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Hyperlipidemic Effects of Dietary Saturated Fats Mediated through PGC-1β Coactivation of SREBP

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Summary

The PGC-1 family of coactivators stimulates the activity of certain transcription factors and nuclear receptors. Transcription factors in the sterol responsive element binding protein (SREBP) family are key regulators of the lipogenic genes in the liver. We show here that high-fat feeding, which induces hyperlipidemia and atherogenesis, stimulates the expression of both PGC-1β and SREBP1c and 1a in liver. PGC-1β coactivates the SREBP transcription factor family and stimulates lipogenic gene expression. Further, PGC-1β is required for SREBP-mediated lipogenic gene expression. However, unlike SREBP itself, PGC-1β reduces fat accumulation in the liver while greatly increasing circulating triglycerides and cholesterol in VLDL particles. The stimulation of lipoprotein transport upon PGC-1β expression is likely due to the simultaneous coactivation of the liver X receptor, LXRα, a nuclear hormone receptor with known roles in hepatic lipid transport. These data suggest a mechanism through which dietary saturated fats can stimulate hyperlipidemia and atherogenesis.

Introduction

Obesity and type 2 diabetes are associated with increased risk for the development of cardiovascular disease, a leading cause of morbidity and mortality in developed countries (Flier, 2004; Reaven et al., 2004; Zimmet et al., 2001). The predisposition to atherosclerosis appears to be the consequence of pathogenic dyslipidemia in insulin-resistant states, which is characterized by hypertriglyceridemia, as well as increased concentrations of low-density lipoprotein (LDL) cholesterol and reduced levels of high-density lipoprotein (HDL) cholesterol (Betteridge, 1999; Goldberg, 2001). Genetic and epidemiological studies have provided compelling evidence that plasma LDL cholesterol correlates positively with risk for the development of cardiovascular disease (Breslow, 2000; Sacks and Katan, 2002). In addition, increased plasma triglyceride levels have been shown to be an independent risk factor for coronary heart disease. Although genetic factors, environmental influences, and importantly, the interaction of the two all contribute to the progression of this complex disease, it is now clear that dietary intake of saturated and trans fats significantly raises plasma LDL cholesterol while it lowers HDL cholesterol (Sacks and Katan, 2002; Spady et al., 1993). In fact, dietary intake of saturated and trans fats has a much greater hyperlipidemic effect than intake of cholesterol itself. Despite the strong connection between dietary intake of saturated and trans fats and atherogenic lipid profiles, the mechanisms leading from these lipids to elevated cholesterol levels remain poorly understood.

The liver plays a central role in the maintenance of systemic lipid homeostasis. Hepatocytes are responsible for the synthesis and secretion of very low-density lipoprotein (VLDL); the latter redistributes lipids, primarily triglycerides, for storage and utilization by peripheral tissues, and it is the precursor for the atherogenic LDL particles. Several transcription factors in the sterol responsive element binding protein (SREBP) family have been shown to be key regulators of the transcriptional activation of lipogenic genes (Horton et al., 2002). All SREBP isoforms are synthesized as precursor proteins in the endoplasmic reticulum membrane and undergo two steps of proteolytic cleavage (Brown and Goldstein, 1997). This leads to release of the N-terminal active forms which subsequently translocate into nucleus and stimulate the expression of target genes. SREBP1a and 1c (also called ADD1) isoforms are derived from a single gene by alternative usage of transcription start sites, resulting in two proteins with different amino termini (Shimomura et al., 1997; Tontonoz et al., 1993; Yokoyama et al., 1993), while SREBP2 is encoded by a different gene (Hua et al., 1993). SREBP1c mRNA is highly inducible in both fat cells and liver by insulin (Kim et al., 1998; Shimomura et al., 1999), whereas the proteolytic processing of SREBP2 in cells is stimulated in response to sterol-depletion (Brown and Goldstein, 1997; Sakai et al., 1996). Studies in cell culture or mouse liver revealed that SREBP1c and SREBP2 preferentially regulate the expression of genes involved in fatty acid and cholesterol synthesis, respectively (Horton et al., 1998; Kim and Spiegelman, 1996); in contrast, SREBP1a appears to activate both pathways (Horton et al., 2003; Pai et al., 1998). Notably, all three SREBPs induce a severe fatty liver phenotype in transgenic mice with

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abundant accumulation of triglycerides and cholesterol, suggestive of an imbalance between lipid synthesis and secretion (Horton et al., 1998; Shimano et al., 1996, 1997). In contrast, hepatic lipogenesis in healthy animals and humans is tightly coupled to lipoprotein secretion, and hepatic steatosis does not usually develop.

Transcription factors function via docking of coactivator proteins, and the coactivators that function with the SREBPs in hepatic lipogenesis are largely unexplored. Previous studies have shown that the CBP/p300 coactivators are able to bind to and coactivate the SREBPs (Oliner et al., 1996), but the biological significance of this interaction in the regulation of endogenous genes remains unclear. Recent studies indicate that the PGC-1 family of coactivators is of particular importance in the control of liver metabolism (Puigserver and Spiegelman, 2003). PGC-1α stimulates mitochondrial biogenesis and respiration in multiple cell types and modulates biological programs normally associated with increased oxidative metabolism, such as hepatic gluconeogenesis (Herzig et al., 2001; Yoon et al., 2001) and myofiber determination (Lin et al., 2002b). It does so by coactivating transcription factors in a tissue-specific manner, such as HNF4α and FOXO1 in the liver (Puigserver et al., 2003; Yoon et al., 2001), and the MEF2 family members in skeletal muscle (Lin et al., 2002b).

PGC-1β is a recently identified transcriptional coactivator closely related to PGC-1α whose biological activities are largely unknown (Kressler et al., 2002; Lin et al., 2002a). Although PGC-1β shares a similar tissue distribution with PGC-1α, they are differentially regulated during development and in response to changes in nutritional status (Kamei et al., 2003; Lin et al., 2002a, 2003). Like PGC-1α, PGC-1β strongly activates mitochondrial biogenesis and cellular respiration in differentiated myotubes and hepatocytes (Lin et al., 2003; St-Pierre et al., 2003). In this report, we have found that PGC-1β is coinduced with SREBP1c/1a in response to short-term high-fat feeding of mice. PGC-1β coactivates the SREBP and LXR families of transcription factors as it induces a broad program of lipid metabolism, including de novo lipogenesis and lipoprotein secretion. Our studies reveal a novel mechanism whereby consumption of saturated fats may alter hepatic lipid metabolism and lead to hypertriglyceridemia and hypercholesterolemia.

Results

Stimulation of a Program of Hepatic Lipogenesis by High-Fat Feeding

Metabolic pathways leading to increased total and LDL cholesterol from consumption of saturated and trans fats are incompletely understood. To investigate this, we fed mice a diet rich in saturated fats but with little or no cholesterol (58% fat, mainly from hydrogenated coconut oil; D12331, Research Diets). We concentrated on early changes, as these diets are known to bring about many chronic effects, such as insulin resistance and obesity, which may confound the study of regulatory events leading to hyperlipidemia. Clustering analysis of Affymetrix arrays revealed that the expression of a large number of genes involved in de novo lipid synthesis are strongly induced following this high-fat feeding, including those responsible for fatty acid, cholesterol, and triglyceride synthesis (Figure 1A). Notably, mRNA levels for many enzymes in the cholesterol synthesis pathway, such as *HMG-CoA reductase*, phosphomevalonate kinase, and lanosterol synthase, are significantly elevated in response to dietary saturated fats. Interestingly, the activation of this hepatic lipogenic program is accompanied by increased expression of two potentially important hepatic transcriptional regulators, SREBP1c, a central regulator of lipogenic gene expression, and PGC-1β, a transcription coactivator in the PGC-1 family (Figure 1A). The mRNA level of SREBP1c is elevated more than 7-fold at day 1 of high-fat feeding, as determined by quantitative real-time PCR analysis (Figure 1B). The expression of SREBP1a is also induced approximately 2-fold. In contrast, SREBP2 expression remains unchanged. Unlike PGC-1α, which is only slightly induced by the dietary switch, the expression of PGC-1β mRNA is stimulated more than 4-fold in response to high-fat feeding, paralleling that of SREBP1c (Figure 1B). Several-fold induction of mRNA for lipogenic genes, such as fatty acid synthase (*FAS*) and *HMG-CoA reductase*, is also shown by real-time PCR analysis (Figure 1B and data not shown). The expression of PGC-1β is not altered in skeletal muscle and white adipose tissue under these conditions (data not shown). Dietary intake of cholesterol has little effect on the expression of PGC-1β (Supplemental Figure S1A at http://http://www.cell.com/cgi/content/full/120/2/261/DC1/). These results demonstrate that dietary saturated fats and cholesterol have distinct effects on the expression of mRNA for the SREBPs and PGC-1β. This striking induction of the genetic program of hepatic lipogenesis following acute intake of a high saturated fat diet has not been previously noted to our knowledge.

