Diminished Hepatic Gluconeogenesis via Defects in TCA Cycle Flux in PPARγ Coactivator-1α (PGC-1α)-Deficient Mice

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SUMMARY

The peroxisome proliferator-activated receptor γ (PPARγ) coactivator 1α (PGC-1α) is a highly-inducible transcriptional coactivator implicated in the coordinate regulation of genes encoding enzymes involved in hepatic fatty acid oxidation, oxidative phosphorylation, and gluconeogenesis. The present study sought to assess the effects of chronic PGC-1α deficiency on metabolic flux through the hepatic gluconeogenic, fatty acid oxidation, and TCA cycle pathways. To this end, hepatic metabolism was assessed in wild-type (WT) and PGC-1α−/− mice using isotopomer-based NMR with complimentary gene expression analyses. Hepatic glucose production was diminished in PGC-1α−/− livers coincident with reduced gluconeogenic flux from phosphoenolpyruvate. Surprisingly, the expression of PGC-1α target genes involved in gluconeogenesis was unaltered in PGC-1α−/− compared to wild-type mice under fed and fasted conditions. Flux through TCA cycle and mitochondrial fatty acid β-oxidation pathways was also diminished in PGC-1α−/− livers. The expression of multiple genes encoding TCA cycle and oxidative phosphorylation enzymes was significantly depressed in PGC-1α−/− mice and was activated by PGC-1α overexpression in liver in wild-type mice. Collectively, these findings suggest that chronic whole-animal PGC-1α deficiency results in defects in hepatic glucose production that are secondary to diminished fatty acid β-oxidation and TCA cycle flux rather than abnormalities in gluconeogenic enzyme gene expression per se.

INTRODUCTION

Flux through hepatic gluconeogenesis, fatty acid oxidation (FAO), TCA cycle, and mitochondrial oxidative phosphorylation (OXPHOS) pathways can be modulated at multiple regulatory levels. Substrate availability, post-translational modification, and transcriptional regulation of genes encoding enzymes at various points can influence the capacity for, and the rate of flux through, each of these pathways. Moreover, flux through one pathway has an inevitable impact on the flux of the others. For instance, mitochondrial FAO is the principal source of energy in the hepatocyte, impacting the
amount of chemical work that can be performed by the liver. Furthermore, the TCA cycle not only oxidizes acetyl-CoA generated by β-oxidation and produces reducing equivalents for ATP synthesis, but also supplies carbons necessary for gluconeogenesis through pyruvate carboxylase (PC) and phosphoenolpyruvate carboxykinase (PEPCK). Thus, the TCA cycle is a critical hub linking FAO with gluconeogenesis and OXPHOS pathways.

Recent work has shown that the peroxisome proliferator-activated receptor γ (PPARγ) coactivator-1α (PGC-1α) is a highly-inducible transcriptional coactivator that integrates multiple interconnected metabolic pathways in liver (1). PGC-1α controls transcription of genes involved in hepatic gluconeogenesis, fatty acid catabolism, oxidative phosphorylation (OXPHOS), and mitochondrial biogenesis (1-3). Although PGC-1α was originally identified in a yeast two-hybrid screen of PPARγ-interacting factors in a brown adipocyte cDNA library (4), it is now known to coactivate myriad nuclear receptor and non-nuclear receptor transcription factors in a variety of cell types (1). Expression is enriched in tissues with high capacity for mitochondrial OXPHOS including heart, skeletal muscle, and brown adipose tissue (1,4). Although hepatic PGC-1α levels are relatively low in normal, ad libitum-fed mice, its expression is robustly induced by acute food deprivation or diabetes mellitus (5,6), states when rates of fatty acid oxidation and gluconeogenesis are increased. Overexpression of PGC-1α in liver transcriptionally activates genes involved in hepatic gluconeogenesis, fatty acid catabolism and OXPHOS (2,3,6,7) whereas acute loss-of-function (adenoviral-driven RNAi) markedly down-regulates expression of genes involved in each of these processes (8). Similarly, liver-specific PGC-1α gene deletion in mice impairs expression of gluconeogenic genes in response to acute food deprivation (9).

