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Accessibility
Sensitivity of Lipid Metabolism and Insulin Signaling to Genetic Alterations in Hepatic Peroxisome Proliferator–Activated Receptor-γ Coactivator-1α Expression

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OBJECTIVE—The peroxisome proliferator–activated receptor-γ coactivator (PGC-1) family of transcriptional coactivators controls hepatic function by modulating the expression of key metabolic enzymes. Hepatic gain of function and complete genetic ablation of PGC-1α show that this coactivator is important for activating the programs of gluconeogenesis, fatty acid oxidation, oxidative phosphorylation, and lipid secretion during times of nutrient deprivation. However, how moderate changes in PGC-1α activity affect metabolism and energy homeostasis has yet to be determined.

RESEARCH DESIGN AND METHODS—To identify key metabolic pathways that may be physiologically relevant in the context of reduced hepatic PGC-1α levels, we used the Cre/Lox system to create mice heterozygous for PGC-1α specifically within the liver (LH mice).

RESULTS—These mice showed fasting hepatic steatosis and diminished ketogenesis associated with decreased expression of genes involved in mitochondrial β-oxidation. LH mice also exhibited high circulating levels of triglyceride that correlated with increased expression of genes involved in triglyceride-rich lipoprotein assembly. Concomitant with defects in lipid metabolism, hepatic insulin resistance was observed both in LH mice fed a high-fat diet as well as in primary hepatocytes.

CONCLUSIONS—These data highlight both the dose-dependent and long-term effects of reducing hepatic PGC-1α levels, underlining the importance of tightly regulated PGC-1α expression in the maintenance of lipid homeostasis and glucose metabolism. Diabetes 58:1499–1508, 2009
notype because of loss of PGC-1α in other metabolically active tissues, including skeletal muscle, brain, brown fat, and heart (14,15,18). Additionally, adenosivial knockdown of PGC-1α can only address the acute effects of losing coactivator activity. Most importantly, the expression levels of PGC-1α and other coactivators are tightly regulated, often changing only mildly in response to physiological cues (16,19–21). Therefore, the above-mentioned models may not appropriately reflect the effects of physiological fluctuations of PGC-1α expression on tissue-specific target pathways.

We were interested in observing the consequences of moderate, long-term changes in hepatic PGC-1α expression. To do this, we used a tissue-specific gene-targeting approach to create mice with only one functional allele of PGC-1α within the liver. Using this mouse model, we have identified liver-specific PGC-1α–regulated pathways highly sensitive to quantitatively reduced coactivator expression. Loss of only one allele of hepatic PGC-1α was sufficient to cause significant dysfunctions in fasting lipid oxidation, ketogenesis, and the regulation of circulating triglyceride levels. These data underline the importance of PGC-1α expression to hepatic lipid metabolism and indicate that even moderate decreases in PGC-1α function may contribute to the development of liver disease.

**RESULTS**

Generation of liver-specific PGC-1α heterozygous mice. To investigate the effects of chronically reduced PGC-1α within the liver, we crossed mice harboring one floxed PGC-1α allele (PGC-1αfl/fl) with transgenic mice expressing Cre recombinase under the control of the albumin promoter (alb-cre transgene) (Fig. 1A). The resulting mice possessed one functional and one disrupted PGC-1α allele within the liver (PGC-1αfl/fl) and other coactivators are tightly regulated, often changing only mildly in response to physiological cues (16,19–21). Therefore, the above-mentioned models may not appropriately reflect the effects of physiological fluctuations of PGC-1α expression on tissue-specific target pathways.

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**RESEARCH DESIGN AND METHODS**

Floxed PGC-1α alleles are previously described (14). To create liver-specific heterozygous (LH) animals, female mice with one floxed PGC-1α allele (PGC-1αα/β) were crossed with mice transgenically expressing Cre recombinase under control of the rat albumin promoter (Jackson Laboratory). Control mice were a mixed population of PGC-1αα/β and PGC-1αα/α,α/β littermates. All mice were on a mixed background of C57BL/129, which is similar to the background of other PGC-1α models (11,14,15). Animals were fed a regular diet or provided high-fat diet (58% kcal fat; D12331; Research Diets). All experiments were performed in accordance with animal facility institutional animal care and use committee regulations.

