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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

Imbalances in hepatic lipid metabolism, leading to accumulation of hepatic triglycerides, insulin resistance, inflammation, and apoptosis, are intimately related to diseases of energy imbalance; these include obesity, diabetes, hyperlipidemia, and atherosclerosis (1,2). Changes in hepatic energy balance are often modulated at the transcriptional level by hormonal signals acting on nuclear receptors and forkhead box O (FoxO) proteins (3). In addition to ligand-mediated receptor activation, physiological stimuli promote recruitment of coactivators to the transcriptional machinery, adding an additional layer of regulation by selective amplification of specific gene sets. Peroxisome proliferator-activated receptor (PPAR)-γ coactivator-1 (PGC-1α) is one such transcriptional coactivator shown to play a particularly important role in liver biology.

Hepatic PGC-1α binds to and activates multiple transcription factors, including FoxO1, glucocorticoid receptor, hepatic nuclear factor-4α, estrogen-related receptor-α, and PPAR-α, resulting in increased expression of genes important for gluconeogenesis, fatty acid oxidation, lipid transport, and oxidative phosphorylation (4,5). Glucagon increases hepatic PGC-1α expression during a fast, whereas insulin potently inhibits PGC-1α expression and activity (6–10). Thus, alterations in hormone activity, such as the insulin resistance or hyperglucagonemia associated with diabetes, may lead to dysregulation of PGC-1α. Hepatic PGC-1α expression levels are increased in multiple rodent models of diabetes and obesity, including liver insulin receptor knockout (11,12), high-fat–fed (13), leptin-deficient (ob/ob), and streptozotocin-administered mice (7). Given its role in promoting gluconeogenesis, inappropriate high levels of hepatic PGC-1α may exacerbate hyperglycemia. Therefore, reducing hepatic PGC-1α may be an attractive therapeutic strategy for improving hepatic insulin signaling and preventing inappropriate glucose production in diabetic patients.

Though highlighting the importance of PGC-1α within liver biology, previous studies have been limited to gain/loss-of-function strategies using adenoviral vectors or complete loss-of-function knockout mouse models. These models suggest that although complete loss of PGC-1α within the liver results in fasting-induced steatosis, it improves glucose tolerance concomitant with decreased gluconeogenesis and increased insulin sensitivity (11,14–17). However, PGC-1α knockout mice exhibit multiple metabolic abnormalities contributing to their overall phe-
not because of loss of PGC-1α in other metabolically active tissues, including skeletal muscle, brain, brown fat, and heart (14,15,18). Additionally, adenoviral 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. Consistent with this, analysis of hepatic gene expression suggested that fatty acid oxidation and lipid processing pathways were most affected by loss of PGC-1α. More strikingly, chronic reduction of PGC-1α reduced hepatic insulin sensitivity, likely contributing to the alterations in hepatic glucose and lipid metabolism. These data underlie 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.

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-1αfl/+ ) 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-1αfl/+ and PGC-1αfl/fl,alb-cre 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 chow diet (5008I; PharmaServ) or a 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 (Picutum II; Lunar). Body composition was analyzed in fed, overnight-fasted, and long-term–fasted mice, respectively.