Surprisingly, recent studies of two independently-derived strains of mice in which the PGC-1α gene was constitutively disrupted in a whole-animal fashion (PGC-1α−/− mice) have shown that the expression of many known PGC-1α target genes was unaltered (10) or only modestly altered in liver (11). Despite this, rates of fatty acid β-oxidation (10), mitochondrial respiration (10), and oxygen consumption (11) were significantly diminished in hepatocytes from PGC-1α−/− mice. Moreover, one PGC-1α−/− mouse line exhibited significant hepatic steatosis following a 24 h fast, likely due to diminished capacity for FAO (10).

Although previous studies have provided significant evidence implicating PGC-1α in the transcriptional control of genes encoding enzymes involved in gluconeogenesis, FAO, and the TCA cycle, less is known about the impact of this coactivator on metabolic flux through these key pathways in intact liver. PGC-1α−/− mice provide a unique opportunity to address this issue. Accordingly, we studied the isolated perfused liver of PGC-1α−/− mice by deuterium and 13C NMR spectroscopy isotopomer analysis. These studies were complimented with gene expression analyses examining multiple genes encoding enzymes in the relevant hepatic metabolic pathways. Surprisingly, we found that despite marked deficits in rates of gluconeogenic flux in liver of fasted PGC-1α−/− mice, gluconeogenic gene expression was normal under fed and fasted conditions. Rates of fatty acid β-oxidation and TCA cycle flux were also defective in PGC-1α−/− mice, which correlated with diminished expression of TCA cycle enzymes and genes involved in OXPHOS. Taken together, these data suggest that gluconeogenic deficits in PGC-1α−/− mice are secondary to deficits in mitochondrial oxidative metabolism and TCA cycle activity, but not gluconeogenic enzyme expression.

RESEARCH DESIGN AND METHODS

Animal Studies. The generation and general characterization of PGC-1α−/− mice was recently described (10). Six week old PGC-1α−/− mice, with age- and sex-matched wild-type (WT) control mice, were employed. Short term fasting studies were performed with individually-housed mice which were either food deprived for 24 h or given ad libitum access to normal mouse chow. For adenoviral injection, C57BL/6 mice were injected i.v. with adenovirus driving expression of GFP or PGC-1α as previously described (12) and sacrificed 5 d later for tissue collection.
Liver Glycogen. Hepatic glycogen content was determined as described by Passonneau and Lauderdale (13) using freeze-clamped liver tissue from WT or PGC-1α KO mice fasted for 24 h.

Liver Perfusion Experiments. Livers were isolated and perfused from 24-hour fasted mice as previously described (14). Briefly, a mid-line laparotomy was performed to expose the liver and portal circulatory system. The liver was heparinized and the portal vein was cannulated. The hepatic vein and inferior vena cava were dissected and the perfusate flow through the portal vein was started simultaneously with a peristaltic pump at 8 ml/min in a non-recirculation circuit. The liver was suspended in a beaker containing effluent perfusate at 37 °C. Per fusate was siphoned off and stored on ice. The perfusate was composed of Krebs-Henseleit bicarbonate buffer containing 1.5 mM lactate, 0.15 mM pyruvate, 0.25 mM glyc erol, 0.2 mM octanoate, 0.2 mM [U-13C]propionate and 3% v/v D2O. Oxygen consumption was measured by oxygen electrode. Fractions (2 ml) of perfusate were collected at 10 minute intervals and stored at -80° C until assay for glucose. Liver perfusions were performed for 60 minutes and the last 30 minutes of perfusate was combined for NMR analysis (n = 5 WT and 6 PGC-1α −/−). Acetoacetate and β-hydroxybutyrate production was measured in a separate group of animals under the exact same conditions (n=5 WT and 6 PGC-1α −/−). Upon completion, the liver was freeze clamped and stored at -80° C until further analysis.

Sample Preparation and NMR Analyses. Glucose was isolated from the effluent perfusate and then converted to its monoacetone glucose (MAG) derivative as previously described (14). MAG was then analyzed by H (15,16) and 13C (15) NMR spectroscopy at 14.1T using a broadband probe tuned to 92 and 150 MHz respectively. Peak areas in the resulting spectra were measured using the peak fitting routine in the spectral analysis program NUTS (Acorn NMR Inc., Freemont, CA).