**Histology.** Liver tissue was frozen in OCT compound, sectioned, and stained with oil red O.

**Body composition.** Percentage fat mass was determined by dual-energy X-ray absorptiometry scanning in anesthetized mice (Piximus II; Lunar). Hepatic lipid levels. Hepatic lipids were extracted as previously described (22). Triglycerides (Sigma), nonesterified free fatty acids (NEFAs; Wako), and cholesterol (Pointe Scientific) were measured by colorimetric assay and fractionalization of palmitate oxidation in cultured hepatocytes was performed as previously described (25). The purified fraction was used for lipid chromatography/mass spectrometry/mass spectrometry analysis using an API 300 mass spectrometer (Applied Biosystems).

**Triglyceride secretion rate.** Mice were fasted 4 h before tail vein injection of 250 mg/kg tyloxapol (Sigma). Triglyceride concentration in blood, collected before and every hour for 4 h after injection, was measured by colorimetric assay.

**Protein isolation and Western blotting.** Hepatocyte or liver protein was solubilized in radioimmunoprecipitation assay buffer containing protease and phosphatase inhibitors. PGC-1α was immunoprecipitated from 500 μg (deoxymethasone/forskolin-treated) or 2 mg (fresh hepatocytes) of protein using an anti–PGC-1α antibody. Protein samples were resolved by SDS-PAGE, blotted, and incubated with anti–PGC-1α (gift from Dr. Thomas Getts, Pennington Biomedical Research Center), phospho-Akt (Ser473), or total Akt antibodies (Cell Signaling). Equal loading was confirmed using anti-actin or 90-kDa heat shock protein.

**In vivo insulin signaling and pyruvate tolerance tests.** Animals were fasted for 16 or 6 h before intraperitoneal injection of 2 g/kg sodium pyruvate or 0.8 units/kg insulin (Humulin; Eli Lilly), respectively. Glucose was measured in tail vein blood taken at 15 min intervals. For in vivo measurement of phospho-Akt, animals fed a high-fat diet for 16 weeks were fasted 4 h before intravenous injection of 0.5 units/kg insulin via the inferior vena cava. Liver was harvested 5 min after injection.

**Statistical analysis.** Statistical significance (P < 0.05, P < 0.01, and P < 0.001) was assessed by ANOVA, Student’s t test, or linear regression analysis using GraphPad Prism, as indicated.

**RESULTS**

Generation of liver-specific PGC-1α heterozygous mice. To investigate the effects of chronically reduced PGC-1α within the liver, we crossed mice harboring one floxed PGC-1α allele (PGC-1αfl/fl) with transgenic mice expressing Cre recombinase under the control of the albumin promoter (alb-cre transgene) (Fig. 1A). The resulting mice possessed one functional and one disrupted PGC-1α allele within the liver (PGC-1αfl/fl) and other coactivators are tightly regulated, often changing only mildly in response to physiological cues (16,19–21). Therefore, the above-mentioned models may not appropriately reflect the effects of physiological fluctuations of PGC-1α expression on tissue-specific target pathways.
PGC-1α within wild-type and LH hepatocytes correlated with hepatic mRNA levels.

LH mice were born in the expected Mendelian ratio, with no obvious growth abnormalities observed at birth. Although they were slightly lighter, this difference was not statistically significant. Importantly, they exhibited no differences in fat mass compared with control mice at 24 weeks of age (supplementary Fig. A2, panels A and B).

**Levels of hepatic PGC-1α correlate inversely with fasting hepatosteatosis.** Because PGC-1α plays a key role in modulating the hepatic response to nutrient deprivation (7,16,17), we fasted wild-type and LH mice to identify potential differences in glucose and lipid metabolism. Although PGC-1α is known to play a significant role in the regulation of hepatic gluconeogenesis (7,9,17), mice lacking only one allele of PGC-1α in the liver did not show evidence of fasting hypoglycemia or impaired pyruvate metabolism on a regular chow diet (supplementary Fig. A2, panels A and B). However, oil red O staining of liver sections indicated that LH livers had a higher lipid content than wild-type controls after a 24-h fast (Fig. 2A). Quantitatively, fasted LH livers accumulated significantly more triglycerides and cholesterol than wild-type controls, whereas there was no difference in NEFA levels (Fig. 2B). Furthermore, the relative level of hepatic PGC-1α transcripts in fasted mice inversely correlated with the concentration of triglycerides and cholesterol, but not NEFA (Fig. 2C). Fed levels of hepatic triglycerides, cholesterol, and NEFA were similar between groups (Fig. 2B and supplementary Fig. A2, panel E). These data indicate that
FIG. 2. Accumulation of hepatic lipids in fasted LH mice. A: Oil red O staining of liver sections isolated from 24-h–fasted mice. B: Average triglyceride, cholesterol, and NEFA levels in fed or 24-h–fasted wild-type (WT) and LH livers after chloroform/methanol lipid extraction, n = 6 (fed) or 9–10 (fasted) mice per group. C: Individual fasted lipid concentrations were plotted against the relative PGC-1α mRNA value for each mouse and subjected to linear regression analysis. *P < 0.05. (A high-quality digital representation of this figure is available in the online issue.)
fasting lipid metabolism is highly sensitive to reductions in PGC-1α expression and that even modest reductions can cause significant defects in lipid processing, leading to fatty liver disease.