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 enzyme-linked immunosorbent assay. Solid-phase extraction of hepatic fatty acyl-CoAs and mass spectrometry. Concentrations of acyl-CoAs was performed as previously described (25). The purified fraction was used for liquid chromatography/mass spectrometry/mass spectrometry analysis using an API 3000 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 phosphorylation, 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/+ ) 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 liver heterozygotes, LH mice) confirmed by PCR analysis of both tail and liver genomic DNA (Fig. 1A). mRNA levels of PGC-1α in LH livers were, on average, 57, 42, and 34% of wild-type levels in fed, overnight-fasted, and long-term–fasted mice, respectively (Fig. 1B and supplementary Fig. A1). The >50% reduction was expected in fasted LH mice given that PGC-1α acts in an autoregulatory positive feedback loop (26,27). Hepatic levels of the structurally related transcriptional coactivator PGC-1β remained unchanged (Fig. 1B). LH mice expressed wild-type levels of PGC-1α in brown fat, white fat, muscle, and heart (Fig. 1C), confirming tissue specificity of PGC-1α heterozygosity. PGC-1α protein was difficult to detect by Western blot of whole-liver extracts (not shown). To visualize hepatic PGC-1α protein levels, we immunoprecipitated endogenous PGC-1α from protein extracts of freshly isolated hepatocytes from wild-type, LH, and whole-body PGC-1α knockout mice (Fig. 1D). These data show that relative protein levels of...
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 (VLCD), 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 Fig. A3). Interestingly, the levels of medium-chain acyl-CoA dehydrogenase, carnitine palmitoyl transferase-1α, and PPAR-α, known PGC-1α target fatty acid oxidation genes, were not significantly affected by chronic knockdown of hepatic PGC-1α (Fig. 3B and supplementary Fig. A3). To confirm that the regulation of these genes by PGC-1α was cell autonomous, we overexpressed PGC-1α using adenovirus in primary hepatocytes and measured the levels of gene transcripts by quantitative RT-PCR. PGC-1α overexpression significantly increased the mRNA levels of many genes specifically involved in mitochondrial β-oxidation of fatty acids (Fig. 3C).

Consistent with a hepatic defect in hepatic fatty acid oxidation, 14C-palmitate oxidation was significantly lower in LH primary hepatocytes (Fig. 3D). Moreover, LH mice had lower levels of circulating β-hydroxybutyrate in both the fed and fasted state, suggesting a deficiency in ketogenesis (Fig. 3E). Analysis of liver samples by mass spectrometry revealed that LH livers accumulated significantly higher levels of medium- to long-chain fatty acyl-CoAs, specifically C12:2, C12:1, C14:2, C14:1, and C18:2, after a 24-h fast (Fig. 3F). This pattern is similar to that found in mice lacking LCAD or VLCD enzymes (28–30).

**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 APOCI (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 tyloxyap 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 tyloxyap 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.

**Liver 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 and 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; **P < 0.01.

decreased expression of tribbles-3 (TRB-3), an inhibitor of Akt (11). To address this, we measured TRB-3 mRNA expression in our mouse model and found no differences between mice on either regular chow or high-fat diet (Fig. 6F and 7B). Thus, our study clearly illustrates the potential differences of chronic versus acute reduction of PGC-1α expression on hepatic metabolic function.

High-fat feeding of LH mice unmasks defects in gluconeogenesis. Although PGC-1α is known to increase the expression of the gluconeogenic program (7,9,31), mice with a global knockout of the PGC-1α gene have constitutively increased PEPCk and G6P gene expression likely caused by increased CCAAT/enhancer binding protein-β (C/EBP-β) expression (14). Interestingly, these same PGC-1α knockout mice exhibit defects in the ability to convert pyruvate to glucose, demonstrating that gluconeogenesis remains impaired. Although LH mice fed a regular chow diet exhibited normal gluconeogenic gene expression and pyruvate tolerance (supplementary Table A1 and supplementary Fig. A2), we addressed whether a high-fat diet could reveal defects in hepatic gluconeogenesis within LH mice.

Under these dietary conditions, we observed a mild, yet significant, reduction in fasting glycemia in LH mice after short-term food deprivation (Fig. 7A). In contrast to LH mice fed ad libitum (Fig. 6F), fasted LH mice exhibited decreased PEPCk gene expression, consistent with the observed fasting hypoglycemia and dependence on PGC-1α to potentiate the gluconeogenic response during a fast (Fig. 7B). We observed no differences in the expression of C/EBP-β (Fig. 6F and 7B, supplementary Table A1).

To directly assess hepatic gluconeogenesis, we monitored the appearance of glucose in the blood after injection of pyruvate after 12 weeks of high-fat feeding. Fasted LH mice had significantly reduced area under the curve in the pyruvate tolerance test, further suggesting a defect in hepatic gluconeogenesis (Fig. 7C). Taken together, it is apparent that reduced levels of PGC-1α can affect hepatic glucose metabolism at the level of both insulin signaling in the fed state and glucose production in the fasted state.