Metabolic Profile. Metabolic fluxes were calculated from the NMR peak areas and biochemical assay of glucose as previously described (14,17). Deuterium NMR spectra of MAG were used to determine the relative 2H enrichments of glucose at the H2, H5 and H6s positions. In turn, these enrichments were used to calculate the relative fractions of glucose production from glycolysis (GLY), gluconeogenesis from glycerol (GNGglycerol) and gluconeogenesis from phosphoenolpyruvate (PEP) originating from lactate or amino acids via the TCA cycle (GNGPEP) (15,18,19). Absolute fluxes were determined by multiplying the relative fluxes by total glucose production (14,17).

Anaplerosis and pyruvate cycling fluxes were determined from the C2 multiplets in the 13C NMR spectra of MAG using previously reported equations (14,17,20). Hepatic anaplerosis must be balanced by disposal pathways which, under typical conditions, in the liver is dominated by flux through PEPCK, but may also have minor contributions, for instance from the malic enzyme. For simplicity we refer to this measurement as PEPCK, though it represents the total disposal fluxes whose sum must equal anaplerosis, and thus is a maximal estimate of PEPCK. The portion of anaplerosis contributed by pyruvate cycling could be, again, from the malic enzyme (Pyr → OAA → Mal → Pyr) or from pyruvate kinase (Pyr → OAA → PEP → Pyr) and it should be pointed out that these two pathways cannot be distinguished from each other using the tracer technique employed here. The difference between anaplerosis and pyruvate cycling is equal to gluconeogenesis from PEP which allows the absolute fluxes determined by glucose production and the deuterium NMR data to be extended to the fluxes intersecting the TCA cycle.

β-oxidation (octanoate units) was calculated from ketogenesis and citrate synthase flux (CS). Rates of fatty acid β-oxidation were calculated under the assumption that citrate synthase flux and ketogenesis represent the only fate of β-oxidation derived acetyl-CoA (see equation 1).

(1) β-oxidation = (CS + 2 x ketogenesis) / 4

Where CS is in 2 carbon units of acetyl-CoA, ketogenesis is in 4 carbon units (2 acetyl-CoA) and β-oxidation is in 8 carbon (octanyl) units. The sum of CS and 2 times ketogenesis is divided by 4 because there are 4 acetyl-CoA generated per octanoate.

Analytical measurements. Perfusate fractions designated for analytical analysis (2ml
fractions) were thawed and extracted with perchloric acid prior to assay. Glucose was assayed by standard enzyme coupled reactions (21) and this data was used to determine the rate of hepatic glucose production (GP). Acetoacetate (ACAC) and β-hydroxybutyrate (BHB) were measured by the method of Williamson et al. (22). The rate of ACAC and BHB production were summed to represent the rate of ketogenesis.

Frozen liver tissue was divided and PCA extracted for analysis of ACAC, BHB (100mg), HPLC analysis of adenylate nucleotides (100 mg) and 13C NMR analysis (1 g). Ketone concentrations in tissue extracts were determined by enzyme linked assays (22). The HPLC assay for ATP, ADP and AMP was performed as described by Stochi et al. (23) on a Dionex (Palo Alto, Ca) HPLC system equipped with UV detector and Supelco C18 reverse phase column.

Hepatocyte Isolation and Metabolic Analyses. Primary cultures of mouse hepatocytes were obtained from WT and PGC-1α−/− mice as described (24). For gene expression analyses with isolated hepatocytes, cells were stimulated for 6 h with vehicle or dexamethasone (1 μM) and 8-bromo-cAMP (1 mM).

Quantitative Real-time RT-PCR. First-strand cDNA was generated by reverse transcription using total hepatic RNA. Real-time RT-PCR was performed using the ABI PRISM 7500 sequence detection system (Applied Biosystems, Foster City, CA) and the SYBR green kit. Primer sets were designed to span exon splice borders and are shown in Supplemental Table 1. Arbitrary units of target mRNA were corrected by measuring the levels of 36B4 RNA.

Statistical Analyses. For quantitative data, statistical comparisons were made using analysis of variance (ANOVA) coupled to Scheffe’s test or students t-test assuming unequal variances. All data are presented as means ± SEM, with a statistically significant difference defined as a P value < 0.05.

