4α knock-out mice also suffer from a loss of these same apolipoproteins and dramatically reduced serum lipid levels (19). It is likely that PGC-1α coactivation of HNF4α serves as a critical regulatory step in hepatic apolipoprotein induction and lipoprotein export during fasting.

The screen used to identify the targets of HNF4α and PGC-1α cooperative action was performed in pluripotent F9 teratocarcinoma cells. In this unbiased cellular context, there is presumably little expression of other liver-enriched transcription factors. In essence, we detect only those genes whose induction minimally requires the ectopic expression of PGC-1α and HNF4α. Although apolipoproteins A-I, A-IV, C-II, and C-III have been previously identified as HNF4α targets (31, 35–37), they are induced robustly *only* with co-expressed PGC-1α. These data suggest that HNF4α requires PGC-1α to realize its full transcriptional potential. Interestingly, other known targets of PGC-1α and HNF4α, such as glucose-6-phosphatase and phosphoenolpyruvate carboxykinase, did not score high in our screen. One possible explanation is that these gluconeogenic genes have more complex promoters that require the presence of other transcription factors for their activation. For instance, cAMP-response element-binding protein, glucocorticoid receptor, and FOXO1 all bind gluconeogenic promoters, and PGC-1α cannot induce phosphoenolpyruvate carboxykinase or glucose-6-phosphatase if FOXO1 is inactive (11).

Another important consideration is that several HNF4α targets, such

as apoB, apoE, and E-cadherin, are not further increased by expression of PGC-1α. Although the mechanism for this selective effect of PGC-1α on HNF4α target genes is unknown, PGC-1α may confer a subtle conformational change upon its partner transcription factor that affects its ability to bind certain sequences of DNA. Alternatively, small differences in response elements in promoters may determine whether a productive interaction occurs between a bound transcription factor and a coactivator. It has recently been shown that DNA sequence variation in κB sites dictates which coactivators will interact with bound NF-κB (38). This implies that promoter response elements hold transcription factors in very specific conformations.

These results underscore the roles of apolipoproteins and lipoprotein assembly in the fasted liver. During fasting, mobilization of free fatty acids from adipose tissue and their influx into the liver produce a marked steatosis. Whereas free fatty acids serve as substrate for β-oxidation to acetyl CoA and subsequent conversion into ketone bodies, excess free fatty acids can be re-esterified into triglycerides for lipoprotein export and extrahepatic utilization (39). In fact, triglyceride synthesis is actually *increased* in the fasted liver (40, 41). In addition, fasting presumes a complete halt in the exogenous supply of dietary lipids from the intestine. In this case, *de novo* synthesis of VLDL from the liver becomes the primary source of triglyceride and cholesterol delivery to peripheral tissues. Increased lipoprotein assembly and export in the liver during fasting are reflected in the elevated levels of apolipoproteins A-I, A-IV, and C-II, in part mediated by PGC-1α.

It has been shown previously that alterations in apolipoprotein synthesis *per se* can affect lipid homeostasis. ApoA-IV knock-out mice have greatly reduced plasma triglyceride and VLDL levels (42). Transgenic studies in which human apolipoproteins A-IV, C-II, and C-III are overexpressed in mice all lead to severe hypertriglyceridemia (43–45). Furthermore, apoC-III regulates liver VLDL synthesis in an insulin-responsive manner. Insulin normally inhibits the secretion of VLDL into the systemic circulation (46), and diabetic hypertriglyceridemia is due in part to the loss of insulin down-regulation of apoC-III (47, 48). Concomitant defects in both glucose and lipid homeostasis are common in the diabetic state and increase the risk of cardiovascular disease in the "metabolic syndrome" (49). Indeed, variation in the *apoA-I/C-III/A-IV* gene cluster has been heavily associated with familial combined hyperlipidemia, estimated to cause 10–20% of all premature coronary heart disease (50–52).

Because PGC-1α coactivation of HNF4α is an important step in hepatic triglyceride release, modulation of PGC-1α binding to HNF4α may provide a novel therapy to manage the dyslipidemia of insulin resistance. Chemical inhibitors that selectively disrupt the docking of HNF4α to PGC-1α may have therapeutic benefit in preventing diabetic hyperglycemia, hyperlipidemia, and other cardiac risk factors. Paradigms for such inhibition already exist in normal physiology; it has been reported that bile acids disrupt the binding of HNF4α to PGC-1α as part of a negative feedback loop in bile acid synthesis (3). It is possible that small molecules that bind the ligand pocket of HNF4α may alter its recruitment to PGC-1α, just as some agonists of the glucocorticoid receptor affect its docking to the coactivator GRIP1 (53).

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