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 were the metabolic

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. Values are the means ± SD and representative of two individual experiments. *P < 0.05. ns, nonsignificant; siScr, scrambled siRNA.
consequences of modulating PGC-1α within physiological levels. Importantly, the expression of PGC-1α is quantitatively dysregulated in a variety of disease states. Increased PGC-1α expression has been shown in livers of diabetic mice (7,13), reduced levels are found in the muscle of insulin-resistant humans (33), and hepatic PGC-1α levels are inversely correlated with insulin resistance in humans (34). We show here that hepatic heterozygosity for PGC-1α, leading to a corresponding reduction in PGC-1α mRNA and protein, caused a substantial change in hepatic metabolism that is manifested primarily as hepatic steatosis and insulin resistance.

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 expression and increased insulin sensitivity in vivo. In contrast, we observed no differences in TRB-3 mRNA levels.

![Image](https://example.com/image.png)
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 show marked hepatosteatosis 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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REFERENCES

1. Yeh MM, Brunt EM. Pathology of nonalcoholic fatty liver disease. Am J Clin Pathol 2007;128:837–847.
2. Adiels M, Taskinen MR, Boren J. Fatty liver, insulin resistance, and dyslipidemia. Curr Diab Rep 2008;8:50–64.
3. Staudinger JL, Lichti K. Cell signaling and nuclear receptors: new opportunities for molecular pharmaceuticals in liver disease. Mol Pharm 2008;5:17–34.
4. Lin J, Handschin C, Spiegelman BM. Metabolic control through the PGC-1 family of transcription coactivators. Cell Metab 2005;1:361–370.
5. Finck BN, Kelly DP. PGC-1 coactivators: inducible regulators of energy metabolism in health and disease. J Clin Invest 2006;116:615–622.
6. Herzig S, Long F, Jhala US, Hedrick S, Quinn R, Bauer A, Rudolph D, Schutz G, Yoon C, Puigserver P, Spiegelman B, Montminy M. CREB regulates hepatic gluconeogenesis through the coactivator PGC-1α. Nature 2001;413:139–183.
7. Yoon JC, Puigserver P, Chen G, Donovan J, Wu Z, Rhee J, Adelmant G, Stafford J, Kahn CR, Grammer DK, Newgard CB, Spiegelman BM. Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1α. Nature 2001;413:131–138.
8. Daitoku H, Yamagata K, Matsuzaki H, Hatta M, Fukamizu A. Regulation of PGC-1α promoter activity by protein kinase B and the forkhead transcription factor FKHR. Diabetes 2003;52:642–649.
9. Puigserver P, Rhee J, Donovan J, Walkley CJ, Yoon JC, Oriente F, Kitamura Y, Almendez J, Dong H, Acili D, Spiegelman BM. Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1α-interaction. Nature 2003;425:520–550.
10. Li X, Monks B, Ge Q, Birnbaum MJ. Akt/PKB regulates hepatic metabolism by directly inhibiting PGC-1α transcription coactivator. Nature 2007;447:1012–1016.
11. Koo SH, Satoth H, Herzig S, Lee CH, Hedrick S, Kulkarni R, Evans RM, Olefsky J, Montminy M. PGC-1α promotes insulin resistance in liver through PPAR-alpha-dependent induction of TRB-3. Nat Med 2004;10:530–534.
12. Biddinger SB, Hernandez-Ono A, Rask-Madsen C, Haas JT, Aleman JO, Suzuki R, Scapa EF, Agarwal C, Carey MC, Stephanopoulos G, Cohen DE, King GL, Ginsberg HN, Kahn CR. Hepatic insulin resistance is sufficient to produce dyslipidemia and susceptibility to atherosclerosis. Cell Metab 2008;7:125–134.
13. Biddinger SB, Almind K, Miyazaki M, Kokkotou E, Nimbati JM, Kahn CR. Effects of diet and genetic background on sterol regulatory element-binding protein-1c, stearyl-CoA desaturase 1, and the development of the metabolic syndrome. Diabetes 2005;54:1314–1323.
14. Lin J, Wu PH, Tarr PT, Lindenberg KS, St-Pierre J, Zhang CY, Mootha VK, Jager S, Vianna CR, Reznick RM, Cui L, Manieri M, Donovan MX, Wu Z, Cooper MP, Fan MC, Bohas LM, Zavacki AM, Cinti S, Shulman GI, Lowell BB, Krainc D, Spiegelman BM. Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1α null mice. Cell 2004;119:121–135.
15. Leone T, Lehman J, Finck B, Schaeffer PJ, Wende AR, Boudina S, Courtois M, Wozniak DF, Sambandam N, Bernal-Mizrachi C, Chen Z, Holloszy JO, Medeiros DM, Schmidt RE, Saffitz JE, Abel ED, Semenkovich CF, Kelly DP. PGC-1α deficiency causes multi-system energy metabolic derangements: muscle dysfunction, abnormal weight control and hepatic steatosis. PLoS Biol 2005;3:e101.
16. Handschin C, Lin J, Rhee J, Peyer AK, Chinn S, Wu PH, Meyer UA, Spiegelman BM. Nutritional regulation of hepatic heme biosynthesis and porphyria through PGC-1α. Cell 2005;122:505–515.
17. Burgess SC, Leonc TC, Wende AR, Croce MA, Chen Z, Sherry AD, Malloy CR, Finck BN. Diminished hepatic gluconeogenesis via defects in triglycerolysis and release of fatty acids. Proc Natl Acad Sci USA 2003;100:2380–2385