RESULTS

PGC-1α-deficient livers have diminished gluconeogenic flux. Livers from 24-hour fasted PGC-1α−/− mice and their littermate controls were isolated and perfused with a non-recirculating perfusion media for 60 min. The PGC-1α−/− livers produced 60% less glucose over the last 45 min of the perfusion (Figure 1a). To determine the source of the glucose produced in these experiments, deuterated water was included in the perfusion media and the effluent glucose was analyzed by 2H NMR (Figure 1b). A lower H5/H2 ratio suggests a lower fractional contribution of gluconeogenesis and a higher contribution of glycogenolysis to glucose production (18) in PGC-1α−/− livers compared to WT controls (Figure 1c). There was no difference in the (H5-H6s)/H2 ratio (15) between groups, indicating that the fraction of glucose production due to gluconeogenesis from glycerol was unchanged. However, the H6s/H2 (19) ratio was significantly decreased in PGC-1α−/− livers, indicating a decrease in gluconeogenesis from PEP as a fraction of glucose production (Figure 1c). The finding that glycogenolysis was a significant source of glucose after a 24-hour fast was surprising, but agreed with a two-fold elevation in liver glycogen content in fasted PGC-1α−/− mice versus fasted WT mice (Figure 1d). These data suggest that hepatic glycogen cycling is altered in PGC-1α−/− mice, perhaps to allow for significant glycogenolysis to compensate for a relative reduction in gluconeogenic flux following prolonged fasting. Nevertheless, absolute glycogen levels were still low compared to the fed state.

Therefore, to determine which pathways contributed to decreased glucose production (v1 in Figure 2a) in the PGC-1α−/− liver, the absolute flux through glycogenolysis, GNGglycerol, and GNGPEP (v2, v3, and v4, respectively in Figure 2a) pathways was quantified. Despite the increased fraction of glucose derived from glycerol in the PGC-1α−/− livers, there was no difference in absolute rates of glycogenolysis between PGC-1α−/− and WT livers (Figure 2b) due to the overall decrease in glucose production. In addition, the flux from glycerol to glucose (GNGglycerol) was not significantly different between the WT and PGC-1α−/− livers. However, absolute flux through GNGPEP was dramatically decreased in PGC-1α−/− livers (Figure 2b) indicating that the primary defect in glucose output was at the level of gluconeogenesis from substrates which pass through the TCA cycle (e.g. lactate, pyruvate or
amino acids) via the combined activity of PC and PEPCK.

To investigate flux through the PEP pathways, we measured TCA cycle anaplerosis and pyruvate cycling (as illustrated in Figure 2a) by 13C NMR isotopomer analysis of the effluent glucose from PGC-1α−/− and control livers. Absolute flux through the pathway mal/OAA → pyr/PEP (v6 in Figure 2a), was halved in PGC-1α−/− livers compared to WT livers, indicating that PEPCK flux was remarkably impaired (Figure 2c). Figure 2c also shows that PGC-1α−/− livers had decreased flux through pyruvate kinase or malic enzyme catalyzed pyruvate cycling (v5) (Figure 2c) as determined by 13C NMR isotopomer analysis. This may be a compensatory response to decreased PEPCK flux to augment GNG by sparing PEP from this “futile cycle”. Without the attenuated pyruvate cycling, gluconeogenesis would be close to zero in the PGC-1α−/− livers.

PGC-1α is not required for basal or fasting-induced expression of gluconeogenic enzymes. Since NMR isotopomer analyses indicated that gluconeogenesis, especially via the PEPCK pathway, is defective in PGC-1α−/− mice, we examined the fasting-induced expression of genes encoding PEPCK, glucose-6-phosphatase (G-6-P), and PC in PGC-1α−/− livers. Surprisingly, the hepatic expression of PEPCK, G-6-P, and PC was equally and robustly induced by fasting in WT and PGC-1α−/− mice (Figure 3). The expression of gluconeogenic enzymes was also evaluated in isolated hepatocytes stimulated ex vivo with 8-bromo cAMP and dexamethasone, which is known to induce PGC-1α and gluconeogenic gene expression. PGC-1α deficiency again did not affect the activation of PEPCK or G-6-P gene expression in response to this stimulus (Figure 4). Collectively, these data indicate that PGC-1α is not required for activation of gluconeogenic gene expression in response to acute fasting or gluconeogenic stimuli and suggest the existence of PGC-1α-independent regulatory mechanisms.