Previous studies have demonstrated that fatty acids, especially polyunsaturated species, suppress both the expression of SREBP1c and the generation of cleaved nuclear isoforms (Hannah et al., 2001). To determine whether dietary fats have a direct impact on PGC-1β expression, we treated primary hepatocytes with various saturated, unsaturated, and trans fatty acids and examined the levels of PGC-1β mRNA. While monounsaturated (oleic acid, $C_{18:1}$n-9) and polyunsaturated (linoleic acid, $C_{18:2}$n-6; EPA, $C_{20:5}$n-3; DHA, $C_{22:6}$n-3; and arachidonic acid, $C_{20:4}$n-6) slightly induce PGC-1β expression, saturated fatty acids of varying chain length ($C_{10:0}$ to $C_{18:0}$) elevate PGC-1β mRNA levels much more strongly (Figure 1C). Strikingly, trans fatty acids such as elaidic acid (*trans*-$C_{18:1}$n-9) and *trans*-vaccenic acid (*trans*-$C_{18:1}$n-7), abundantly present in hydrogenated vegetable oil and dairy products, respectively, also robustly induce the expression of PGC-1β (3.2-fold). In contrast, fatty acid treatments have no effect on the expression of PGC-1α mRNA under these conditions, except stearic acid ($C_{18:0}$), which also induces PGC-1α mRNA 2.2-fold (Figure 1C). These results indicate that certain fatty acids, especially saturated and trans species, can stimulate PGC-1β expression in a cell-autonomous manner.

Coactivation of the SREBP Family of Transcription Factors by PGC-1β

Coinduction of PGC-1β and SREBP1c in the liver of high-fat-fed mice suggested that PGC-1β might modulate

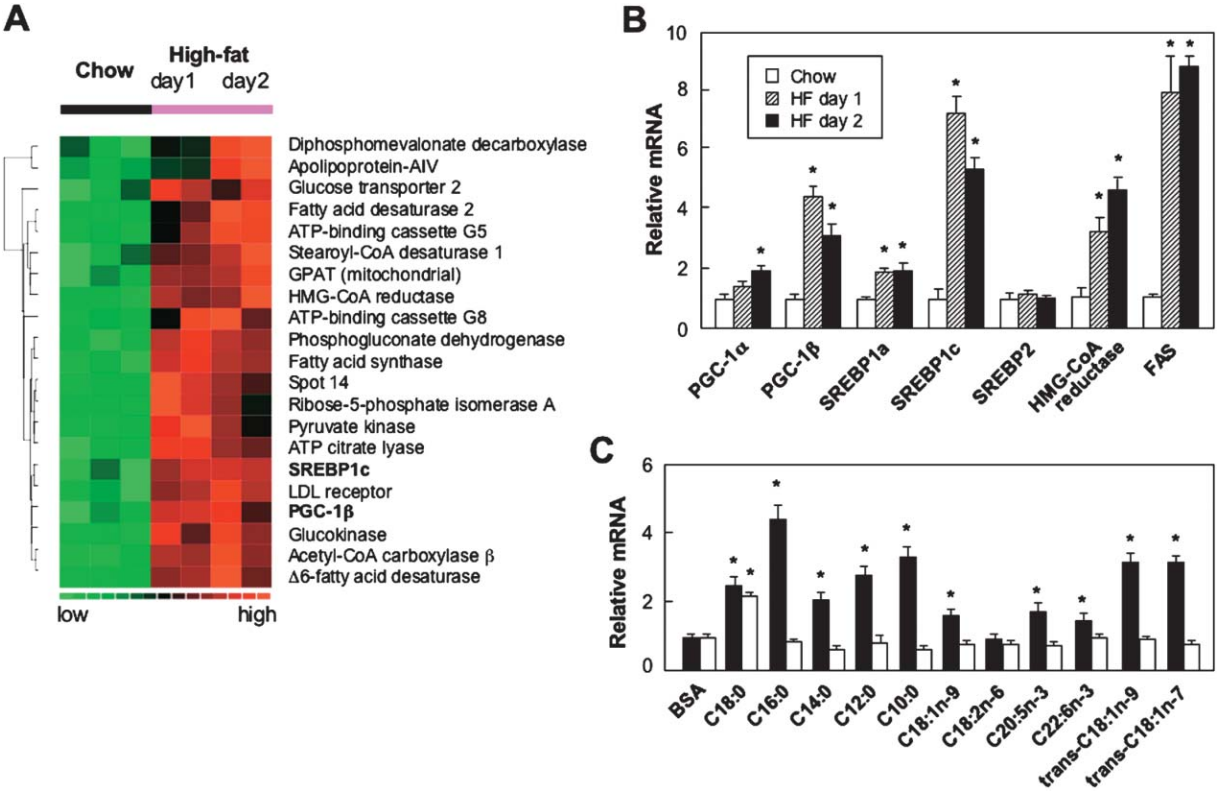

Figure 1. Induction of PGC-1β Expression by Dietary Intake of Saturated Fats

(A) Cluster analysis of liver gene expression in response to high-fat feeding. Dietary changes and transcriptional profile analysis were performed as described in the Experimental Procedures. Shown are genes involved in hepatic lipogenesis that are induced more than 1.8-fold in response to high-fat feeding. Note that SREBP1c and PGC-1β are included in this lipogenic cluster.

(B) Real-time PCR analysis of total liver RNA from mice fed a high-fat diet for 24 (hatched box) or 48 hr (filled box). Relative mRNA abundance was calculated by normalization to the control chow values (open box). $n = 4$; *: $p < 0.01$.

(C) Regulation of PGC-1β expression by free fatty acids in cultured hepatocytes. Primary hepatocytes were treated with 400 μM of various fatty acids for 4 hr as described in the Experimental Procedures. Total RNA was extracted and analyzed by real-time PCR using primers specific for PGC-1β (filled box) and PGC-1α (open box). *: $p < 0.01$.

the transcriptional activity of SREBP1c. As expected, SREBP1c activates the *FAS* promoter (Joseph et al., 2002; Magana and Osborne, 1996; Tontonoz et al., 1993) by approximately 5-fold in transiently transfected H2.35 mouse hepatoma cells (Figure 2A). PGC-1β greatly augments the transcriptional activity of SREBP1c on this promoter, as shown by a 17-fold increase in the luciferase activity compared to the basal levels. In contrast, PGC-1α has minimal effect on the activity of SREBP1c. Mutation of the SREBP binding site on the promoter completely abolishes its activation by both SREBP1c alone and the combination of SREBP1c and PGC-1β, indicating that PGC-1β coactivates SREBP1c through the SRE on this promoter. PGC-1β also strongly increases the activity of SREBP2 and SREBP1a in these coactivation assays (Figure 2B and data not shown).

To determine whether PGC-1β is recruited to SREs present in the promoter/enhancer region of endogenous SREBP target genes, we performed chromatin immunoprecipitation (ChIP) assay. As shown in Figure 2C, PGC-1β, but not PGC-1α, is present in proximity to the SRE on the *FAS* promoter. Interestingly, the recruitment of PGC-1β to SREBP binding sites seems to depend on their promoter context; PGC-1β is not recruited to the SRE on the *LDLR* promoter. No PCR product was detected when control IgG was used in the immunoprecipitations (Figure 2C). These results indicate that SREBP1c is able to directly recruit PGC-1β to the proximity of its binding sites on the target promoters. In fact, these two proteins physically interact in cells as shown by coimmunoprecipitation assays. SREBP1c is able to interact with and precipitate PGC-1β, but not PGC-1α, when these proteins are coexpressed in hepatoma cells (Figure 2D). In order to identify domains of PGC-1β that interact with SREBP1c, we utilized a fusion protein between GST and the processed form of SREBP1c. Full-length PGC-1β interacts well with SREBP1c (Figure 2E), and analysis of PGC-1β mutants revealed that a domain (amino acids 350–530) unique for PGC-1β, but absent in PGC-1α, is required for interaction between SREBP1c and PGC-1β (Figures 2E–2F). These data indicate that PGC-1β, but not PGC-1α, coactivates the SREBP family of transcription factors by direct physical association.