**Genetic reduction of hepatic PGC-1α diminished the gene program of fatty acid oxidation.** Next, we investigated the effects of having reduced PGC-1α on the expression of mRNAs encoding key metabolic enzymes in livers of fed and fasted mice. We observed modest yet significant reductions in the expression of select genes involved in fatty acid oxidation, including lipin-1 (LPIN-1), very-long-chain acyl-CoA dehydrogenase (VLCAD), long-chain acyl-CoA dehydrogenase (LCAD), and short-chain acyl-CoA dehydrogenase (SCAD) (Fig. 3A and supplementary Table A1). Similarly, reduced levels of VLCAD, LCAD, and SCAD and increased hepatic lipid content were also observed after long-term fasting of 24–72 h (supplementary Table A1), which, taken together, may suggest an increase in lipoprotein synthesis.

**Dysregulation of hepatic PGC-1α leads to hypertriglyceridemia.** Defects in hepatic lipid catabolism can directly affect levels of circulating lipids. Circulating levels of triglycerides were significantly higher in fed LH mice, whereas fasting levels showed no differences (Fig. 4A). There were no differences in the fed or fasted concentrations of circulating free fatty acids, and levels of cholesterol in the VLDL, LDL, or HDL fractions were similar between wild-type and LH mice (supplementary Fig. A4).

Increases in serum triglycerides may arise because of dysregulation of hepatic lipid assembly, secretion, or catabolism. Because PGC-1α regulates key genes involved in lipid transport (31), we investigated whether hepatic PGC-1α heterozygosity affected lipid secretory pathways. We saw no differences in the expression of APOAI, APOAV, or APOCIII (Fig. 4B and supplementary Table A1), previously characterized PGC-1α target genes. However, in both fed and fasted mice, there was significantly increased expression of microsomal triglyceride transfer protein (MTP), a protein essential for LDL assembly (Fig. 4B and supplementary Table A1). We also observed increased APOB expression levels in LH mice (Fig. 4B and supplementary Table A1), which, taken together, may suggest an increase in lipoprotein synthesis.

To assess whether high triglyceride levels were caused by increased triglyceride secretion, we inhibited lipoprotein lipase using tyloxapol and measured the rate of triglyceride accumulation in the serum. Consistent with previous findings, LH mice exhibited higher levels of circulating triglycerides before and at all points after tyloxapol injection (Fig. 4C). However, the rate of triglyceride accumulation in the serum of wild-type and LH mice was similar (Fig. 4C), suggesting that the higher levels of circulating triglycerides were not caused by increased hepatic lipoprotein secretion.

**LH hepatocytes exhibit defects in insulin signaling.** Defects in fatty acid oxidation and high hepatic triglycerides have been linked to hepatic insulin resistance (32). Isolated primary hepatocytes from LH mice showed markedly reduced levels of phosphorylated Akt after incubation with insulin (Fig. 5A). Furthermore, insulin pretreatment did not suppress the induction of PEPCK or glucose-6-phosphatase (G6P) by glucagon in primary LH hepatocytes (Fig. 5B). Acute knockdown of PGC-1α in primary hepatocytes with siRNA did not affect insulin signaling in primary hepatocytes (Fig. 5C and supplementary Fig. A5). Thus, it appeared that only chronic reduction of hepatic PGC-1α diminished the ability of liver cells to respond to insulin.