PGC-1α-deficient livers have diminished energy production. Since PGC-1α is a well-recognized transcriptional regulator of fatty acid catabolism, OXPHOS, and mitochondrial biogenesis (2,3), flux through biochemical pathways important for hepatic energy homeostasis was also measured. We found that hepatic oxygen consumption in the isolated perfused PGC-1α−/− livers was reduced by 25% compared to control livers (Figure 5a). In addition, total ketone (ACAC and BHB) production (v8 in Figure 2a) was decreased 30% in the PGC-1α−/− liver compared to controls (Figure 5a). Carbon-13 isotopomer analysis of the effluent glucose revealed a two-fold impairment of TCA cycle citrate synthase flux (v7 in Figure 2a) in PGC-1α−/− livers versus WT controls (Figure 5b). β-oxidation of octanoate (v9 in Figure 2a) was also decreased by 25% in the PGC-1α−/− liver (Figure 5b), which is consistent with our previous report using 3H-palmitate in isolated hepatocytes (10). These findings collectively indicate a significant energetic disadvantage due to loss of PGC-1α function.

Decreased flux through β-oxidation and the reactions of the TCA cycle suggested decreased mitochondrial NADH production. To investigate whether mitochondrial redox state was impacted, liver tissue was extracted after perfusion and assayed for the redox pair ACAC and BHB. Surprisingly, PGC-1α−/− livers had a 2-fold higher BHB/ACAC ratio compared to control livers (Figure 5a). In PGC-1α−/− liver, but did not reach statistical significance and we found no difference in the calculated adenosine energy charge (AEC) (Figure 5d).

PGC-1α controls the expression of TCA cycle and OXPHOS enzymes in liver. We previously demonstrated that, despite reduced rates of FAO in hepatocytes isolated from PGC-1α−/− mice, the expression of several genes involved in FAO (carnitine palmitoyltransferase 1α, very long-chain acyl-CoA dehydrogenase, and
medium-chain acyl-CoA dehydrogenase) was unaffected by PGC-1α deficiency (10), which is notable given the significant defect in β-oxidation flux. Therefore, we examined the expression of genes encoding TCA cycle and OXPHOS enzymes. We found that the expression of the TCA cycle enzymes, citrate synthase (CS), isocitrate dehydrogenase 3α (IDH), and succinate dehydrogenase subunit A (SDH) was significantly diminished in liver of PGC-1α KO mice (Figure 6a), whereas the expression of malate dehydrogenase 2 (MDH) was not altered. As has been reported in other tissues of PGC-1α-/- mice, the expression of several genes encoding enzymes involved in OXPHOS, including cytochrome C (CytC), CytC oxidase 2 (COX2), COX4, and the β-subunit of ATP synthase was also diminished (Figure 6b).

Given the apparent crucial role of TCA cycle deficiency in the control of hepatic energy and glucose homeostasis by PGC-1α, we examined whether PGC-1α activation was sufficient to induce the expression of TCA cycle genes. Wild-type mice were injected i.v. with an adenovirus driving expression of murine PGC-1α and/or GFP (vector control) and hepatic gene expression examined 5 days post-infection. As predicted from the loss-of-function studies, the expression of CS, SDH, IDH, MDH was robustly activated by PGC-1α overexpression in liver of mice (Figure 7a). The expression of CytC, COX2, and COX4 was also strongly induced by PGC-1α (Figure 7b). Collectively, these combined gain-of-function and loss-of-function studies identify multiple enzymes in the TCA cycle as target genes of PGC-1α in liver.

DISCUSSION

A flurry of recent studies has shown that the PGC-1 family of coactivators transcriptionally regulates enzymes involved in mitochondrial OXPHOS, FAO, and glucose homeostasis (1-3). Consistent with this, we demonstrate that chronic PGC-1α deficiency leads to significant impairments in hepatic β-oxidation, TCA cycle, and gluconeogenic flux. Altered metabolic flux through these pathways correlated to decrements in the expression of multiple enzymes in the TCA cycle and OXPHOS pathways. However, deficits in hepatic gluconeogenic and mitochondrial fatty acid β-oxidation flux observed in PGC-1α-/- mice did not correlate with altered expression of key enzymes involved in gluconeogenesis or fatty acid catabolism. Based on these findings, we postulate that the gluconeogenic and β-oxidation defects in PGC-1α-/- mice are secondary to TCA cycle, OXPHOS, or generalized mitochondrial dysfunction and suggest that these findings elucidate novel mechanisms by which diminished PGC-1α activity impacts glucose and fatty acid homeostasis.