Activation of Hepatic Lipogenesis and Hyperlipidemia by PGC-1β

To examine the effects of PGC-1β on the expression of endogenous lipogenic genes, we infused rats via tail vein injections with recombinant adenoviruses expressing β-galactosidase (β-gal), PGC-1α, or PGC-1β. Adenovirus

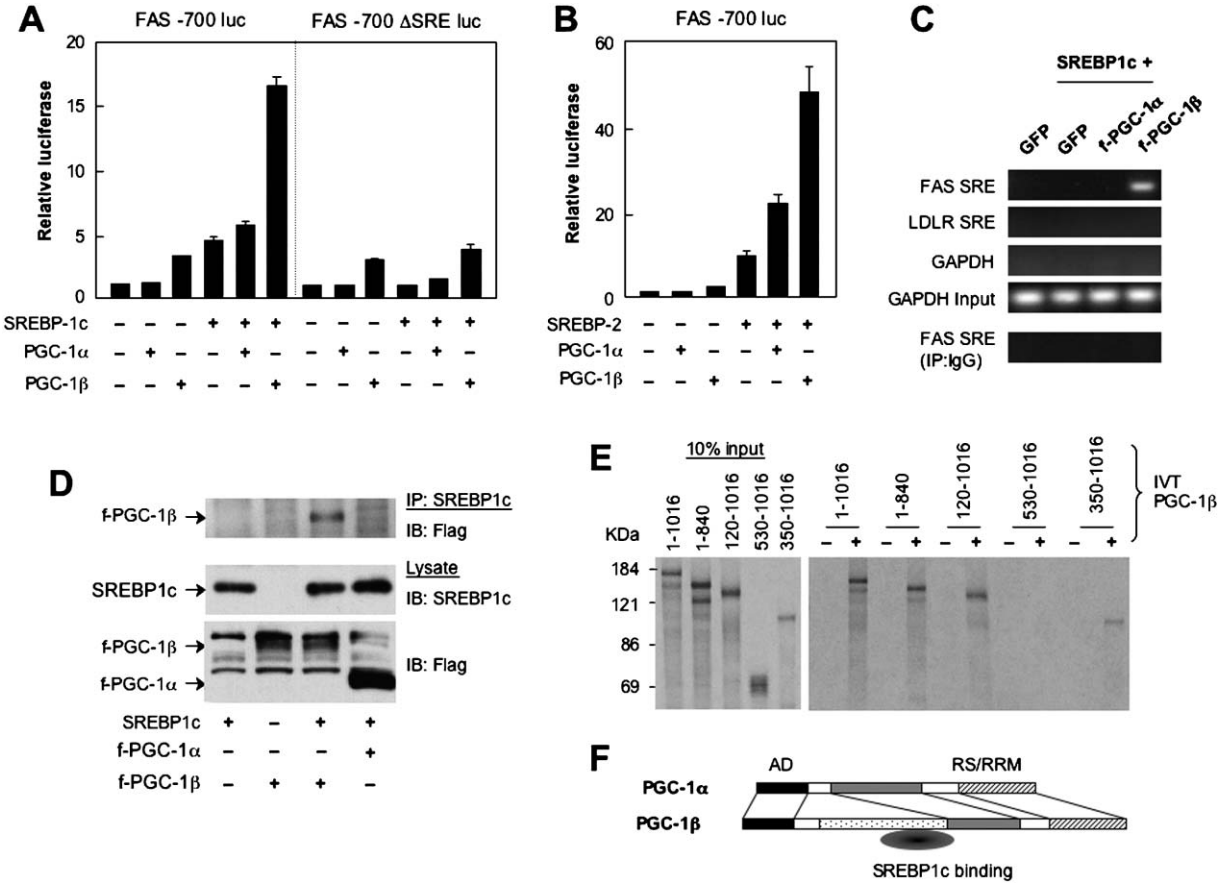

Figure 2. Coactivation of the SREBP Family of Transcription Factors by PGC-1β

(A) H2.35 mouse hepatoma cells were transiently transfected with either the wild-type *FAS* promoter (FAS −700 luc) or the SRE mutant (FAS −700 ΔSRE luc) reporter constructs, in combination with SREBP1c in the presence or absence of the PGC-1.

(B) Transient transfection of FAS −700 luc reporter plasmid with SREBP2 in the presence or absence of the PGC-1.

(C) ChIP analysis on SREBP target genes. Hepatoma cells were infected with adenoviruses for 48 hr as indicated and harvested for ChIP analysis with anti-Flag antibody or control IgG (bottom). The precipitated genomic fragments were amplified using primers flanking SREs on the FAS and LDLR promoters or control GAPDH promoter. Genomic DNA from total chromatin lysates was included as an input control.

(D) Coimmunoprecipitation of PGC-1β and SREBP1c. Cultured 293 cells were transfected with plasmids as indicated. Total lysates from transfected cells were subjected to immunoprecipitation using antibodies specific for SREBP1c. Both lysates and precipitates were analyzed by immunoblotting with antibodies specific for SREBP1c or the Flag epitope tag. Arrows indicate the bands corresponding to Flag-PGC-1α (f-PGC-1α), Flag-PGC-1β (f-PGC-1β) and SREBP1c.

(E) Mapping of PGC-1β domains that interact with SREBP1c. Glutathione beads containing immobilized GST (−) or GST-SREBP1c (+) were incubated with in vitro translated ^{35}S-labeled full-length PGC-1β or truncated PGC-1β mutants. Shown in the left are in vitro translated PGC-1β mutants equivalent to 10% of input for the interaction assay. The numbers above the gel denote amino acid positions of the mutants. Note that a domain between amino acids 350 and 530 of PGC-1β is necessary for binding to SREBP1c.

(F) Diagram of the structure of PGC-1α and PGC-1β showing a domain unique for PGC-1β that provides the docking site for SREBP1c.

almost exclusively transduces hepatocytes when introduced through the tail vein. As expected, both PGC-1α and PGC-1β stimulate the expression of mitochondrial genes such as *cytochrome c*, *βATPase*, and medium chain acyl-CoA dehydrogenase (*MCAD*) when ectopically expressed in liver (Figure 3A). PGC-1β also powerfully stimulates the expression of genes involved in lipid synthesis, such as *FAS*, *SCD-1*, *HMG-CoA reductase*, *DGAT*, and *GPAT*, all of which are well-known SREBP targets. In striking contrast, PGC-1α has little or no effect on the expression of these genes. The expression of microsomal triglyceride transfer protein (*MTTP*), a gene that regulates VLDL secretion and is mutated in familial abetalipoproteinemia (Sharp et al., 1993), is induced by both PGC-1α and PGC-1β. Gene expression analysis by real-time PCR revealed that in addition to modulating *HMG-CoA reductase*, PGC-1β also increases mRNA level of multiple enzymes in the cholesterol synthesis pathway, while PGC-1α has much weaker effects (Figure 3B). In fact, many of these PGC-1β target genes are also highly induced in response to high-fat feeding, suggesting that PGC-1β may be a key factor in mediating the effects of dietary saturated fats on hepatic lipogenesis including cholesterol biosynthesis. Surprisingly, the expression of *LDLR*, a classic SREBP target, is not elevated by PGC-1β (Figure 3C). This induction of SREBP target genes by PGC-1β does not appear to be due to increased levels of SREBP transcription factors as shown by mRNA and protein analysis (Figures 3B and 3D). Rather, these results are consistent with the obser-