18. Handschin C, Spiegelman BM. Peroxisome proliferator-activated receptor gamma coactivator 1 coregulators, energy homeostasis, and metabolism. Endocr Rev 2006;27:728–735

19. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. A cold-induced coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 1998;92:829–839

20. Goto M, Terada S, Kato M, Katoh M, Yokozeki T, Tabata I, Shimokawa T. Hepatic lipin 1beta expression is diminished in insulin-resistant obese subjects and is reactivated by marked weight loss. Diabetes 2007;56:2385–2390

21. Pilegaard H, Saltin B, Neufer PD. Exercise induces transient transcriptional activation of the PGC-1alpha gene in human skeletal muscle. J Physiol 2001;546:751–758

22. Lin J, Yang R, Tarr PT, Wu PH, Handschin C, Li S, Yang W, Pei L, Uldry M, Handschin J, Kohane I, Costello M, Saccone R, Landaker EJ, Goldfine AB, Mun E, DeFronzo R, Finlayson J, Kahn CR, Mandarino LJ. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: potential role of PGC1 and NRF1. Proc Natl Acad Sci USA 2003;100:8466–8471

23. Handschin C, Rhee J, Lin J, Tarr PT, Spiegelman BM. An autoregulatory loop controls peroxisome proliferator-activated receptor gamma coactivator 1alpha expression in muscle. Proc Natl Acad Sci USA 2003;100:7111–7116

24. Handschin C, Rhee J, Lin J, Tarr PT, Spiegelman BM. An autoregulatory loop controls peroxisome proliferator-activated receptor gamma coactivator 1alpha expression in muscle. Proc Natl Acad Sci USA 2003;100:7111–7116

25. Neschen S, Morino K, Hammond LE, Zhang D, Liu ZX, Romanelli AJ, Cline GW, Pongratz RL, Zhang XM, Choi CS, Shulman GL. Prevention of hepatic steatosis and hepatic insulin resistance in mitochondrial acyl-CoA:glycerol-sn-3-phosphate acyltransferase 1 knockout mice. Cell Metab 2005;2:55–65

26. Handschin C, Rhee J, Lin J, Tarr PT, Spiegelman BM. An autoregulatory loop controls peroxisome proliferator-activated receptor gamma coactivator 1alpha expression in adipocytes via peroxisome proliferator-activated receptor-gamma coactivation. Endocrinology 2006;147:2829–2838

27. Handschin C, Rhee J, Lin J, Tarr PT, Spiegelman BM. An autoregulatory loop controls peroxisome proliferator-activated receptor gamma coactivator 1alpha expression in adipocytes. J Clin Invest 2007;119:S10–S16