The hepatic PGC-1α system is activated in both type 1 and type 2 models of diabetes mellitus (5,6). Because PGC-1α transcriptionally activates the expression of genes encoding gluconeogenic enzymes, PGC-1α overactivity is thought to contribute to uncontrolled hepatic glucose production in the diabetic state. In support of this, RNAi-mediated knockdown of hepatic PGC-1α improved glucose homeostasis in a rodent model of diabetes (8). In contrast to the strong activation observed in liver, the expression of PGC-1α and several downstream target genes involved in OXPHOS is actually diminished in skeletal muscle of diabetic patients (26). Whether skeletal muscle PGC-1α system inactivity plays a causative role in the development of diabetes or is a secondary consequence of metabolic perturbations of the disease is still unclear. However, it has been postulated that PGC-1α system deficiencies may exacerbate lipid accumulation and drive the development of skeletal muscle insulin resistance. Interestingly, PGC-1α-/- mice exhibit enhanced insulin sensitivity on standard chow and are protected against high fat diet-induced insulin resistance (10,11). These findings prompted us to examine the effects of PGC-1α deficiency on hepatic metabolic flux; particularly because PGC-1α influences hepatic glucose production, a principal constituent of whole-body glucose homeostasis. The marked impairment we observed in hepatic glucose production may explain, in part, the enhanced insulin-sensitivity of PGC-1α-/- mice.

The hepatic metabolic phenotype of PGC-1α-deficient mice is reminiscent of mice nullizygous for PPARα, a liver-enriched transcription factor partner of PGC-1α, which also exhibit defects in hepatic fatty acid oxidation and
glucose production (27) and are insulin sensitive (28-30). PPARα−/− mice exhibit normal hepatic PEPCK and G-6-P expression under fasting conditions (27,31), but are severely hypoglycemic (32). We postulate that defects in mitochondrial metabolism underlie the observed defects in hepatic gluconeogenesis in both PGC-1α−/− and PPARα−/− mice. This notion is supported by other genetic models of altered mitochondrial energy metabolism. For example, ablation of β-oxidation enzymes leads to hypoglycemia during fasting (33,34) while children with inborn errors in mitochondrial FAO or OXPHOS often present with hypoglycemia secondary to defects in gluconeogenesis (35-37). The precise lesion (i.e., β-oxidation, TCA cycle, or OXPHOS) that leads to this metabolic bottleneck in this and other models is unclear and will require further study. Conversely, in mice with a liver-specific knockout of PEPCK, TCA cycle flux is impaired (14) despite up-regulation of some TCA cycle enzymes (38). Collectively, these studies indicate that cataplerosis related to GNGPEP and TCA cycle flux are exquisitely interdependent (14,39,40) and support the existence of bi-directional cross-talk between hepatic energy generation and gluconeogenic pathways. We propose that in the PGC-1α−/− liver, impaired hepatic energy production necessarily inhibits the energetically costly process of gluconeogenesis. Interestingly, GNGglycerol (the conversion of glycerol to glucose), which occurs in the cytosol and results in net production of ATP, was unaffected in PGC-1α−/− livers.

Given the strong activation of gluconeogenic enzymes following PGC-1α overexpression (6), our finding that PGC-1α is not required for full expression of these enzymes, especially during fasting when PGC-1α is induced, is surprising. Previous work demonstrated that liver-specific PGC-1α deficiency attenuated the fasting-induced activation of PEPCK and G-6-P (9). Acute RNAi-mediated knockdown of PGC-1α in liver also caused a profound down-regulation of gluconeogenic enzyme gene expression (8). In contrast, the expression of gluconeogenic enzymes in the two models of constitutive whole-animal PGC-1α deficiency was unaltered (current study) or actually increased (11). However, it should be noted that gluconeogenic gene expression in response to dexamethasone and forskolin was defective in the other constitutive PGC-1α−/− mouse strain (11). It is likely that the differences in gluconeogenic gene expression amongst the various models of PGC-1α deficiency are explained by whole-animal versus liver-specific deficiency or related to the developmental timing of PGC-1α deactivation. The data obtained from the two chronic, whole-animal PGC-1α-deficient models suggest compensatory adaptations by other transactivators. Two related proteins with regions of homology to PGC-1α (PGC-1β and PGC-related coactivator (PRC)) have been identified as part of the PGC-1 family. Whereas PGC-1β functionally overlaps with PGC-1α in its effects on mitochondrial FAO and OXPHOS, the β isoform has distinct effects on gluconeogenic and lipogenic gene expression (7,41). Overexpression of PGC-1β fails to drive a gluconeogenic response and interacts poorly with HNF4α and FOXO1, transcription factors controlling PEPCK and G-6-P expression (7). To our knowledge, the effects of PRC on gluconeogenesis have not yet been characterized. Additionally, TORC2, a transcriptional coactivator outside of the PGC-1α family, has recently been shown to stimulate gluconeogenesis in fasted liver (42). In the context of the constitutive PGC-1α-deficient liver, other transcriptional coactivators likely compensate for PGC-1α to transactivate the expression of gluconeogenic genes.