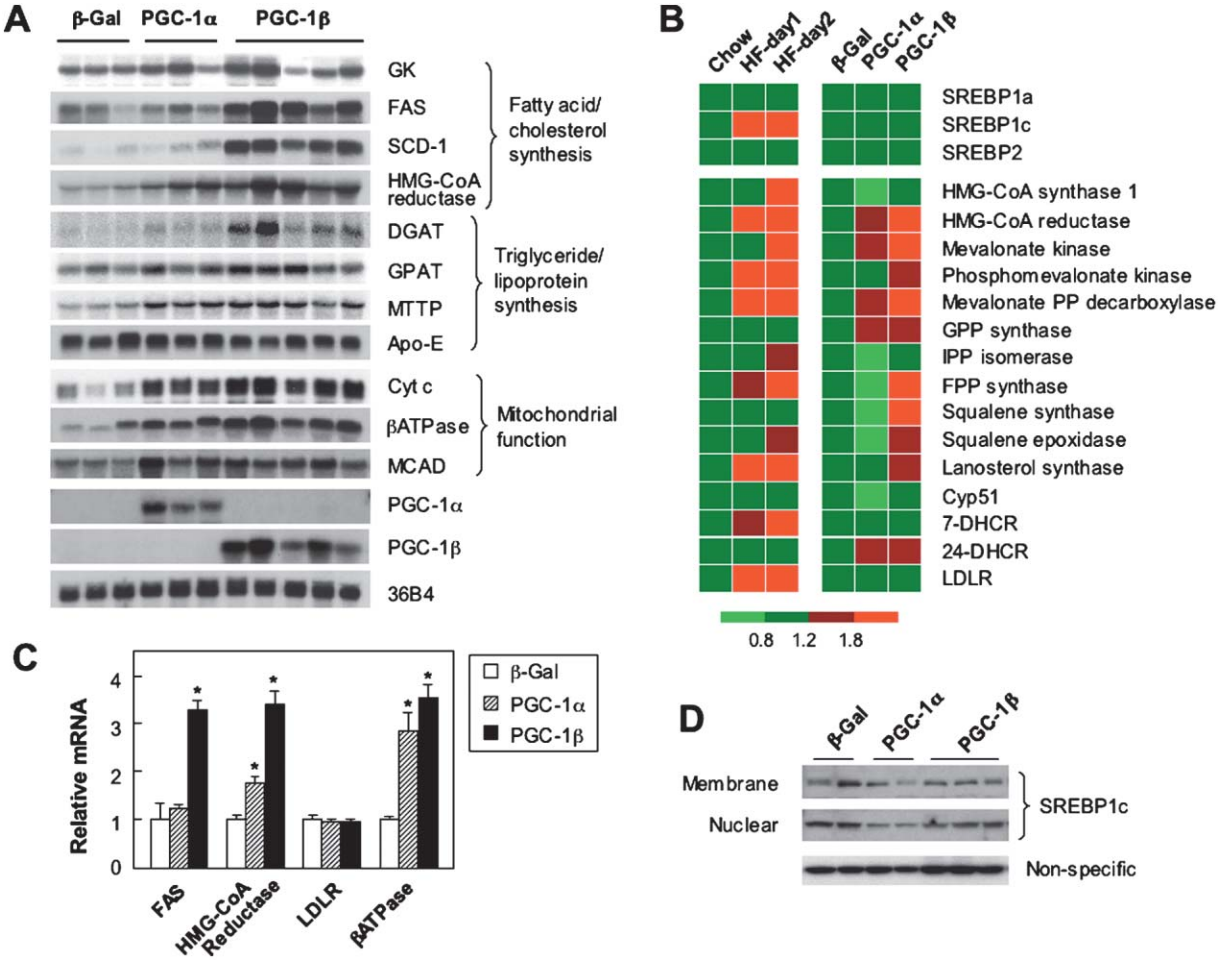

Figure 3. Induction of mRNAs Encoding Enzymes in the Pathways of Fatty Acid and Cholesterol Biosynthesis by PGC-1β.

(A) Hybridization analysis of total liver RNA from rats transduced with the control adenovirus (Ad-β-gal), Ad-PGC-1α or Ad-PGC-1β. Probe specific for ribosomal protein 36B4 was included as a loading control.

(B) Induction of hepatic mRNAs encoding enzymes in the cholesterol biosynthesis pathway by PGC-1β compared to the high-fat-fed mouse liver. The expression levels for high-fat-fed mouse liver represent normalized values obtained from the Affymetrix arrays. The expression of these genes in response to PGC-1s was determined by real-time PCR analysis of total RNA from rat liver transduced with adenoviral vectors as indicated. Note that PGC-1β stimulates the expression of multiple enzymes involved in cholesterol biosynthesis, which are also induced in response to high-fat feeding.

(C) Real-time PCR analysis of hepatic gene expression. Relative mRNA levels were obtained after normalization to 18S rRNA. Note that the LDLR mRNA levels are not altered by PGC-1β expression. *: $p < 0.05$.

(D) PGC-1β has no effects on the expression and processing of SREBP1c in rat liver. Shown are membrane and nuclear forms of rat SREBP1c protein.

vation that PGC-1β coactivates SREBPs through direct physical association and augmentation of their transcriptional activity.

Transgenic expression of SREBP in mouse liver activates lipogenic gene expression, and lipids accumulate in liver and fail to be exported to peripheral tissues (Horton et al., 1998; Shimano et al., 1996, 1997). Indeed, plasma triglyceride levels are actually *reduced* in the transgenic mice compared to wild-type controls, probably due to increased LDLR levels in liver (Shimano et al., 1997). Surprisingly, adenoviral-mediated PGC-1β expression in liver *lowers* hepatic triglyceride content by more than 50% in the high-fat-fed rats (Figure 4A). This decrease in hepatic lipid storage may be explained by an increase in lipid export as PGC-1β expression in liver caused profound plasma hypertriglyceridemia in rats, with plasma triglyceride concentrations elevated more than 6-fold compared to control rats receiving Ad-β-gal (Figure 4B). PGC-1α also lowers liver triglyceride content while slightly raising plasma triglyceride levels (Figures 4A and 4B). Analysis of plasma cholesterol indicated that total cholesterol is increased approximately 55% and 200% by PGC-1α and PGC-1β, respectively (Figure 4C). The increase in plasma cholesterol is mainly a result of accumulation of cholesterol in the VLDL fractions as shown by FPLC analysis of lipoprotein profiles (Figure 4D). In fact, the level of triglycerides and cholesterol in VLDL is increased 6.2- and 5.3-fold, respectively, in response to PGC-1β compared to the β-gal control (Figure 4E). In contrast, the level of HDL cholesterol is largely

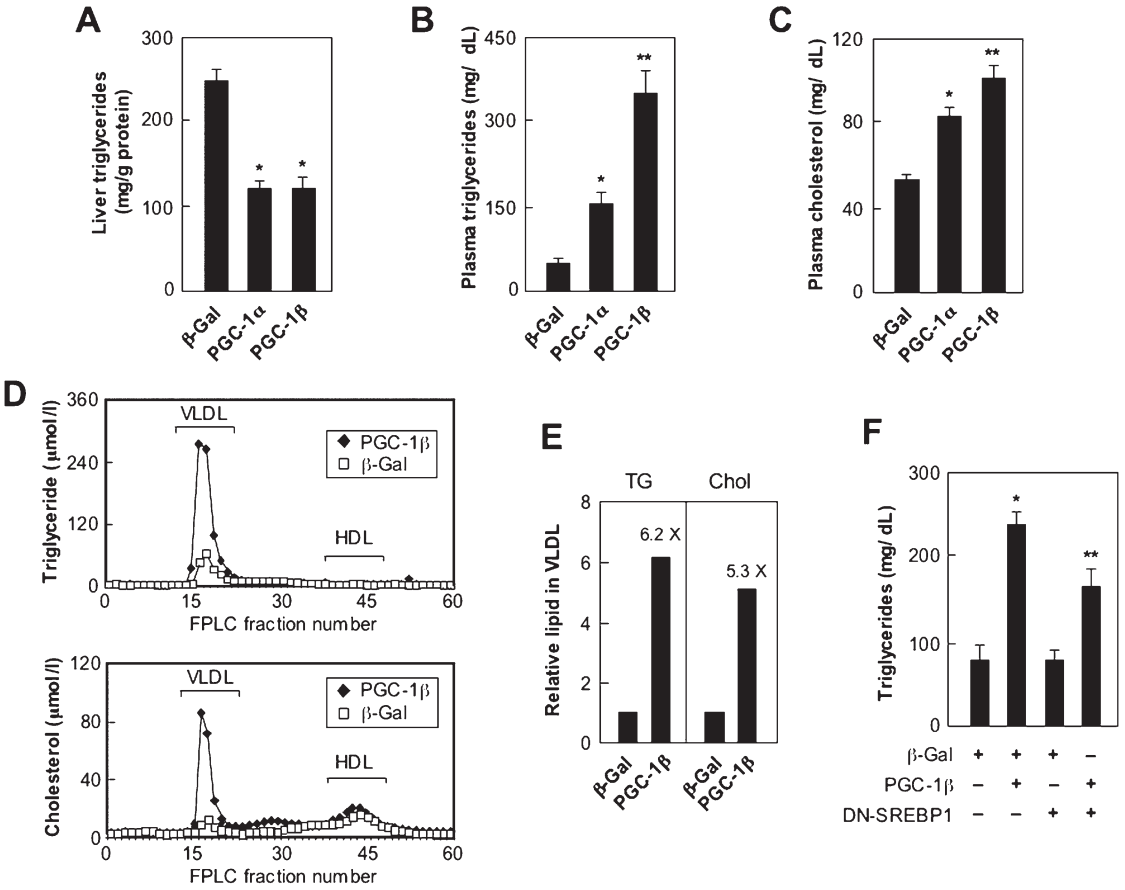

Figure 4. Induction of Hyperlipidemia by PGC-1β

Rats were transduced via tail vein injection with adenoviruses as described in the Experimental Procedures.

(A–C) Liver triglycerides (A), plasma triglycerides (B), and total plasma cholesterol (C) in rats transduced with Ad-β-gal, Ad-PGC-1α or Ad-PGC-1β. (A) *: $p < 0.0003$; (B) *: $p < 0.0001$, **: $p < 0.007$; (C) *: $p < 0.0004$, **: $p < 10^{-6}$.

(D) Analysis of lipoprotein profiles. Plasma from rats transduced with the control Ad-β-gal or Ad-PGC-1β was fractionated by FPLC. Triglyceride and cholesterol concentrations in each fraction were measured. Fractions corresponding to VLDL and HDL are indicated.

(E) Relative lipid content in VLDL fractions was calculated using areas under curve in (D). Shown are relative amounts of triglycerides (TG) and cholesterol (Chol) in VLDL.