28. Kurtz DM, Rinaldo P, Rhead WJ, Tian L, Millington DS, Vockley J, Pinkert CA, Rhead WJ, Lindsey JR, Wood PA. Gestational, pathologic and biochemical differences between very long-chain acyl-CoA dehydrogenase deficiency and long-chain acyl-CoA dehydrogenase deficiency in the mouse. Hum Mol Genet 2001;10:2009–2077

29. Cox KB, Hamann DA, Millington DS, Matern D, Vockley J, Rinaldo P, Pinkert CA, Rhead WJ, Lindsey JR, Wood PA. Gestational, pathologic and biochemical differences between very long-chain acyl-CoA dehydrogenase deficiency and long-chain acyl-CoA dehydrogenase deficiency in the mouse. Hum Mol Genet 2001;10:2009–2077

30. Zhang D, Liu ZX, Choi CS, Tian L, Kibbey R, Dong J, Cline GW, Wood PA, Shulman GI. Mitochondrial dysfunction due to long-chain acyl-CoA dehydrogenase deficiency causes hepatic steatosis and hepatic insulin resistance. Proc Natl Acad Sci USA 2007;104:17075–17080

31. Rhee J, Ge H, Yang W, Fan M, Handschin C, Cooper M, Lin J, Li C, Spiegelman BM. Partnership of PGC-1alpha and HNF4alpha in the regulation of lipoprotein metabolism. J Biol Chem 2006;281:14683–14690

32. Petersen KF, Shulman GI. Etiology of insulin resistance. Am J Med 2006;119:S10–S16

33. Patti ME, Butte AJ, Crunkhorn S, Cusi K, Berria R, Kashyap S, Miyazaki Y, Kohane I, Costello M, Saccone R, Landaker EJ, Goldfine AB, Mun E, DeFronzo R, Finlayson J, Kahn CR, Mandarino LJ. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: potential role of PGC1 and NRF1. Proc Natl Acad Sci USA 2003;100:8466–8471

34. Croce MA, Eagon JC, LaRiviere LL, Korenblat KM, Klein S, Finck BN. Hepatic lipid beta expression is diminished in insulin-resistant obese subjects and is reactivated by marked weight loss. Diabetes 2007;56:2385–2390

35. Idiels M, Olofsson SO, Taskinen MR, Boren J. Overproduction of very low-density lipoproteins is the hallmark of the dyslipidemia in the metabolic syndrome. Arterioscler Thromb Vasc Biol 2008;28:1225–1236

36. Allister EM, Borradaile NM, Edwards DY, Huff MW. Inhibition of microsomal triglyceride transfer protein expression and apolipoprotein B100 secretion by the citrus flavonoid naringenin and by insulin involves activation of the mitogen-activated protein kinase pathway in hepatocytes. Diabetes 2005;54:1676–1683

37. Vakkilainen J, Steiner G, Ansquer JC, Aubin F, Rattier S, Foucher C, Hamsten A, Taskinen MR. Relationships between low-density lipoprotein particle size, plasma lipoproteins, and progression of coronary artery disease: the Diabetes Atherosclerosis Intervention Study (DAIS). Circulation 2003;107:1733–1737

38. Handschin C, Choi CS, Chin S, Kim S, Kawamori D, Kupad AJ, Neubauer N, Hu H, Mootha VK, Kim YB, Kulkarni RN, Shulman GI, Spiegelman BM. Abnormal glucose homeostasis in skeletal muscle-specific PGC-1alpha knockout mice reveals skeletal muscle-pancreatic beta cell crosstalk. J Clin Invest 2007;117:3463–3474

39. Savage DB, Petersen KF, Shulman GI. Disordered lipid metabolism and the pathogenesis of insulin resistance. Physiol Rev 2007;87:507–520

40. Schuler AM, Gower BA, Matern D, Rinaldo P, Vockley J, Wood PA. Gestational diabetes: the Diabetes Atherosclerosis Intervention Study (DAIS). Circulation 2003;107:1733–1737

41. Wolf G. Role of fatty acids in the development of insulin resistance and type 2 diabetes mellitus. Nutr Rev 2008;66:597–600