**High-fat feeding exacerbates defects in hepatic insulin signaling in LH mice.** We next investigated whether mice with chronically reduced levels of PGC-1α exhibited alterations in insulin sensitivity in vivo. Differences in insulin action were not immediately evident in LH mice fed a chow diet (supplementary Fig. A6). Because hepatic insulin insensitivity is a hallmark of metabolic syndrome linked to the consumption of a diet high in fat, we challenged the LH mice with a diet consisting of 58% fat for up to 16 weeks and monitored hepatic metabolic function. Consistent with previous reports (13), high-fat feeding resulted in increased expression of hepatic PGC-1α in both wild-type and LH mice (Fig. 6A). Importantly, no significant differences in body weight, body composition, growth, or food intake were noted between the groups (Fig. 6B and data not shown). We observed reduced expression levels of select fatty acid oxidation genes in fasted and refed high-fat–fed LH mice (supplementary Fig. A7). Levels of hepatic lipids were extremely high in both fed and fasted mice, and although a trend toward higher triglycerides in LH mice was noted, it did not reach statistical significance (supplementary Fig. A7, panels C and D).

Levels of phosphorylated Akt in response to exogenous insulin administration were lower in LH mice after 16 weeks on a high-fat diet (Fig. 6C), suggesting a mild decrease in hepatic insulin signaling. Interestingly, although circulating insulin concentrations were similar in 24-h fasted mice, refed producing higher insulin levels in LH mice (Fig. 6D). Regardless, refed LH mice did not decrease hepatic PEPCK mRNA levels to the same extent as wild-type controls (Fig. 6E), suggesting a defect in the ability of endogenous insulin to shut down fasting-induced gluconeogenesis. A similar trend was also observed for G6P mRNA, though not reaching statistical significance ($P = 0.055$). Consistent with decreased hepatic insulin sensitivity, high-fat–fed LH livers had inappropriately high levels of gluconeogenic gene expression (Fig. 6F). Thus, hepatic insulin resistance was apparent at the level of both insulin signaling and target gene expression. Taken to-
together, these data show a significant defect in hepatic insulin signaling that manifests physiologically in response to the metabolic stress of high-fat feeding.

Our data are in striking contrast to a previous study showing that acute knockdown of PGC-1α within the liver enhances insulin-mediated Akt phosphorylation through...
FIG. 4. LH mice exhibit increased circulating triglycerides and altered expression of genes involved in VLDL production. A: Concentrations of circulating triglycerides in fed or 24-h-fasted mice. Data are the means ± SE (n = 11). □, Wild-type; ■, LH. B: Lipid transport genes. Hepatic mRNA levels were quantified by RT-PCR in mice fed or fasted overnight. Data are the means ± SE (n = 6). Values are expressed relative to wild-type (WT) fed levels. C: Time-course of serum triglyceride accumulation after inhibition of lipolysis with intravenously injected Tyloxapol. Values are the means ± SE (n = 3) and are representative of two independent experiments. *P < 0.05 by two-way ANOVA. **P < 0.01.

FIG. 5. Decreased insulin signaling in primary LH hepatocytes. Levels of phosphorylated Akt (pAkt) and total Akt were measured in protein extracts from primary wild-type (WT) or LH hepatocytes (A) or wild-type hepatocytes infected with either siPGC-1α or control virus (C) treated with media alone (control) or 100 nmol/l insulin for 10 min before addition of vehicle or 25 nmol/l glucagon, as indicated. Western blots show biological duplicates and are representative of two individual experiments. *P < 0.05, ns, nonsignificant; siScr, scrambled siRNA.

DISCUSSION

It is clear that the PGC-1 coactivators play important roles in various aspects of energy homeostasis. Using gain-of-function and complete-loss-of-function studies, these proteins were shown to be dominant regulators of oxidative metabolism, particularly mitochondrial biogenesis, skeletal muscle biology, brown fat thermogenesis, and the hepatic fasting response (4,5,7,11,14,16). However, what remained unclear from these studies was the metabolic
Our data demonstrate for the first time that chronically and mildly reduced hepatic PGC-1α causes hepatic insulin resistance. Primary LH hepatocytes and high-fat–fed LH livers exhibited decreased insulin-stimulated Akt activation (Figs. 5 and 6). Moreover, LH mice on a high-fat diet had increased fed gluconeogenic gene expression that could not be efficiently reduced after fasting/refeeding (Fig. 6). Interestingly, the decrease in hepatic insulin sensitivity shown here is in contrast to results from Koo et al. (11), who demonstrated that a sharp, adenoviral-mediated reduction of hepatic PGC-1α caused hepatic insulin resistance. Our data suggest that this discrepancy is likely due to the fact that our mice were chronically fed a high-fat diet, whereas Koo et al. used a short-term adenoviral injection.