(F) Plasma triglyceride concentrations in rats transduced with adenoviral vectors expressing control β-gal, PGC-1β, dominant-negative SREBP1c (DN), or the combination of PGC-1β and DN. *: $p < 0.0001$ (PGC-1β versus β-gal), **: $p < 0.02$ (PGC-1β versus PGC-1β+DN).

unaffected. This is significant in light of the fact that VLDL cholesterol is the precursor of LDL cholesterol. These data strongly suggest that although PGC-1β coactivates the SREBPs and increases expression of the lipogenic SREBP target genes, it also modulates lipid transport pathways and alters the balance between hepatic and plasma lipids.

To determine whether SREBP is necessary for mediating the effects of PGC-1β on lipogenic gene expression, we infused rats with Ad-PGC-1β in combination with a well-characterized dominant-negative mutant of SREBP (Foretz et al., 1999; Kim et al., 1995). While DN-SREBP alone has no effect on the levels of plasma triglycerides compared to β-gal controls, the DN mutant significantly diminishes hypertriglyceridemia caused by PGC-1β (Figure 4F). The induction of several lipogenic genes, such as *FAS*, *SCD-1*, and *HMG-CoA reductase*, are also reduced when rats are transduced with both Ad-PGC-1β and Ad-DN-SREBP (Supplemental Figure S1B on the *Cell* website). Thus, a significant portion of the effects of PGC-1β on lipogenic gene expression is mediated through the SREBP family of transcription factors.

Modulation of the LXRα Pathway by Both PGC-1α and PGC-1β

The hypertriglyceridemia induced by PGC-1β is reminiscent of that caused in animals by activation of liver-X receptor (LXR) with an agonist ligand (Grefhorst et al., 2002; Schultz et al., 2000). LXRα has also been shown to play an important role in the regulation of lipid export and reverse cholesterol transport in macrophages (Chawla et al., 2001). To examine whether PGC-1β affects transcriptional activity of LXRα, we cotransfected a reporter plasmid that contains multimerized LXR binding sites (4× LXRE-luc) with PGC-1α and PGC-1β. As shown in Figure 5A, PGC-1β strongly augments the activation of reporter gene expression by LXRα and RXRβ in a ligand-dependent manner. Similarly, both PGC-1 coactivators are able to augment the transcriptional activity of LXRβ when assayed on this reporter construct (Sup-

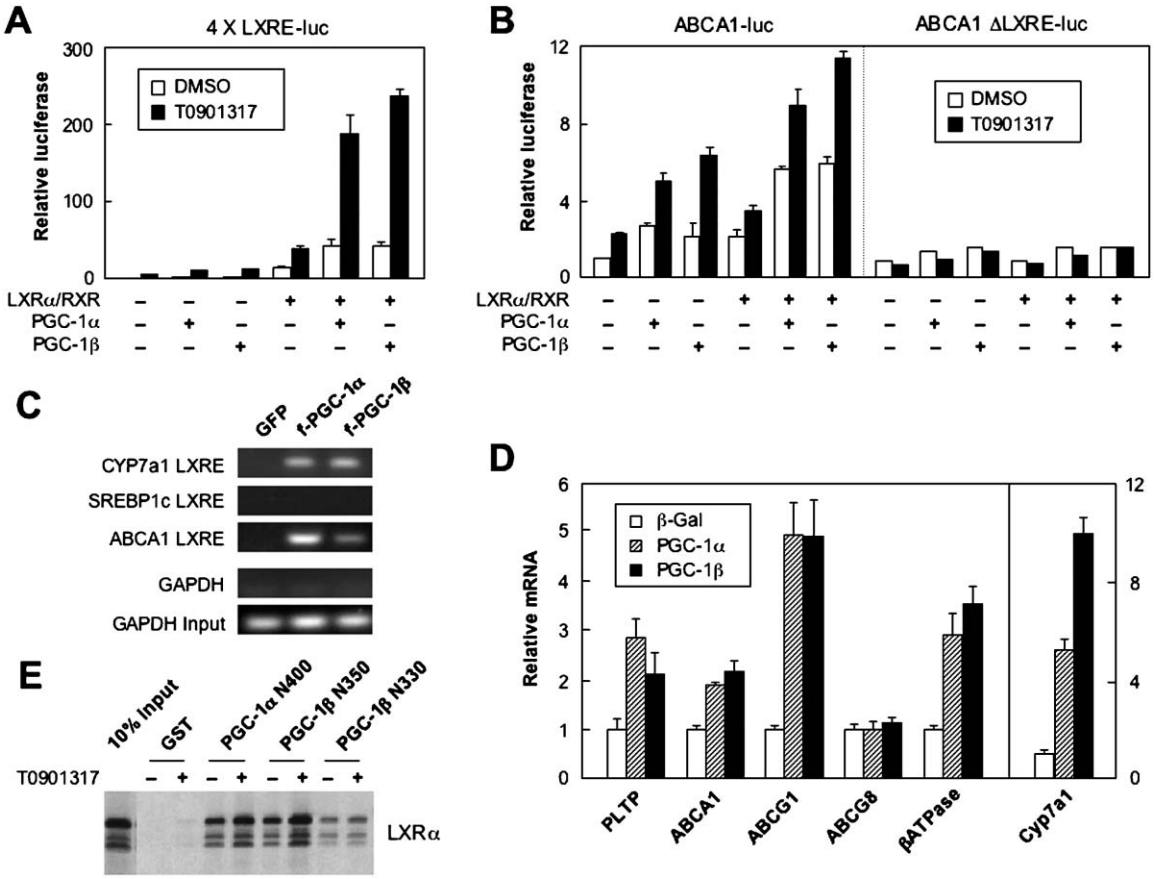

Figure 5. Coactivation of LXRα by PGC-1α and -1β

(A) H2.35 hepatoma cells were transiently transfected with 4× LXRE-luc in combination with plasmids as indicated. Transfected cells were treated with either vehicle DMSO (open box) or 10 μM of the LXR agonist T0901317 (filled box) for 24 hr before luciferase assay.

(B) H2.35 hepatoma cells were transiently transfected with wild-type ABCA1 promoter reporter plasmid (ABCA1-luc) or a mutant lacking the LXRE (ABCA1 ΔLXRE-luc) in combination with plasmids as indicated.

(C) ChIP analysis on LXR target genes. H2.35 cells were infected with adenoviruses expressing GFP, flag-PGC-1α, or flag-PGC-1β. Cells were treated with 10 μM of T0901317 for 3 hr before harvesting. PCR was performed on input or precipitated DNA using primers as indicated.

(D) Induction of endogenous LXR target genes by PGC-1α and PGC-1β. Real-time PCR analysis of total liver RNA isolated from rats transduced with Ad-β-gal (open box), Ad-PGC-1α (hatched box) or Ad-PGC-1β (filled box).

(E) Interaction between LXRα and the N termini of the PGC-1 proteins. In vitro translated LXRα was incubated with GST or fusion proteins of GST and the N termini of PGC-1s as indicated. The binding reactions were incubated in the presence (+) or absence (−) of 10 μM T0901317. Shown in the left is 10% of LXRα input for the reactions.

plemental Figure S2A on the *Cell* website). Cotransfection of PGC-1s increases the promoter activity of ATP binding cassette transporter A1 (*ABCA1*) by approximately 2-3 fold compared to LXRα/RXRβ alone (Figure 5B). Mutation of the LXR binding site on the promoter completely abolishes its regulation by LXR and PGC-1s, indicating that LXR binding to its response element on the *ABCA1* promoter is required for mediating effects of the PGC-1s. In fact, both PGC-1α and PGC-1β are recruited to the proximity of the LXREs present on the promoters of *CYP7a1* and *ABCA1* and induce the expression of several endogenous LXR target genes (Figures 5C and 5D). Furthermore, these two coactivators are able to directly bind LXRα in an in vitro interaction assay (Figure 5E). Deletion of a small region that contains the conserved LXXLL motif (PGC-1β N350) reduced the binding between PGC-1β and LXR and completely abolished the effects of ligand.

Since mice deficient in the LXRs are known to express SREBP1c very poorly (Repa et al., 2000), it is not possible to genetically determine the role of the LXRs in the PGC-1β responses independent of SREBP1c. However, introduction of adenoviral PGC-1β into mice lacking both LXRα and LXRβ shows a complete loss of the hyperlipidemic response shown in wild-type animals, consistent with a role of the LXRs in this pathway (Supplemental Figure S2B on the *Cell* website).