The finding that TCA cycle enzymes are direct targets of PGC-1α is not surprising given that PGC-1α controls many other aspects of mitochondrial oxidative metabolism. As was recently demonstrated in skeletal muscle (43), PGC-1α activation coordinately induces multiple pathways (β-oxidation, TCA cycle, and OXPHOS) to synchronize the capacity of the entire ATP synthesis pathway of the mitochondrion. Our findings suggest that PGC-1α deficiency leads to a coordinate deactivation of each of these metabolic pathways and reaffirm the critical role that PGC-1α plays in controlling energy homeostasis in liver.

**Summary**

In summary, PGC-1α loss of function caused decreased hepatic expression of enzymes in
the TCA cycle and the electron transport chain. However, PGC-1α deficiency did not impact the expression of known PGC-1α target genes involved in fatty acid β-oxidation or gluconeogenesis, suggesting compensatory changes in the transcriptional control of these pathways in response to chronic PGC-1α deficiency. Nevertheless, primary defects of the TCA cycle and OXPHOS pathways caused impaired flux through fatty acid β-oxidation, the TCA cycle, anaplerotic pathways, and GNGPEP. These studies unveil novel mechanisms of PGC-1α action and identify biochemical pathways that may be most impacted by altered PGC-1α activity in pathologic states, including obesity-related insulin resistance and diabetes.

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FIGURE LEGENDS

Figure 1. Sources of substrate used for glucose production in the PGC-1α−/−mice. [a] The graph depicts mean (± SEM) glucose production by isolated perfused livers from WT and PGC-1α−/−mice determined by glucose assay of the perfusate. [b] Deuterium NMR spectra of MAG derived from glucose produced by the isolated perfused liver. The peak area of H2, H5 and H6s are used to determine the relative contributions of glycogenolysis, gluconeogenesis from glycerol and gluconeogenesis from PEP. [c] The NMR data indicates that PGC-1α−/−livers form a greater fraction of their glucose production from glycogen and less from PEP compared to control livers. [d] The graph depicts mean (± SEM) hepatic glycogen levels in WT or PGC-1α−/−mice given ad libitum access to food or after a 24 h fast. *p < 0.05 versus WT fasted mice.

Figure 2. Defects in hepatic gluconeogenesis and altered flux through gluconeogenic pathways in PGC-1α−/−mice. [a] The diagram illustrates the metabolic pathways under investigation by the combination of deuterium and 13C tracers. The three major sources of glucose production in liver are denoted v2 (glycogenolysis, GLY), v3 (gluconeogenesis from glycerol, GNGglycerol), v4 (gluconeogenesis from phosphoenolpyruvate, GNGPEP). PEPCK flux is a major constituent of total efflux from the hepatic TCA cycle, which is estimated as total anaplerosis by the 13C NMR spectra of perfusate glucose and represented by v6. Pyruvate cycling, v5, denotes pathways such as pyruvate kinase or the malic enzyme which regenerate pyruvate rather than contributing to gluconeogenesis. [b and c] The graphs depict mean (± SEM) absolute flux through the pathways shown in [a]. [b] The graph shows the absolute flux through pathways leading to glucose production: GLY (hexose units), GNGglycerol (triose units) or GNGPEP (triose units) as determined by deuterium NMR of perfusate glucose. [c] Fluxes contributing to GNGPEP determined from 13C NMR. Mice were fasted 24-hours before the liver perfusion experiment. *p < 0.05 versus WT mice.