Conclusions

In conclusion, we have shown that chronic PGC-1α reduction causes hepatic insulin resistance, reduced insulin sensitivity, and increased hepatic gluconeogenesis. These findings support the role of PGC-1α in regulating glucose metabolism and highlight the importance of considering the chronic effects of PGC-1α reduction.

FIG. 7. Fasting hypoglycemia and deficient gluconeogenesis in LH mice fed a high-fat diet. A: Blood glucose levels in fed, fasted (6 or 16 h), or refed (fasted overnight and refed 2 h) wild-type (WT) or LH mice maintained on a high-fat diet for 6 weeks. Data are the means ± SE (n = 4–14). B: Hepatic mRNA levels in high-fat diet–fed wild-type and LH mice after a 24-h fast. Data are the means ± SE (n = 9, expressed relative to wild-type levels). C: Pyruvate tolerance test. Blood glucose levels were measured in 16-week high-fat–fed wild-type or LH mice after an overnight fast (time 0) and at the indicated times after an intraperitoneal injection of pyruvate (pyruvate tolerance test). Values represent the means ± SEM (n = 10), P < 0.01 by two-way ANOVA, indicating a significance difference between wild-type versus LH curves. *P < 0.05; **P < 0.01.
expression in fed or fasted LH mice (Fig. 6F and 7B, supplementary Table A1). These differences may be attributable to the degree of PGC-1α loss in these two sets of experiments, the method of knockdown, or the differential effects of chronic versus transient decreases in PGC-1α expression.

Chronic reductions in hepatic PGC-1α affected triglyceride assembly and/or production (Fig. 4), which can also be attributed to hepatic insulin resistance. Insulin reduces the amount of circulating VLDL particles by directly suppressing hepatic VLDL production (2), and hepatic insulin resistance contributes to both increased hepatic VLDL production and decreased VLDL uptake in patients with type 2 diabetes (rev. in 35). Insulin has been shown to inhibit the expression of MTP, a protein that initiates the production of VLDL (36). Consistent with decreased insulin action, we observed increased expression of MTP in LH mice (Fig. 4F). We also detected increased expression of apoB, the major protein constituent of VLDL, which, along with high serum triglycerides, is associated with coronary artery disease (35,37). Thus, it is likely that hepatic insulin resistance contributed to the hypertriglyceridemia in fed LH mice. However, there remains the possibility that long-term reduction of hepatic PGC-1α has extrahepatic effects on triglyceride lipolysis or absorption.

Interestingly, we observed increased circulating insulin levels in LH mice after refeeding. In contrast to the muscle-specific PGC-1α knockout mice, we observed no difference in gross islet morphology (38) (data not shown). Thus, it is likely that chronically reduced hepatic PGC-1α has effects on peripheral tissue metabolism through currently unidentified mechanisms.

The most striking and clear-cut consequence of quantitatively decreasing hepatic PGC-1α was impairment of the fatty acid oxidation gene program. Decreased hepatic fatty acid oxidation and concomitant lipid accumulation have been shown to negatively affect insulin signaling (39). Our study showed that the fatty acid oxidation genes VLCAD, LCAD, and SCAD are highly sensitive to changes in PGC-1α expression levels. Other studies have shown that mice deficient in these fatty acid oxidation genes showed hepatic steatosis and hepatic insulin resistance (30). Studies also suggest there is a synergistic effect of having reduced function in two or more of the acyl-CoA dehydrogenases (40). Given that PGC-1α is crucial for maintaining the expression levels of multiple enzymes within this family, it is likely that long-term dysregulation of lipid metabolism in LH mice contributes to the development of hepatic insulin resistance, particularly under the challenge of a high-fat diet. Interestingly, hepatic steatosis was not significantly worse in high-fat–fed LH mice. However, because insulin directly downregulates fatty acid oxidation (41), insulin resistance may mask the effects of reduced PGC-1α on fatty acid oxidation in these mice.

Our study clearly demonstrates that modest changes in hepatic PGC-1α expression can have significant effects on energy homeostasis. Furthermore, although chronic reduction of hepatic PGC-1α had only a modest effect on reducing gluconeogenesis, multiple aspects of hepatic metabolism were significantly disrupted by loss of the transcriptional coactivator. Because there is growing interest in the therapeutic potential of targeting this transcriptional coactivator during the development of metabolic diseases, it will be of interest to investigate how chemical modulators of PGC-1α activity affect liver function in diabetic and obese patients.

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