Requirement for PGC-1β in the Transcriptional Activity of the SREBPs

The fact that PGC-1β is highly induced along with SREBP1a/1c in response to high-fat feeding suggests that the concentration of PGC-1β in hepatocytes may be a necessary and limiting factor for SREBP activity. To examine this possibility, we constructed RNAi vectors (RNAi #1 and #2) that specifically knock down PGC-1β

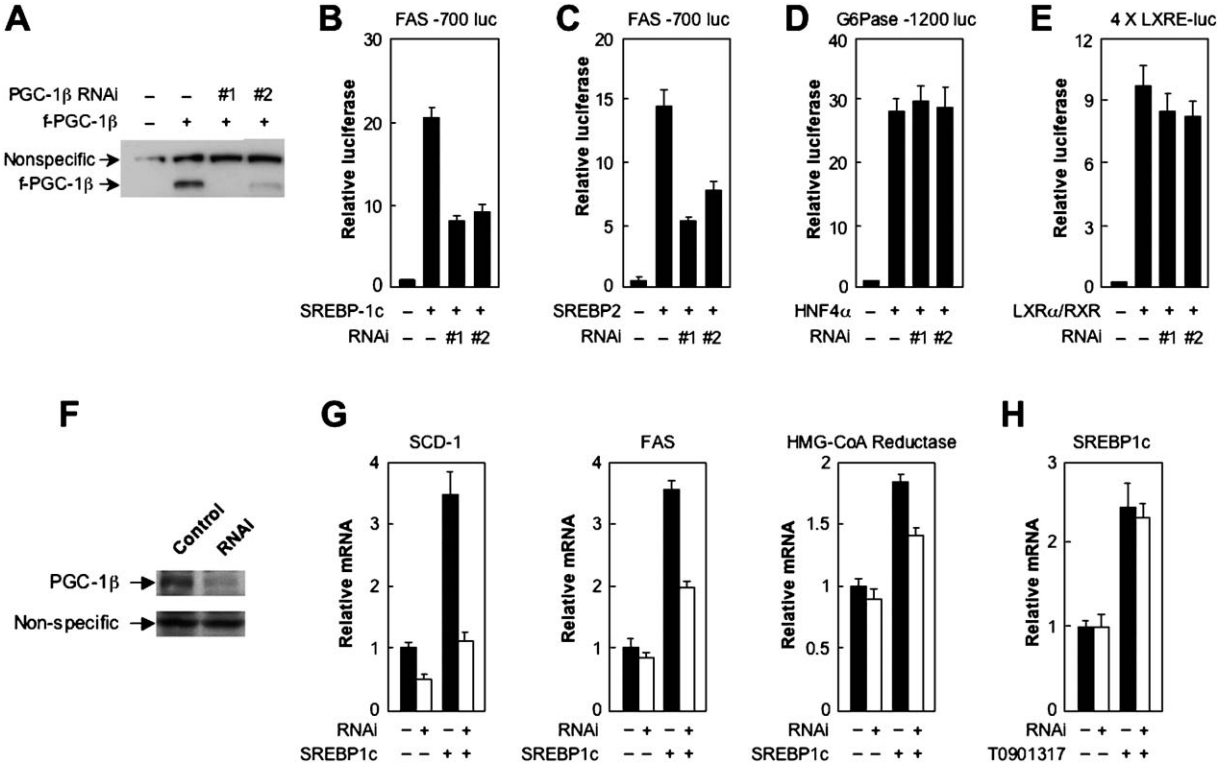

Figure 6. Requirement for PGC-1β in SREBP-Mediated Transcription

(A) Knockdown of PGC-1β protein levels by RNAi constructs. Cultured 293 cells were transiently transfected with PGC-1β expression plasmid (f-PGC-1β) in the presence of RNAi constructs or vector control. Transfected cells were harvested for immunoblotting using a monoclonal antibody against the Flag epitope. Note that both RNAi constructs decrease PGC-1β protein levels compared to the vector control.

(B–E) Hepatoma cells were transiently transfected with FAS −700 luc (B and C), G6Pase −1200 luc (D) or 4× LXRE-luc (E) in combination with plasmids as indicated. For RNAi, either vector control or RNAi constructs directed toward PGC-1β were included in the transfection experiments. Luciferase activity was measured 48 hr after transfection.

(F) Knockdown of endogenous PGC-1β protein by adenoviral expression of RNAi. H2.35 hepatoma cells were infected with a control RNAi adenovirus or Ad-RNAi for two days. Total lysates were prepared for immunoblotting analysis using polyclonal antibodies raised against PGC-1β.

(G) A requirement of PGC-1β for the induction of endogenous SREBP target genes. H2.35 hepatoma cells were infected with Ad-GFP (filled box) or Ad-RNAi (open box) for 48 hr and then infected with Ad-SREBP1c for 20 hr. Total RNA was isolated from infected cells and analyzed by real-time PCR.

(H) H2.35 hepatoma cells were infected with Ad-GFP (filled box) or Ad-RNAi (open box) for 48 hr, and then treated with 10 μM of T0901317 as indicated. Relative SREBP1c expression was determined by real-time PCR.

levels in cells. As shown in Figure 6A, both RNAi vectors directed toward PGC-1β reduce the protein level of PGC-1β by 60%–90% when tested in transient transfection assays, compared to the control vector. We first analyzed the effects of PGC-1β RNAi on SREBP transcriptional activity in transient transfections. Activation of the *FAS* promoter by SREBP1c is reduced more than 60% by PGC-1β RNAi constructs compared to the control vector or a vector expressing random RNAi sequence (Figure 6B). A very similar reduction of transcriptional activity is also observed with these RNAis when combined with SREBP2 and SREBP1a (Figure 6C and data not shown). Importantly, these PGC-1β RNAi vectors have little or no effect on the regulation of *G6Pase* promoter activity by PGC-1α and HNF4α (Figure 6D). These RNAi vectors also do not alter LXRα/RXRβ transcriptional activity when assayed on a reporter containing multimerized LXR responsive elements (Figure 6E), perhaps due to the presence of PGC-1α and/or other coactivator proteins for LXRs in hepatocytes. Taken together, these data strongly suggest that PGC-1β activity is required for the full transcriptional activity of SREBP.

To determine whether PGC-1β is required for the expression of endogenous SREBP1c targets, we used an adenoviral RNAi vector (Ad-RNAi) toward PGC-1β. As shown in Figure 6F, treatment of hepatoma cells with this Ad-RNAi reduces endogenous PGC-1β protein by approximately 80%. No effect on PGC-1α expression was detected (data not shown). As shown in Figure 6G, SREBP1c expression in hepatoma cells strongly stimulates mRNA abundance of several lipogenic genes such as *SCD-1* (3.5-fold), *FAS* (3.6-fold), and *HMG-CoA reductase* (1.8-fold). The induction of all these genes in response to SREBP1c, however, is greatly impaired in the cells infected with Ad-RNAi compared to the control GFP. Notably, while the induction of *FAS* and *HMG-CoA reductase* is decreased more than 50%, the expression of *SCD-1* mRNA is reduced to near the basal level even in the presence of SREBP1c. In contrast, the induction

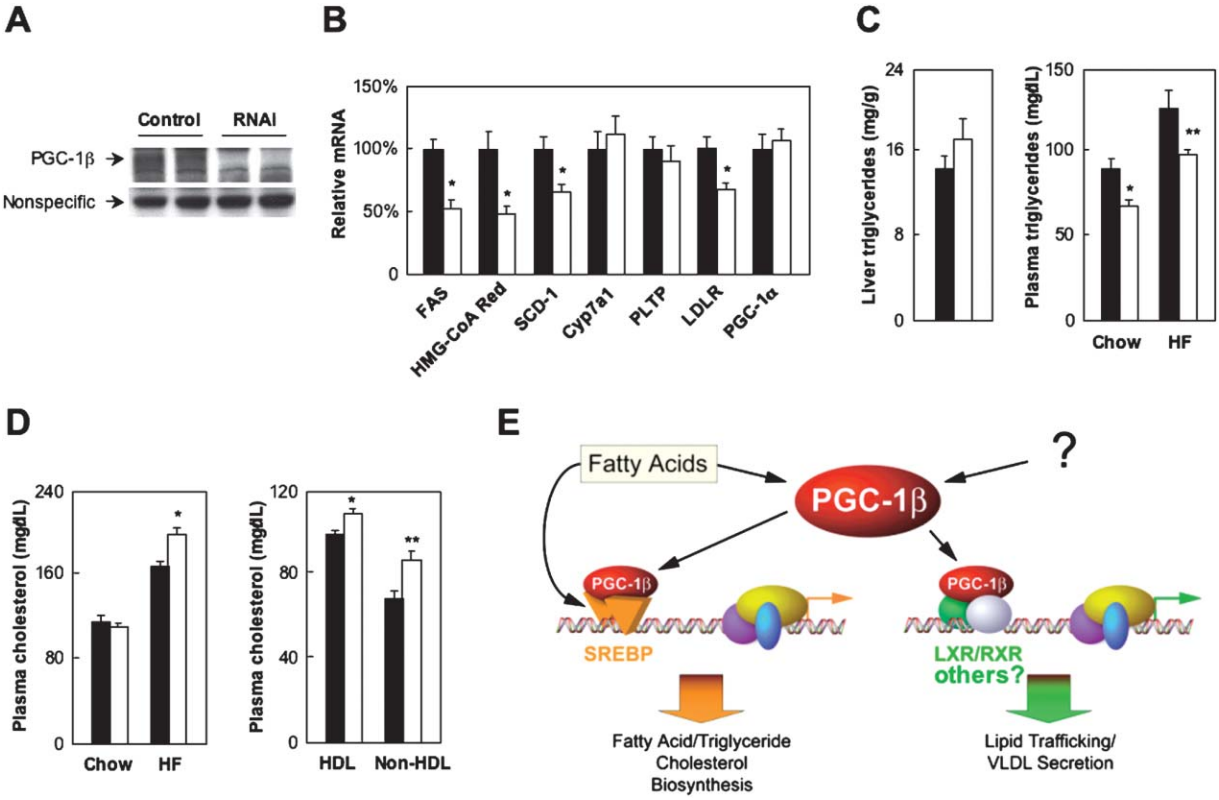

Figure 7. PGC-1β Is Required for Lipogenic Gene Expression and Lipid Homeostasis In Vivo