Figure 3. Fasting-induced activation of genes involved in gluconeogenesis is normal in PGC-1α−/−liver. Graphs depict mean (± SEM) levels of phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G-6-P), and pyruvate carboxylase (PC) mRNA collected from liver of fed and fasted WT
and PGC-1α-/- mice determined by SYBR green RT-PCR (n=7). Values are normalized to fed WT mice (= 1.0) and corrected to 36B4 RNA levels. *p < 0.05 versus fed mice.

**Figure 4. Normal induction of gluconeogenic enzymes in PGC-1α-/- hepatocytes.** Graphs depict mean levels of PEPCK and G-6-P mRNA collected from hepatocytes isolated from WT and PGC-1α-/- mice determined by SYBR green RT-PCR (n=6). Hepatocytes were treated in culture with vehicle or dexamethasone (1 μM) and 8-bromo-cAMP (1 mM) for 6 h prior to RNA collection. Values are normalized to vehicle-treated WT hepatocytes (= 1.0) and corrected to 36B4 RNA levels in the same sample. *p < 0.05 versus vehicle-treated hepatocytes.

**Figure 5. Defective TCA cycle activity and β-oxidation in isolated perfused livers from fasted PGC-1α-/- mice.** Graphs depict mean (± SEM) values in isolated perfused livers from 24-fasted mice. [a] Perfusate was assayed to measure hepatic oxygen consumption and ketogenesis. The rate of oxygen consumption was measured by oxygen electrode and ketogenesis was determined from the sum of effluent acetoacetate and β-hydroxybutyrate. [b] TCA cycle activity or citrate synthase activity (CS) was measured using a combination of 2H and 13C NMR data from perfusate glucose. Total β-oxidation was determined from ketone production and TCA cycle activity. [c] Perfused livers were extracted and assayed for acetoacetate and β-hydroxybutyrate as an indication of mitochondrial redox state. [d] Total high energy nucleotides in liver extracts were determined by HPLC. *p < 0.05 versus WT liver. **p < 0.1 versus WT liver.

**Figure 6. Diminished expression of genes encoding enzymes in TCA cycle enzymes and genes involved in mitochondrial OXPHOS in PGC-1α-/- mice.** Graphs depict mean (± SEM) levels of [a] citrate synthase (CS), isocitrate dehydrogenase (IDH), succinate dehydrogenase (SDH), and malate dehydrogenase (MDH) or [b] cytochrome C (CytC), CytC oxidase (COX2), COX4, and the β subunit of ATP synthase mRNA collected from liver of WT and PGC-1α-/- mice determined by SYBR green RT-PCR (n=7). Values are normalized to WT mice (= 1.0) and corrected to 36B4 RNA levels. *p<0.05 versus WT mice.

**Figure 7. PGC-1α overexpression drives transcriptional activation of TCA cycle enzymes.** Graphs depict mean (± SEM) levels of [a] CS, IDH, SDH, and MDH or [b] CytC, COX2, and COX4 mRNA collected from liver of WT mice infected with virus driving expression of GFP or PGC-1α as determined by SYBR green RT-PCR (n=7). Values are normalized to GFP-infected mice (= 1.0) and corrected to 36B4 RNA levels. *p<0.05 versus GFP mice.
REFERENCES


Figure 3

**PEPCK**

- **WT**
- **PGC-1α**

**G-6-P**

- **Fed**
- **24 h fast**

**PC**

- **Fed**
- **24 h fast**
Figure 4

**PEPCK**
- **WT**
- **PGC-1α−/−**

**G-6-P**
- **WT**
- **PGC-1α−/−**

Normalized AU

<table>
<thead>
<tr>
<th>Condition</th>
<th>WT</th>
<th>PGC-1α−/−</th>
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<tbody>
<tr>
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</tr>
<tr>
<td>Dex</td>
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<tr>
<td>8 Br cAMP</td>
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</tbody>
</table>

* indicates significant difference.
Figure 6

(a) Normalized AU

- CS
- IDH
- SDH
- MDH

(b) Normalized AU

- CytC
- COX2
- COX4
- ATP synthase β subunit

Legend:
- WT
- PGC-1α⁻/⁻