(A) Knockdown of endogenous PGC-1β in the livers from mice transduced with Ad-RNAi. Adenoviral transduction was performed as described in the Experimental Procedures. Eighty micrograms of total liver lysates from mice receiving control or RNAi adenoviruses were subjected to immunoblotting analysis using PGC-1β antibody.

(B) Hepatic gene expression in the mice transduced with control (filled box, n = 5) or RNAi (open box, n = 6) adenoviruses. *: $p < 0.03$.

(C) Concentrations of liver and plasma triglycerides in the mice transduced with control (filled box) or RNAi (open box) adenoviruses. Plasma triglyceride concentrations were measured before (chow) or after (HF) two days of high-fat feeding as indicated. *: $p < 0.03$; **: $p = 0.0002$.

(D) Concentrations of total and HDL/non-HDL plasma cholesterol in the mice transduced with control (filled box) or RNAi (open box) adenoviruses. HDL cholesterol was measured in animals after two days of high-fat feeding. Non-HDL cholesterol was calculated by subtracting HDL from total cholesterol. *: $p < 0.03$; **: $p = 0.02$.

(E) Coordination of lipid synthesis and lipoprotein secretion through transcriptional coactivation by PGC-1β. PGC-1β stimulates hepatic lipid synthesis by coactivating the SREBP family of transcription factors. PGC-1β also promotes lipoprotein secretion through its coactivation of transcription factors including LXR.

of LXR target genes, such as SREBP1c, is not affected by PGC-1β knockdown (Figure 6H), perhaps reflecting the observation that PGC-1α is also capable of coactivating LXR and stimulating the expression of its targets (Figure 5).

To examine whether PGC-1β is required for the activation of lipogenic gene expression in vivo, especially in the context of high-fat feeding, we transduced mice with Ad-RNAi directed against PGC-1β or a control random RNAi for four days and then switched animals to a high-fat diet for two days. As expected, the Ad-RNAi directed against PGC-1β greatly reduced endogenous PGC-1β protein in the liver (Figure 7A). Analysis of hepatic gene expression indicates that the mRNA level of several key lipogenic enzymes, including *FAS*, *SCD-1*, and *HMG-CoA reductase*, is significantly decreased in the liver from mice receiving Ad-RNAi compared to the control RNAi vector (Figure 7B). The expression of *CYP7a1*, *PLTP, and PGC-1α* is similar between the two groups. Surprisingly, the mRNA level of *LDLR* is also reduced by approximately 40% in response to PGC-1β knockdown, suggesting that this coactivator may be a limiting factor, directly or indirectly, for optimal expression of *LDLR* in the liver. These data clearly illustrate that PGC-1β is indeed necessary for the full activation of the lipogenic program by the SREBPs and perhaps other as yet undefined transcription factors in the mouse liver.

Consistent with a key role of PGC-1β in the regulation of lipoprotein synthesis and secretion, plasma triglyceride concentration is significantly reduced (15%) in Ad-RNAi-transduced mice when fed either chow or a high-fat diet (Figure 7C). Hepatic triglyceride levels tend to be higher in the RNAi group, but the difference does not reach statistical significance. As expected, plasma cholesterol level is increased in mice following high-fat feeding (Figure 7D). Somewhat surprisingly, total plasma cholesterol concentration is slightly but significantly higher in Ad-RNAi-transduced mice following high fat

feeding. This is due to an increase in both HDL and non-HDL cholesterol (Figure 7D). This could be due, at least in part, to the decreased expression of *LDLR* observed when PGC-1β is knocked down in live animals (Figure 7B).

Discussion

PGC-1α and PGC-1β have similar effects on mitochondrial biogenesis and respiration, but the range of biological functions of PGC-1β is largely unexplored. However, it is already clear that the expression of PGC-1β is apparently controlled by distinct physiological and cellular signals. For example, the expression of PGC-1β, but not PGC-1α, is strongly increased during differentiation of both brown and white adipocytes (Kamei et al., 2003; Lin et al., 2002a). In contrast, PGC-1α is highly inducible in brown fat in response to cold temperature, while PGC-1β is not (Lin et al., 2002a; Puigserver et al., 1998). In this paper, we have identified a novel mode of regulation and function for PGC-1β, working through transcription factors in the SREBP and LXR families.

First, PGC-1β strongly coactivates the entire SREBP family of transcription factors in reporter gene assays. Importantly, the effect of PGC-1β on the SREBPs is not restricted to isolated promoters; PGC-1β also strongly induces the expression of SREBP target genes in rat liver when ectopically expressed via adenoviral vectors. These include targets of both SREBP1c and SREBP2, namely enzymes of fatty acid, triglyceride synthesis, and cholesterol biosynthesis. In addition, PGC-1β appears to be required for full transcriptional activity of the SREBPs, as shown by analysis of the *FAS* promoter using PGC-1β RNAi vectors, as well as for the induction of endogenous lipogenic targets in cells and in vivo. The ability of PGC-1β to augment the stimulatory effects of SREBP on the *FAS* promoter appears to be mediated through direct association between PGC-1β and the SREBPs. Interestingly, SREBP1c docks on PGC-1β at a domain that has no counterpart in PGC-1α, and hence, PGC-1α does not coactivate the SREBPs.

While the effects of adenoviral vectors expressing PGC-1β and RNAi against PGC-1β substantially mirror each other's effects on plasma triglycerides, the effects of these agents on blood cholesterol appear somewhat paradoxical. The viral-mediated elevation of PGC-1β from the baseline levels seen in chow-fed animals stimulates the elevation of VLDL cholesterol and total cholesterol, but the RNAi against PGC-1β, which knocked down the level of this coactivator to undetectable levels, also raised both HDL and non-HDL cholesterol. Although a role for PGC-1α acting in a manner independent of coactivation of the SREBPs cannot be ruled out, this role seems rather unlikely since knockdown of PGC-1β caused no change in the expression of PGC-1α (Figure 7B). A much more plausible molecular explanation for these results can be gleaned from the effects of these agents on the expression of the LDLR. This receptor, which is largely responsible for removal of LDL cholesterol from the blood, is not increased at all when PGC-1β levels are raised above baseline levels by adenoviral vectors (Figure 3C). In contrast, the near total suppression of PGC-1β levels by the RNAi (Figure 7A) causes a significant decrease in the expression of mRNA for the LDLR (Figure 7B). These data suggest that PGC-1β is indeed involved in control of the LDLR promoter and that suppression of hepatic LDLR expression may play an important role in the elevation of blood cholesterol in RNAi-treated mice.

Perhaps the most interesting observation from these in vivo studies is that PGC-1β highly induces the lipogenic genes targeted by SREBPs, but the induction of these genes does not lead to lipid accumulation in the liver. Indeed, lipid levels in the liver are actually decreased while plasma triglyceride and cholesterol levels are significantly elevated. The ability of PGC-1β to alter the balance between hepatic versus plasma lipids is extremely robust and clearly indicates that PGC-1β must be activating additional pathways linked to lipoprotein synthesis and secretion. In fact, the effects of PGC-1β on plasma triglycerides are very reminiscent of what has been observed with administration of synthetic LXR agonist to animals (Grefhorst et al., 2002; Schultz et al., 2000).

Several pieces of data link PGC-1β with the LXR pathway. PGC-1β coactivates LXR on both a synthetic reporter containing multimerized binding elements and an endogenous promoter (*ABCA1*) and does so in an LXR ligand-dependent manner (Figure 5). More importantly, PGC-1β is recruited to the promoter region of *CYP7a1* and *ABCA1* and activates the expression of these LXR target genes. Although studies in this field have not elucidated how LXR activates the pathways of lipid transport and VLDL secretion in hepatocytes, the ability of PGC-1β to modulate LXR target gene expression in cultured cells and in vivo suggests that PGC-1β elicits at least a proportion of this hyperlipidemia through coactivation of LXR. However, since most of the data presented here relating to LXR are essentially correlative, it is entirely possible that the effects of PGC-1β on lipoprotein formation and secretion also involve coactivation of other transcription factors. Taken together, it is clear that PGC-1β couples these two important aspects of lipid metabolism in liver, i.e., lipid synthesis via coactivation of the SREBPs and lipoprotein secretion via coactivation of LXR and likely other transcription factors (Figure 7E).

The expression of PGC-1β is highly inducible in response to the dietary intake of saturated fats in vivo and by fatty acids in cultured hepatocytes. Notably, many enzymes involved in cholesterol biosynthesis, such as *HMG-CoA reductase*, a rate-limiting enzyme in this pathway and a target for cholesterol-lowering drugs, are all significantly induced by dietary intake of saturated fats. The stimulation of this pathway is striking in light of the fact that saturated fats have been known to cause increased atherogenic LDL cholesterol and an increased risk for the development of cardiovascular disease. In fact, as shown in Figure 3, adenoviral-mediated expression of PGC-1β in rat liver activates the programs of de novo lipid synthesis that bears close resemblance to the effects of dietary saturated fats. More importantly, PGC-1β expression leads to accumulation of cholesterol in VLDL, the precursor to LDL cholesterol. While rodents do not accumulate much LDL cholesterol because of its high rate of turnover, this pathway is basically similar to that in humans, and LDL cholesterol does build up

in mice when the LDL receptor is mutated. Hence, these findings strongly suggest that the induction of PGC-1β may be a key step linking the dietary intake of saturated and trans fats with the elevation of circulating triglycerides and cholesterol.

It is now clear that dietary intake of saturated and trans fats are crucial in the elevation of LDL cholesterol in humans, and the molecular pathways at work here were previously quite obscure. What is not obscure is the fact that the resulting increase in cholesterol levels by these fats has disastrous consequences for public health: the atherogenic effects of diets rich in saturated and trans fats are now well documented. The role of PGC-1β as both a target of saturated and trans fatty acids and as a regulator of VLDL secretion is clearly illustrated in our studies. Modulation of PGC-1β expression and activity in liver may provide an important therapeutic target for minimizing the deleterious effects of saturated and trans fats on the pathogenesis of atherogenic hyperlipidemia.

Experimental Procedures

High-Fat Feeding and Microarray Analysis

Animals were fed a standard rodent chow and housed in a controlled environment with 12 hr light and dark cycles. For high-fat feeding, three-month-old male C57/Bl6J mice were switched to a diet containing 58% fat-derived calorie (D12331, Research Diets) for 24 or 48 hr. For high-cholesterol feeding, two groups of mice were fed a base diet supplemented with either 0.07% or 2% cholesterol for 24 or 48 hr. Liver was dissected and immediately frozen for RNA isolation. 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 (Li and Wong, 2001).

Free Fatty Acid Treatments

Primary hepatocytes were isolated as previously described (Lin et al., 2004). Free fatty acids were dissolved in ethanol as 100 mM stock solutions for further dilution in DMEM supplemented with 0.5% BSA to a final concentration of 400 μM. Hepatocytes were treated for 4 hr before RNA isolation and analysis.

Adenoviral Transduction

Male Wistar rats (Charles River Laboratories) were fed a high-fat diet (TD96001, Harlan Taklad) for ten weeks. Animals were anesthetized with Nembutal and transduced with purified adenoviruses via tail vein injection (1×10^{12} viral particles per rat). Liver toxicity was monitored by plasma alanine aminotransferase and aspartate aminotransferase levels as determined by ALT/AST assay kit (505-OP, Sigma). Plasma and liver were harvested six days following adenoviral transduction for gene expression and lipid analysis.

Analysis of Liver and Plasma Lipids

Liver triglycerides were extracted using chloroform/methanol (2:1) mix, dried in fume hood overnight, and dissolved in a solution containing 60% butanol and 40% of the Triton-X114/methanol mix (2:1). Liver and plasma triglyceride concentrations were measured using a colorimetric assay kit (337, Sigma). Total plasma cholesterol was determined using the Infinity cholesterol reagent (401, Sigma). For lipoprotein analysis, 300 μl of plasma was fractionated by FPLC. Concentrations of triglycerides and cholesterol in each fraction were determined as described above. Data represent mean ± SEM.

RNA Isolation and Analysis

Total RNA was isolated from liver or cultured hepatocytes using Trizol reagent (Invitrogen). For real-time PCR analysis, cDNA was synthesized by reverse transcription using random primers and subjected to PCR amplification with gene-specific primers in the presence of Cybergreen (Biorad). Relative abundance of mRNA was calculated after normalization to *18S* ribosomal RNA. For hybridization, 20 μg of total RNA was resolved on a formaldehyde gel, transferred to nylon membrane, and hybridized with ^{32}P-labeled gene-specific probes. Hybridization to ribosomal protein 36B4 was included as loading control. Data represent mean ± SEM.

Transient Transfection

Mouse H2.35 hepatoma cells (CRL-1995, ATCC) were maintained in DMEM supplemented with 4% fetal bovine serum in the presence of 0.2 μM dexamethasone. Transient transfection was performed using Superfect (Qiagen). In a typical experiment, 100 ng of reporter plasmids were mixed with 20–50 ng of expression constructs for transcription factors in the presence or absence of 0.5–1.0 μg of PGC-1 expression or RNAi constructs. Equal amounts of DNA were used for all transfection combinations by adding appropriate vector DNA. For LXR agonist treatments, T0901317 (Cayman Chemical) was added to a final concentration of 10 μM 20 hr before luciferase assay. All transfection experiments were repeated at least three times in triplicates. Data represent mean ± SEM.

Chromatin Immunoprecipitation

H2.35 hepatoma cells were infected with Ad-GFP, Ad-flag-PGC-1α, or Ad-PGC-1β in the absence or presence of Ad-SREBP1c for 2 days. Cells were harvested following brief fixation with 10% formalin for preparation of sheared chromatin. Immunoprecipitation was performed using anti-flag or IgG control antibodies. The precipitates were reverse crosslinked for DNA isolation and PCR analysis.

Protein Interaction Assays

Physical association of PGC-1β and SREBP1c in cells was examined by coimmunoprecipitation. Briefly, H2.35 hepatoma cells were infected with Ad-SREBP1c, Ad-Flag-PGC-1α, Ad-Flag-PGC-1β alone or in combinations as indicated. Nuclei were isolated from infected cells 48 hr following infection and extracted in a lysis buffer containing 20 mM HEPES (pH 7.9), 400 mM NaCl, 1.5 mM $MgCl_2$, 0.5 mM DTT, 0.2 mM EDTA, 15% glycerol, and 1 mM PMSF. Immunoprecipitation was performed in the lysis buffer supplemented with 1.5% Triton X-100 and 0.2 mg/ml BSA using polyclonal antibodies against SREBP1 (sc-8984, Santa Cruz Biotechnology). PGC-1s in the complex were revealed by immunoblotting using a monoclonal antibody against the Flag epitope (M2, Sigma). In vitro interaction assays were performed as previously described (Lin et al., 2002a).

PGC-1β RNAi Vectors

The RNAi constructs for PGC-1β were generated using two sequences in the coding region of PGC-1β: 5′-GATATCCTCTGTGATGTTA-3′ (RNAi #1) and 5′-GTACGGAACTGCATAAGCA-3′ (RNAi #2). Oligonucleotides containing these sequences were subcloned into the pSUPER-retro vector (Brummelkamp et al., 2002). For transient transfection, 1.0 μg of pSUPER vector or PGC-1β RNAi constructs were used in combination with 100 ng of reporter plasmids and 50 ng of expression constructs for appropriate transcription factors.

PGC-1β RNAi adenoviruses were generated using the expression cassettes derived from the pSUPER vectors. For knockdown experiments, H2.35 hepatoma cells were infected with either Ad-GFP or Ad-PGC-1β RNAi adenoviruses for 48 hr before incubation with Ad-SREBP1c. Total RNA were harvested from infected cells 20 hr following Ad-SREBP1c infection and analyzed by real-time PCR.

Adenoviral transduction in mice was performed by tail vein injection at 1.5×10^{11} viral particles per mouse (three-month-old C57Bl/6 males). After four days, the animals were switched to a high-fat diet for two more days. Plasma samples were collected before and after high-fat feeding and assayed for triglyceride and cholesterol concentrations. HDL cholesterol was measured using an automated ACE Clinical Chemistry System (ALFA Wassermann, New Jersey). Liver was dissected at the end of high-fat feeding for gene expression and lipid analysis.

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